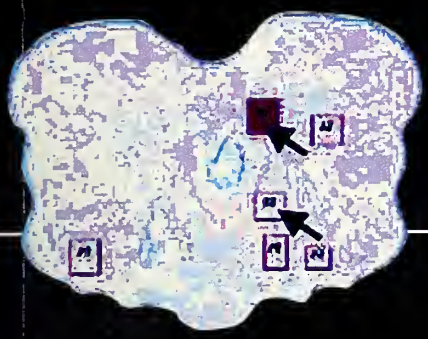
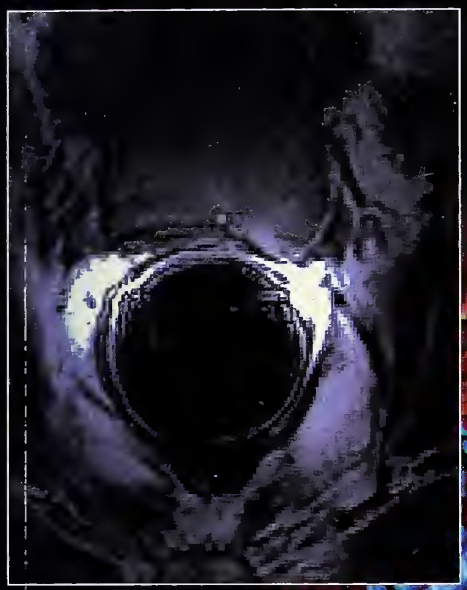
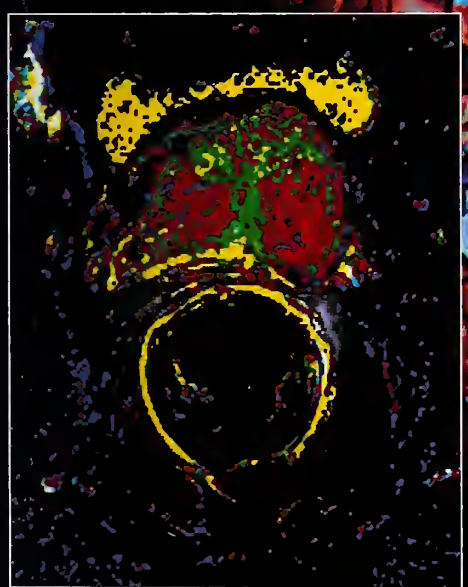
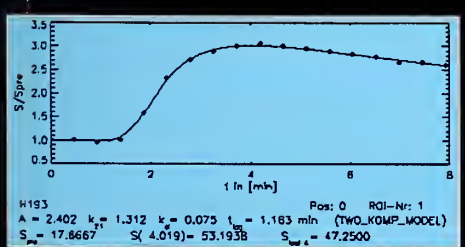


Center for Cancer Research
Annual Research Directory 2002







National Institutes of Health
National Cancer Institute

Cover: *Center: Bovine papillomavirus fully bound by neutralizing monoclonal antibody #9 as seen in a 3D image reconstruction of cryoelectron micrographs. The complex is viewed down a five-fold axis of symmetry. The density due to the antibody is shown in red and the density of BPV1 is shown in blue.*

John Schiller, Laboratory of Cellular Oncology; and Benes Trus, Center for Information Technology

Left, from top to bottom: 3D characterization of the prostate gland by coregistering whole-mount step section histopathology; anatomic MR image; color-encoded Dynamic Contrast Enhanced MRI (DEMRI) depicting areas of vascular perfusion and permeability; and plot of signal intensity over time from prostate cancer.

C. Norman Coleman and Cynthia Menard, Radiation Oncology Branch; Peter Choyke and Michael Knopp, Clinical Center/Diagnostic Radiology Department; reprint permission for the whole-mount step section histopathology obtained from Nature *and from Cole KA, Krizman DB, and Emmert-Buck MR. The genetics of cancer—a 3D model.* Nature Genetics Supplement 1999;21:38–41.

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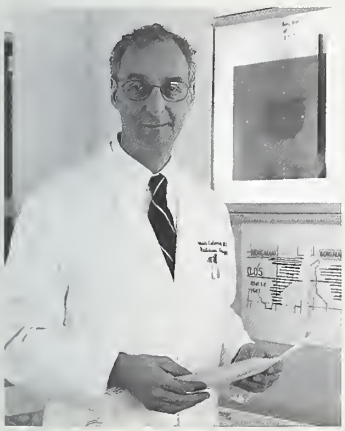
Biography: *Dr. Barrett received his B.S. in chemistry at William and Mary College, Williamsburg, VA, in 1969 and his Ph.D. in biophysical chemistry at Johns Hopkins University, Baltimore, MD, in 1974. Following a 3-year postdoctoral fellowship at Johns Hopkins University, Dr. Barrett began his career at the National Institute of Environmental Health Sciences as leader of the Environmental Carcinogenesis Group, Laboratory of Pulmonary Pathobiology. In 1987, he became chief of the Laboratory of Molecular Carcinogenesis where he conducted research on critical target genes in carcinogenesis molecular mechanisms of environmentally induced cancers. His current research is centered on the relationship between aging and cancer, the genes involved in cellular senescence and differentiation, and the function of KAI–1 and other cancer metastasis suppressor genes. He is editor-in-chief of* Molecular Carcinogenesis, *associate editor of* Cancer Research, *and a member of the editorial boards of 12 journals. He has been a chairperson, organizer, or keynote speaker at various conferences, workshops, and symposia and has authored or coauthored over 400 publications.*



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Biography: *Dr. Coleman graduated from the University of Vermont with a B.A. in theoretical mathematics, then graduated from Yale University School of Medicine in 1970. He completed his internship and residency in internal medicine at the University of California in San Francisco and at the NCI. Board-certified in internal medicine, medical oncology, and radiation oncology, Dr. Coleman was a tenured faculty member of the Departments of Radiology and Medicine at the Stanford University School of Medicine before joining Harvard Medical School in 1985 as professor and chairman of the Joint Center for Radiation Therapy. In 1999, he came to the NCI and became director of the new Radiation Oncology Sciences Program that he created to coordinate radiation oncology activities within the NCI. He also serves the NCI as chief of the Radiation Oncology Branch, deputy director of the Center for Cancer Research, associate director of the Radiation Research Program, and special advisor to the director of the NCI. He has written extensively in his field and has won numerous awards.*

Lee J. Helman, M.D.

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Biography: *Dr. Helman received his M.D.* magna cum laude *in 1980 from the University of Maryland School of Medicine. He completed his clinical training in internal medicine at Barnes Hospital, Washington University, in 1983, and in medical oncology at the NCI Pediatric Oncology Branch and Medicine Branch in 1986. He has held positions of increasing responsibility at the NCI and became branch chief in 1997. Dr. Helman has a wide range of research interests and accomplishments spanning molecular genetics of human cancer and development of new therapeutic approaches to pediatric solid tumors.*

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Biography: *Dr. Lowy received his M.D. from New York University School of Medicine in 1968. Between 1970 and 1973, he was a research associate in the Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, NIH. He trained in internal medicine at Stanford University and dermatology at Yale University, and started his laboratory at the NCI in 1975. He has been chief of the Laboratory of Cellular Oncology since 1983 and a deputy director since 1996. He has received the Wallace Rowe Award for Virus Research and has been a member of many scientific advisory boards, grants committees, and editorial boards.*

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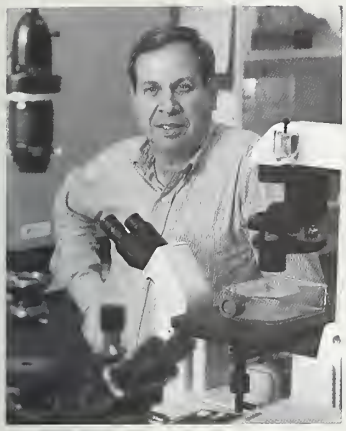
Biography: *Dr. Robert Wiltrout obtained his Ph.D. in immunology from Wayne State University in 1979 and performed postdoctoral studies on the regulation of immune responses with Dr. Ronald Herberman at the NCI. He joined the Laboratory of Experimental Immunology and became head of the Experimental Therapeutics Section in 1986. Dr. Wiltrout has also served as chief of the Basic Research Laboratory since 1999. He has been a member of many scientific advisory boards, extramural review groups, and editorial boards.*



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Biography: *Dr. Yuspa received his B.S. from Johns Hopkins University and his M.D. from the University of Maryland Medical School. He completed his internship and residency at the Hospital of the University of Pennsylvania and has been a senior investigator at the NCI since 1972. Among his honors are the Lila Gruber Award of the American Academy of Dermatology and the Clowes Award from the American Association for Cancer Research. Dr. Yuspa is the author of more than 300 publications in the fields of carcinogenesis and epithelial differentiation.*

Introduction

The NCI's Center for Cancer Research (CCR) was created in March 2001 by merging two vital components of the NCI's Intramural Research Program—the Division of Basic Sciences (DBS) and the Division of Clinical Sciences (DCS). This merger, initiated by former NCI Director Dr. Richard Klausner, is an important step in our goal to promote closer links between basic researchers and clinical investigators, thereby enhancing their opportunities for both scientific discovery and translational research (bench-to-bedside and bedside-to-bench). The CCR is also committed to supporting and training young scientists and clinicians as they launch their careers in basic and clinical research. CCR offers numerous predoctoral, postdoctoral, and clinical training positions with world-class scientists and physicians who are outstanding mentors and experts in their respective fields.

The CCR is composed of over 300 principal investigators in 54 laboratories, branches, and programs. As one of the world's largest cancer research centers, the CCR takes advantage of the breadth of its researchers' training and experience to foster interdisciplinary programs and facilitate translational research. Basic research is a strength of the CCR, with areas of investigation including: immunology; carcinogenesis; human genetics; mouse genetics; viral oncology; HIV; chromatin biology; structural biology; DNA replication and recombination; signal transduction; apoptosis; cell cycle regulation; cytokines and chemokines; cellular, molecular, and developmental biology; medicinal chemistry and natural products chemistry; molecular pharmacology; xenobiotic metabolism; radiation biology; computational biology; and bioinformatics. Areas of excellence in clinical and translational research include: cancer vaccines, clinical proteomics, molecular targets of cancer, molecular imaging, biologic mediators, cell-based therapies, immunotoxin therapy, radiation therapy, cancer genetics, molecular epidemiology, cancer prevention, multidrug resistance, clinical pharmacology, angiogenesis, and molecular pathology. The CCR Web site at **http://ccr.nci.nih.gov** offers detailed descriptions of the basic research and clinical programs as well as links to current CCR clinical trials. The Newsletter and CCR Press section highlight some of the latest published findings from the Center.

New Scientific Opportunities in Interdisciplinary and Translational Research

The CCR mission is to reduce the burden of cancer through exploration, discovery, and translation. The goals of the NCI's restructuring with the creation of the Center for Cancer Research are:

- to foster interdisciplinary research
- to facilitate translational research
- to expedite technology development
- to enhance training, particularly in interdisciplinary and translational research, and
- to build partnerships between NCI and other NIH Institutes, federal agencies, academia, biotechnology companies, and the pharmaceutical industry

The CCR along with the Division of Cancer Epidemiology and Genetics (the other NCI intramural division) has defined new institutional approaches to translate scientific knowledge towards achieving more effective cancer prevention, intervention, and treatment. The goal is to create an integrated, multidisciplinary research environment that brings together scientists from diverse fields to work on translating basic research findings into clinical applications. Collaboration, technology support and development, and access to resources are critical to achieving this goal. The NCI has responded to this challenge by establishing Faculties, which are composed of scientists from diverse laboratories and branches working cooperatively with a common interest in a particular discipline, disease, or approach to scientific discovery. Faculties foster collaboration, open access to new technologies and clinical resources, and challenge NCI researchers to become more involved in clinical research. Within the past year the following Faculties were established:

- Molecular Targets
- Immunology
- Epidemiology, Carcinogenesis, and Prevention
- Chemistry and Structural Biology
- Cellular, Molecular, and Developmental Biology
- Genetics, Genomics, and Proteomics
- Breast Cancer
- Gynecologic Oncology
- Genitourinary Malignancies
- HIV and Cancer Virology
- Bioinformatics, Biostatistics, and Computational Biology

Faculties provide a forum for scientists to engage one another to promote interaction and communication. Their goals are to promote translational and interdisciplinary research; to develop new technologies and resources; to enhance mentoring, recruitment, and training of fellows; to improve communication through retreats and seminars; to sponsor visiting scientists; to provide strategic planning and oversight; and to advise NCI leadership on important and innovative programs critical to the success of the NCI Intramural Program. Visit the Faculties Web site at **http://ccr.nci.nih.gov** for additional information and updates on their activities.

The Medical Oncology Clinical Research Unit (MOCRU) was recently established to enhance the medical oncology clinical infrastructure and to enhance clinical investigation within the CCR. The MOCRU is a group of dedicated physicians who conduct clinical studies on a specific disease or therapeutic area as a team effort. The MOCRU is composed of Clinical Research Sections in breast cancer, genitourinary/gynecologic malignancies, vaccines, lymphoma, transplantation, immunotherapy, AIDS malignancies, lung/gastrointestinal cancer, and clinical genetics. Institutional support for the program is also provided through a Phase I Clinical Research Section, scientific core services, and offices for clinical operations, protocol support, research nurses, nurse practitioners, physicians' assistants, fellowship training, translational research, and Navy-Oncology. The mission of the MOCRU is to provide access to clinical research across the Center, excellence in clinical care, clinical training opportunities, and career growth for clinical staff.

Technology Development and Support

Technology development and support is yet another important goal of the CCR intramural program. Technology initiatives currently under development include clinical proteomics, molecular targets drug discovery, microarray technology, animal models development, and imaging technologies. The proteomics initiative involves the search for new serum markers for cancer; development of antibody chips, protein arrays, and reverse phase chips; a mass spectrometry center; a protein expression laboratory; and bioinformatics support. The molecular targets drug discovery program provides a full range of drug discovery scientific support: advising scientists on molecular target discovery, development of screening assays, conducting screens of pure compound libraries, validation of hits, and assistance in preclinical and clinical development of promising lead compounds. The microarray initiative uses modern lab automation and robotic methods for the production of gene microarrays to allow simultaneous study of the differential expression of large numbers of genes in normal, diseased, or treated cells. The animal models initiative includes transgenic and knockout core services, molecular and comparative pathology support, mouse proteomics, rodent imaging, phenotyping core support, and an animal brain tumor therapeutic and diagnostic core. The imaging initiative incorporates clinical imaging, advanced imaging applications, experimental and innovative technologies, and animal imaging into an interrelated imaging resources program. The CCR Web site at **http://ccr.nci.nih.gov** features an initiatives section with additional details, contact information, and the latest projects.

Mentoring and Training

The CCR places a particular emphasis on training the next generation of investigators in basic, interdisciplinary, and translational cancer research. Programs offered in the CCR include ACGME-accredited residency programs in anatomic pathology, radiation oncology, and dermatology. Additionally, ACGME clinical fellowship training programs in medical oncology, pediatric hematology/oncology, hematology/pathology, and cytology/pathology are available. Fellowships programs in surgical oncology, urological oncology, neuro-oncology, HIV and AIDS malignancy, gynecologic oncology, clinical cancer research for Ph.D.s, cancer epidemiology, cancer genetics, and cancer prevention are also offered. A Senior Clinical Research Fellowship is offered to outstanding clinical fellows to allow a period of intense training in translational research. The Center is involved in the Graduate Program Partnership initiative

that was recently established to attact graduate students to CCR laboratories. Areas of partnership currently under development include bioinformatics, chemistry, and comparative pathology. The Cancer Research Training Award (CRTA) and the Visiting Fellows (VF) program for foreign trainees are available in all the laboratories, branches, and programs. Visit the Web site for career and training opportunities.

Partnerships with Academia and Industry

The CCR is committed to forming partnerships that encourage technology development with industry, academia, and the private sector. CCR scientists and clinicians have a history of successful research collaborations with colleagues nationally and internationally. The CCR is also active in the area of technology transfer and strives to ensure that scientific breakthroughs reach the public through formal agreements between the government and industry. During the last year, there were over 100 active Collaborative Research and Development Agreements (CRADAs) between CCR investigators and outside institutions. For further information, contact the CCR Office of Research and Technology Partnerships at **http://ccr.nci.nih.gov.**

Unique Aspects of the Intramural Research Program

The juxtaposition of basic and clinical researchers in this large, diverse, yet highly interactive Center provides exceptional translational research and training opportunities. With the resources available at the NIH Clinical Center, which houses over 50 percent of the NIH-funded general clinical research center beds in the United States, CCR scientists have a unique environment enabling them to move new drugs and diagnostics quickly from the bench to the bedside. Medical care is provided without charge to patients enrolled on NCI protocols. In addition, patients' travel is covered, and for children and minors participating in intramural studies, travel is also provided for a parent or guardian. One goal of the CCR is to capitalize on this extraordinary clinical resource.

The CCR is a center of excellence for vaccine development and cell-based cancer immunotherapies utilizing specialized expertise, techniques, and facilities that exist within the Intramural Program. An example of the uniqueness of the Intramural Program is seen in the basic and clinical proteomics initiative, which is a collaboration between the NCI and the FDA. The program is built on laser capture microdissection technology, developed in the CCR Laboratory of Pathology, which involves identification and extraction of microscopic homogenous cellular subpopulations from surrounding tissue. This technology is now being used to isolate tumor versus normal cellular subpopulations to identify potential molecular targets for cancer therapies. The long-range commitment needed to develop the technology to accurately identify specific targets for various cancers requires support that is unique to the Intramural Research Program. Another component of the proteomics initiative is the identification of novel markers for early cancer detection.

These types of long-term, high-risk projects can accelerate the pace of medical research with public health importance and have an immeasurable impact on improving the nation's health care.

The Future

With the creation of the CCR, communication, collaborations, and translational research opportunities among the intramural scientists has been increased. To go from bench-to-bedside and bedside-to-bench requires an environment that is not available to most individual investigators or at most research institutions. The CCR is unique in having strong basic and clinical components within the same institutional organization and an institutional infrastructure that facilitates the translation of discoveries from the laboratory to the clinic and, in turn, submits clinical observations back to the laboratory for further analysis.

The CCR and the Intramural Research Program represent an invaluable resource for generating initiatives that will help guide and shape the direction of the NCI. It is expected that the CCR will serve as a model for interdisciplinary and translational biomedical research programs and lead the development of new technologies, provide advanced training for the next generation of cancer scientists, and pioneer new avenues for cancer prevention, diagnosis, and treatment.

J. Carl Barrett, Ph.D.
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National Cancer Institute
National Institutes of Health

Office of the Director Staff

Office of the Director

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C. Norman Coleman	Deputy Director
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Douglas Lowy	Deputy Director
Robert Wiltrout	Deputy Director
Stuart Yuspa	Deputy Director
Greg Curt	Clinical Director
Marquita Broadus	Secretary
Elaine Brown	Secretary
Sandy Carter	Executive Assistant

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Office of the Director (continued)

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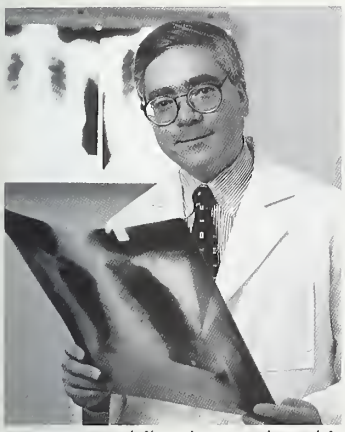
Office of the Director
Office of the Clinical Director



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Biography: *Dr. Curt received his M.D. with distinction in research from the University of Rochester School of Medicine in 1977. He subsequently completed his training in Internal Medicine as an Intern and Resident at the New England Deaconess Hospital and as a Research Fellow at the Peter Bent Brigham Hospital, Harvard Medical School, Boston. Dr. Curt's training in Medical Oncology was completed in the NCI's Medicine Branch, following which he coordinated the intramural Phase I Cancer Drug Development Program while also undertaking basic research into the mechanisms of drug resistance in cancer cells. Dr. Curt served as Deputy Director of the Division of Cancer Treatment before being appointed Clinical Director, NCI, in 1989. His research interests continue in the area of anticancer drug development.*

The Office of the Clinical Director focuses on prioritizing protocols and complementing extramural research efforts within the Division by providing: (1) guidance in navigating investigators through the budgetary, regulatory, governmental, and practical considerations involved in bringing their research from the bench to the bedside; and (2) assistance to CCR researchers in software acquisition, database development, data management and tracking, and protocol analyses. As the primary contact between the NCI and the Clinical Research Center, we have developed a Protocol Resource Office (PRO) to help researchers implement clinical trials with greater efficiency, project long-term clinical research needs, and assure the quality of the NCI's intramural consulting services to the NIH Medical Executive Committee. We regularly evaluate our progress and are pleased to report the following:

- We reorganized the Protocol Review and Monitoring Committee to provide vigorous scientific oversight and to encourage highly innovative clinical trials.
- We are planning to develop new educational modules which define the roles and responsibilities of clinical principal investigators with a new Office of Education.
- We are planning to develop an independent regulatory affairs capacity to file and maintain Investigational New Drug Applications with the FDA for clinical drugs and biological agents developed in the Intramural Program.
- We have begun a comprehensive database development to harmonize the 22 existing clinical informatics systems into a single Web-based system with a standard lexicon.
- We have developed a new capacity for patient outreach and recruitment for accessing patients to the highest priority intramural clinical trials.
- We continue to be the only intramural clinical program to perform real-time quality control and quality assurance through a system of routine audits.

These measures, among many others, have markedly broadened the contributions the CCR is making to the NCI's research—e.g., half of approximately 20 investigational new drug applications filed by the NCI each year are in support of the Intramural Program. The majority of these involve trials with cancer vaccines and new biological agents.

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Jane Cassidy	Senior Nurse Consultant
Doug Hageman	Director, Office of Informatics
John James	Motor Vehicle Operator
Lisa King	Clinical Trials Specialist
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Marianne Owens	Deputy Protocol Assistant
Deb Pearson	Director, Office of Patient Outreach and Recruitment
Diane Reeves	Chief, Office of Clinical Informatics

Cancer Prevention Studies Branch

Philip R. Taylor, M.D., Sc.D.

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The mission of the Cancer Prevention Studies Branch (CPSB) is to develop population- and clinic-based interventions for cancer prevention and early detection, building upon new knowledge of genetic and environmental determinants of risk. Our translational research paradigm in cancer prevention involves the development of preventive interventions and early detection strategies that interface clinical science with molecular science. We focus our translational research efforts on the investigation of gastrointestinal and hormonally-responsive cancers.

There are four selected areas of ongoing research emphasis within the CPSB. These are:

- **Defining the role of select intervention agents in the prevention of epithelial cancers.** Applied research on the role of intervention agents in cancer chemoprevention has been a key emphasis area of the branch since its inception. In addition to our involvement in the design and conduct of randomized clinical trials of cancer prevention, we also conduct mechanistic studies using archived and prospectively collected biological samples to further examine and explain the clinical effects we have observed in these trial populations. Ongoing research places special emphasis on investigating three specific agents or classes of agents: the nonsteroidal anti-inflammatory drugs (NSAIDS), vitamin E, and selenium. A large body of observational and experimental evidence supports an important role for these compounds in cancer prevention. In addition to testing these compounds as cancer chemopreventives in appropriate high risk populations, we are examining their influence on putative molecular mechanisms and measurable intermediate biomarkers of effect.

- **Developing molecular markers for the early detection and prevention of epithelial cancers.** As an extension of our work in the early detection of cancers, we are focused on identifying and developing molecular markers that will improve the sensitivity of current screening methods. Efforts are ongoing to characterize the genetic and proteomic changes that occur during the progression of precursor lesions to invasive cancer of the upper gastrointestinal tract, colon, and prostate. This will enable us to better identify and target lesions that may be more amenable to intervention strategies.

- **Improving clinical methods for the early detection and treatment of epithelial cancers.** Early detection is important for the successful treatment of many cancers, including squamous and glandular carcinomas of the esophagus, which only about 10 percent of patients survive for 5 years after diagnosis principally because most tumors are asymptomatic and go undetected until they are unresectable. Significant reduction in esophageal cancer mortality will require new strategies to detect and treat asymptomatic

precursor lesions and curable early cancers. Research is ongoing to develop and evaluate molecular biomarker-based primary screening tests for both squamous and glandular cancers of the esophagus, to explore approaches for localizing glandular dysplasia and early adenocarcinomas of the esophagus, to evaluate new imaging techniques to distinguish mucosal from submucosal invasion in early esophageal cancers, and to evaluate additional techniques for focal treatment of precursor and early invasive esophageal lesions.

- **Investigating the role of hormonal and dietary factors in the prevention of epithelial cancers in women.** The role of hormones and their interface with diet and other lifestyle factors in relation to cancer risk in women has been an ongoing area of research in the CPSB. We are currently expanding this research to examine the effects of hormones and other exposures throughout the life cycle of women and the effects of hormones on epigenetic mechanisms relevant to cancer risk. Our research focuses on multiple cancer sites in women because of the differential effect of exposures (such as use of exogenous hormone preparations) on risk of colon, uterine, and breast cancers. The goals of this research are to identify both biomarkers of risk and prevention strategies in pregnancy and early childhood that could modify a woman's risk of cancer.

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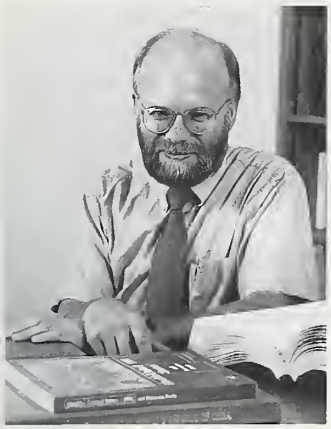

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Biography: *Dr. Taylor received his medical degree from the University of Iowa in 1973 and completed his residency in internal medicine at Vanderbilt University in 1976. He joined the Centers for Disease Control in 1976 as an EIS officer and while there completed a residency in preventive medicine. He received his master's and doctoral degrees in epidemiology from the Harvard School of Public Health, came to the NCI in 1983, and became chief of the Cancer Prevention Studies Branch in 1987. His research interests focus on developing cancer prevention strategies.*

Cancer Prevention Studies Branch
Development of Prevention Strategies for Cancers of the Upper Gastrointestinal Tract and Prostate

Keywords:
breast cancer
cancer prevention
clinical trial
early cancer detection
epidemiology
esophageal cancer
lung cancer
nutrition
prostate cancer

Research: My primary research interests are in the development of prevention strategies for cancers of the upper gastrointestinal tract and prostate, with secondary interests in the prevention of lung and breast cancers. To do this, we have used a variety of different research approaches, including cancer prevention trials, etiologic studies, clinical nutrition studies, early detection, and laboratory-based molecular epidemiology.

Esophageal Cancer

Beginning with initiation of the Nutrition Intervention Trials in over 32,000 residents of Linxian, China, in 1984, our research on esophageal cancer has evolved through the years into an integrated, interdisciplinary collaborative research program that involves studies of etiology, genetics, early detection, and intervention.

The initial goal of the Nutrition Intervention Trials was to evaluate the role of nutrition in the etiology and prevention of esophageal cancer. With the conclusion of the intervention phase in 1991 and the determination that the micronutrient combination of β-carotene/vitamin E/selenium reduced total and cancer mortality as well as stomach cancer, objectives now include evaluation of other (nondietary) environmental exposures (e.g., PAHs, *H. pylori*), genetic susceptibility, and gene-environment interactions. We continue to follow trial participants to ascertain endpoints, conduct nested case-cohort studies using baseline serum (e.g., selenium, vitamin E, and β-carotene), and assess nutritional status; in addition, we have recently collected blood samples to permit expanded DNA and biochemical analyses.

The Esophageal Cancer Genetic Studies were started in 1995 to focus on the role of genetics in upper gastrointestinal cancers. The primary objectives of this project are to identify major susceptibility genes for esophageal cancer and to identify the genetic changes associated with the development of

upper gastrointestinal cancers. Laboratory investigations are being conducted in tumor samples from Shanxi Province in China that include a genome-wide microsatellite scan, loss of heterozygosity deletion mapping, candidate gene mutation analyses, and proteomic analyses for esophageal squamous cell carcinoma. Studies of genetic and proteomic changes associated with neoplastic progression are also ongoing.

The Early Detection of Esophageal Cancer project directed by Dr. Sandy Dawsey includes a series of studies that develop and evaluate different components of an early detection program for esophageal cancer. As part of this project, the Esophageal Squamous Dysplasia Chemoregression Trial in Linxian, China, has also been conducted to determine if selenomethionine and/or a Cox-2 inhibitor will improve regression or reduce progression of dysplasia.

Prostate Cancer

We participate in a formal intramural/extramural clinical consortia that is conducting the SELECT (SELenium and vitamin E Cancer prevention Trial), a large-scale intervention study in 32,400 men to test selenium and vitamin E for the prevention of prostate cancer using extant cancer control clinical networks that are beginning recruitment in 2001.

Utilizing the resources of the Alpha-Tocopherol Beta-Carotene Prevention Trial/Cohort Followup Study, we are evaluating potential mechanisms by which vitamin E might reduce prostate cancer and investigating other nutritional and molecular hypotheses related to prostate cancer.

Lung Cancer

Our research in lung cancer prevention has been based mainly on unique opportunities in foreign countries (i.e., China and Finland) and has involved development of two large cohorts in which we have both evaluated chemoprevention and early detection strategies and searched for etiologic clues. Our most recent activities have focused on nutritional and molecular hypotheses.

The Yunnan Tin Miners Lung Cancer Study was developed in 1992 to evaluate early detection markers for lung cancer in prospectively collected sputum samples from nearly 10,000 miners, to examine the role of environmental (including dietary) and genetic factors in the etiology of lung cancer, and to consider potential intervention strategies.

The Alpha-Tocopherol Beta-Carotene Cancer Prevention Trial is a large intervention study conducted between 1984 and 1993 in Finland in over 29,000 male smokers to test the effect of vitamin E and β-carotene on the prevention of lung and other cancers, and the Alpha-Tocopherol Beta-Carotene Cohort Followup Study is the ongoing followup of trial participants that is primarily evaluating the etiology of nutritional and genetic factors in the development of lung and other cancers.

Breast Cancer

Our research on breast cancer has involved evaluation of modifiable breast cancer risk factors such as alcohol consumption. Recently we completed a controlled alcohol feeding study in postmenopausal women and determined that alcohol ingestion led to increased levels of both estrone sulfate and DHEA sulfate. We are currently exploring alternative mechanisms (e.g., altered expression of DNA repair genes) that might be influenced by alcohol.

Collaborators on this work are Dennis Ahnen, University of Colorado, Denver; Demetrius Albanes, Don Corle, Michael Emmert-Buck, Alisa Goldstein, Victor Kipnis, Maxwell Lee, Steven Mark, Blossom Patterson, Emmanuel Petricoin III, John Schiller, Amy Subar, Fran Thompson, and Ken Tomer, NIH; David Baer, Ellen Brown, Beverly Clevidence, Michael Davies, Joseph Judd, Orville Levander, Kris Patterson, and Claude Veillon, U.S. Department of Agriculture, Beltsville, MD; Martin Blaser, New York University; William Blot, Institute of Cancer Epidemiology, Rockville, MD; Ti Ding, Shanxi Cancer Hospital and Institute, Taiyuan, China; Zhi-Wei Dong, You-Lin Qiao, and Guo-Qing Wang, Cancer Institute, Chinese Academy of Medical Sciences, Beijing; Yener Erozan, Johns Hopkins University, Baltimore, MD; Elaine Gunter and Anne Sowell, Centers for Disease Control, Atlanta, GA; Olli Heinonen, University of Helsinki, Helsinki; Jussi Huttunen, Pirjo Pietinen, and Jarmo Virtamo, KTL, Helsinki; Evelyene Lennette, ViroLab, Berkeley, CA; Paul Limburg, Mayo Clinic, Rochester, MN; Scott Lippman, M.D. Anderson Cancer Center, Houston; Alfred Merrill, Jr., Emory University, Atlanta, GA; Guillermo Perez-Perez, Vanderbilt University, Nashville; Melvyn Tockman, University of South Florida, Tampa; C.S. Yang, Rutgers University, New Brunswick, NJ; Shu-Xiang Yao, Yunnan Tin Corporation, Gejiu, China; and Yingming Zhao, University of Texas Southwestern Medical Center, Dallas.

Recent Publications:

Klein EA, et al. *Prostate Cancer and Prostatic Diseases* 2000;3:145–51.

Hu N, et al. *Clin Cancer Res* 2001;7:883–91.

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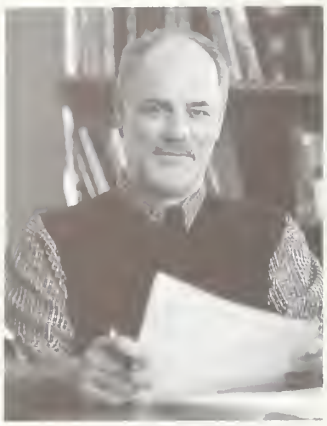

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Biography: *Dr. Tangrea received his M.P.H. and Ph.D. in Epidemiology from the Johns Hopkins University School of Hygiene and Public Health. He joined the Division of Cancer Prevention and Control, NCI, in 1982 and was named deputy chief of the Cancer Prevention Studies Branch in 1989. Dr. Tangrea is a member of the Commissioned Corps of the United States Public Health Service. His research interests include the exploration of molecular targeted interventions in the prevention of cancer, with emphasis on nonsteroidal anti-inflammatory drugs in the prevention of colorectal cancer and the role of peroxisome proliferator-activated receptor activation in prostate cancer prevention. Dr. Tangrea is the recipient of numerous awards including the USPHS Commendation Medal, the Outstanding Service Medal, and the NIH Director's Award.*

Cancer Prevention Studies Branch
Molecular Targeted Interventions in the Prevention of Cancer

Keywords:

chemoprevention
colorectal cancer
NSAIDs
prostate cancer

Research:

Nonsteroidal anti-inflammatory drugs and the prevention of large bowel neoplasia. Over the past 25 years, ample experimental, clinical, and epidemiological evidence has accumulated to suggest that aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) are associated with reduced risk of large bowel neoplasia and cancer. Although experimental support for an antineoplastic effect of NSAIDs has been available since the 1970s, it wasn't until Waddell's observation of a suppressive effect of sulindac on adenomatous polyps in familial adenomatous polyposis (FAP) patients that interest became focused on NSAIDs as potential large bowel neoplasia chemopreventives. Indeed, a number of clinical studies conducted over the past 18 years have confirmed the efficacy of sulindac in FAP polyp regression. In addition, since the first case-control study by Rosenberg et al. in 1991, reports from a number of observational studies have been remarkably consistent in reporting protective associations of 40 to 50 percent for aspirin and/or NSAID use and the risk of large bowel cancer or neoplasia.

We conducted a prospective study examining NSAID use and the risk of recurrent adenomatous polyps among participants in a randomized dietary intervention study, the Polyp Prevention Trial. Information on the use of NSAIDs and other drugs was obtained at baseline and at each subsequent study visit by study coordinator interview and review of participants' current prescription and OTC medications. Adenoma recurrence was based on adenomas detected on complete colonoscopy after 4 years of followup. We observed a significant reduction in overall adenoma recurrence among baseline NSAID users (OR=0.77; 95 percent CI=0.63 to 0.95), with the greatest effect seen in advanced polyps (OR=0.51; 95 percent CI=0.33 to 0.79). We observed a marginal protective effect for NSAIDs on the incidence of multiple polyps at followup (OR=0.84; 95 percent CI=0.65 to 1.10) but saw no

difference in NSAIDs' effect by anatomic location of the polyp (proximal versus distal versus rectal). Aspirin users comprised the majority (69 percent) of those classified as NSAID users. The effect of baseline aspirin alone paralleled that of NSAID use on overall recurrence (OR=0.82; 95 percent CI=0.65 to 1.02) and recurrence of advanced polyps (OR=0.64; 95 percent CI=0.40 to 1.02). We observed a significant dose response effect of aspirin on overall adenoma recurrence, with those taking more than one aspirin (325 mg) per day at baseline exhibiting greater than a 40 percent reduction in risk (OR=0.56; 95 percent CI=0.31 to 0.99). This prospective study provides strong supportive evidence that NSAIDs can play an important role in the chemoprevention of subsequent recurrent adenomas among those patients presenting with adenomatous polyps at screening colonoscopy.

We intend to follow up on this finding by examining the modification of the NSAIDs' protective effect we observed among carriers of the variant alleles for two important metabolizing enzymes, CYP2C9 and UGT1A6. These enzymes are involved in the glucuronidation and hydroxylation of NSAIDs and are polymorphic in the population. The variant alleles result in production of slow-metabolizing enzymes and could influence the bioavailability and elimination of these drugs. One observational study has shown that those who carry the variant allele for one or both of these enzymes have significant attenuation of the protective effect of NSAID use on adenoma risk. We will examine this association in the PPT cohort in a nested case-control study in the near future.

The role of peroxisome proliferator-activated receptor-gamma (PPAR-γ) activation in prostate cancer prevention. Peroxisome proliferator-activated receptors (PPARs) are ligand-inducible transcription factors that belong to the nuclear hormone receptor superfamily, along with receptors for thyroid hormone, retinoids, vitamin D, and steroid hormones. Three different isoforms, PPAR-alpha=α, beta=β (sometimes referred to as delta), and gamma=γ have been identified in humans, each with a specific pattern of expression. PPAR-γ functions as a master regulator of adipogenesis and has been shown to be activated by selected prostaglandins and arachidonic acid metabolites. A new class of antidiabetic drugs, the thiazolidinediones, which have been shown to improve insulin-resistant diabetes and reduce elevated glucose levels, has also been identified as being specific ligands for PPAR-γ.

There is considerable evidence that PPAR-γ ligands may be viable intervention agents for the prevention of prostate cancer progression. The PPAR-γ-specific ligands (troglitazone, rosigliatzone, and PGJ2) have been shown to inhibit the growth of the androgen-insensitive prostate cancer cell line DU–145 (60 percent reduction in cell number compared to control) whereas PPAR-α- or PPAR-β-specific ligands have no effect. In clonal proliferation assays, Troglitazone can inhibit the growth of the highly metastatic PC–3 prostate cancer cells and in concert with a RXR ligand, LG100268, exhibit greater inhibition of PC–3 colony formation. PPAR-γ ligands have also been shown to affect change of prostate-relevant biochemical intermediate endpoints, such as PSA. Thus far, the only animal experimental study has shown that a PPAR-γ–specific ligand can suppress the growth of PC–3 tumor xenografts in immunodeficient mice. In a small, uncontrolled clinical study among patients with locally advanced prostate

cancer, oral troglitazone treatment resulted in prolonged stabilization of serum PSA in a large proportion of patients.

The PPAR-γ ligands exhibit characteristics uniquely suited for potential chemopreventive agents—i.e., preclinical evidence of efficacy, acceptable human toxicity profile, available clinical dosage forms, and measurable molecular and biochemical endpoints. We intend to explore the efficacy and utility of these agents through both preclinical models of efficacy (ex vivo models) and early clinical studies (preprostatectomy model). An ex vivo model using primary prostate cultures will allow the study of prostate tumor characteristics in response to PPAR-γ activation and allow exploration of various molecular pathways as well as phenotypic changes in relation to drug treatment. The preprostatectomy model will allow the validation of these molecular and phenotypic effects taking into account the pharmacokinetics and pharmacodynamics of the specific PPAR-γ ligands.

Our collaborators are Paul Albert, Kevin Camphausen, Norman Coleman, Paul Duray, Michael Emmert-Buck, Elaine Lanza, Marston Linehan, Cynthia Menard, Rita Misra, John Phillips, Duminda Ratnasinghe, Mark Roth, Arthur Schatzkin, and Karen Woodson, NIH; and Judd Moul, Walter Reed Army Medical Center, Washington, DC.

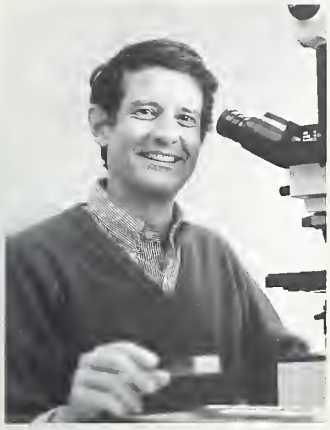
Recent Publications:

Misra RR, et al. *Cancer Lett* 2001;164:161–7.

Ratnasinghe D, et al. *Cancer Epidemiol Biomarkers Prev* 2001;10:119–23.

Woodson K, et al. *Cancer Epidemiol Biomarkers Prev* 2001;10:69–74.

Roth M, et al. *Cancer Lett* 2000;156:73–81.



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Biography: *Dr. Dawsey received his M.D. from Stanford Medical School in 1976 and completed his residency in pathology at the University of Colorado in 1981 and a fellowship in cytology at the University of California, Los Angeles, in 1987. He served as pathologist at the McCormick Hospital in Chiang Mai, Thailand, from 1982 to 1984 and at Saint Joseph Hospital in Denver, Colorado, from 1984 to 1986. He came to the Cancer Prevention Studies Branch at the NCI in 1987 and received tenure in 1995. His main research interest is the prevention and control of upper gastrointestinal cancers.*

Cancer Prevention Studies Branch
Early Detection and Treatment of Esophageal Cancer

Keywords:
chemoprevention
early cancer detection
endoscopic therapy
esophageal cancer
gastric cancer
genetics

Research: Dr. Dawsey's main research activity is the development of clinically useful techniques for the early detection and treatment of esophageal squamous cell carcinoma. Efforts in this area include: (1) histologic studies, to identify the histologic precursors of esophageal squamous cell carcinoma (the target lesions for prevention and control); (2) cytologic studies, to develop an accurate, cost-effective, and patient-acceptable primary screening test for esophageal squamous dysplasia and squamous cell carcinoma; (3) endoscopic localization studies, to endoscopically visualize foci of squamous dysplasia and early squamous cancer; (4) endoscopic staging studies, to develop accurate clinical substaging (T1m versus T1sm) of early esophageal cancer; (5) endoscopic therapy studies, to develop curative endoscopic therapies for high-grade squamous dysplasia and early invasive squamous cell carcinoma; and (6) chemoprevention studies, to evaluate the ability of oral chemopreventive agents to reduce progression or cause regression of low-grade esophageal squamous dysplasia. Most of these studies are carried out in Linxian, China, a county in north central China which has extraordinary rates of squamous esophageal cancer and correspondingly high prevalences of the asymptomatic precursor and early invasive lesions that are needed for these studies.

In addition to his early detection and treatment activities, Dr. Dawsey is also involved in other prevention, etiologic, and genetic studies related to upper gastrointestinal cancers. He is a coinvestigator in the Nutrition Intervention Trials in Linxian, with primary responsibility for endpoint determinations and the evaluation of histologic and cytologic intermediate endpoints, and he actively participates in several nested case-cohort studies correlating baseline NIT measurements with later development of cancer. Dr. Dawsey also supervises studies to evaluate the role of PAH exposure in the etiology of esophageal and gastric cancers in Linxian. He is also a Coinvestigator in a range of genetic studies of upper gastrointestinal cancers in Shanxi Province, China, which may expand understanding of carcinogenesis in these tumors and may provide molecular markers that can be useful for early detection applications.

Many of Dr. Dawsey's research activities are closely integrated with those of other branch members, especially Dr. Philip Taylor, Dr. Mark Roth, Dr. Christian Abnet, and Dr. Nan Hu, and all of his activities support the branch goal of developing new, effective strategies for the prevention and control of cancer.

Recent Publications:

Dawsey SM, et al. *Cancer Epidemiol Biomarkers Prev* 1997;6:121–30.

Roth MJ, et al. *Cancer* 1997;80:2047–59.

Dawsey SM, et al. *Cancer* 1998;83:220–31.

Limburg PJ, et al. *J Natl Cancer Inst* 2001;93:226–33.

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Biography: *In 1977, Dr. Forman received her Ph.D. for studies in nutritional epidemiology at the University of North Carolina, Chapel Hill. Dr. Forman first came to the NIH in 1976 as an epidemiologist in the National Institute of Child Health and Human Development, then spent 3 years as an associate professor at Johns Hopkins University School of Hygiene. In 1989, she returned to the NIH, where she is now a senior investigator in the NCI's Cancer Prevention Studies Branch. Since 1994, Dr. Forman has been a consulting editor with the* American Journal of Clinical Nutrition.

Cancer Prevention Studies Branch
The Role of Early Life Events and Nutritional Status in Reproductive Cancers in Women and in Childhood Cancers

Keywords:
epidemiology
hormones
nutrition

Research: A woman's lifetime exposures to hormones are thought to increase her risk of breast and other reproductive cancers. Epidemiological studies have primarily focused on reproductive risk factors as indicators of adult hormonal status. More recently, research has turned to hormonal and other exposures in the fetal environment, since exposures to hormones in utero are 10-fold higher than in infancy and early childhood. Dr. Forman's research focuses on the effect of birth weight, maternal complications during pregnancy such as preeclampsia, and other perinatal factors as indicators of hormonal exposures in early life of the risk of adult reproductive cancers in offspring. This research, known as the Mother's Study, is being conducted as part of a collaboration with the Nurses' Health Studies at the Harvard School of Public Health, with Dr. Forman and Dr. Walter Willett of Harvard University as the principal investigators. The cohort includes over 100,000 nurse daughters who are currently free of chronic disease and who are being followed to ascertain incident diagnoses of cancer. The mothers of the nurse daughters are asked to report information about early life exposures

including in utero, infant, and early childhood events and practices that might be related (potentially by hormonal, nutritional, and metabolic programming) to risk of adult cancers. The Mother's Study has a retrospective component from the mothers and a prospective followup of their offspring and forms the first prospective cohort study in this field. Currently maternal responses to questionnaires that are either self-administered by the mother or completed with the nurse daughter are being collected, while biannual followup of each Nurses' Health Study cohort occurs.

In collaboration with Drs. Linet and Wacholder of NCI's Division of Cancer Epidemiology and Genetics, Dr. Forman is involved in examining the relation of infant feeding practices to risk of childhood cancer in a case-control study and in examining whether timing of the maternal interview, after diagnosis of a child with cancer, influences the direction and magnitude of maternal reporting of exposures that are currently associated with risk of childhood cancer. The latter study is being piloted and is planned for initial data collection in the late fall of 2001.

Human papillomavirus (HPV) infections are considered the major etiologic risk factor for the development of cervical intraepithelial neoplasia and for invasive cervical cancer. HPV is a transient acute infection in many women, but in others the DNA of the virus becomes integrated into cellular DNA, thereby increasing the risk of developing cancer. As the persistent virus interacts with various host factors, such as immune response, the infection may directly cause cellular and chromosomal damage and the lesion may progress, accumulate additional genetic alterations, and ultimately become invasive. There is an array of HPV cancer-related subtypes because they are associated with increased risk of cervical cancer. As part of a collaboration with colleagues at CICAMS in China and in The Cleveland Clinic, Dr. Forman is examining the relation of vitamin A status and history of bacterial vaginosis to risk of cervical cancer in women. Bacterial vaginosis, like HPV, commonly occurs in women in their twenties and is a transient acute infection that can be treated with antibiotics; however, some women are not responsive to therapy and develop chronic bacterial vaginosis. This condition is associated with alteration of the vaginal environment from an aerobic to anaerobic state, induction of nitrosamine formation, immunological changes, and increased prostaglandin concentrations. Vitamin A, which is indicated by tissue levels of vitamin A and their provitamin A carotenoid precursors, is responsible for epithelial cell proliferation, differentiation, immunological changes, and activation of receptors in cervical cancer. Dr. Forman is examining whether the presence of chronic vaginosis and low levels of vitamin A are associated with increased risk of cervical cancer in a group of women with HPV cancer-related subtypes in a pilot of 1,500 women in Shanxi Province, China.

Dr. Forman's research in the role of nutritional status and its interaction with genetic polymorphisms such as GSTT and GSTM continues as part of a cohort study of tin miners who are at high risk of lung cancer. Data from her diet-biochemical study nested within the cohort study has revealed a seasonal variation in nutrient status (with low folate, vitamins E, A, and C levels) in tin miners as well as identified foods that are associated with serum

levels of these vitamins and other nutrients. Dr. Forman is currently collaborating with members of the Polyp Prevention Trial to examine the relation of serum carotenoid levels to risk of polyp recurrence, the relation of changes in dietary practices to changes in serum carotenoid levels over time, and the analysis of the components of variation in serum carotenoid levels in this population.

Our collaborators are Paul Albert, Barry Graubard, Elaine Lanza, Martha Linet, Luke Ratnasinghe, Susan Steck-Scott, Philip Taylor, and Sholom Wacholder, NIH; Graham Colditz, Karin Michels, and Walter Willett, Harvard University, Boston; and You-lin Qiao and Shu-Xiang Yao, Tin Mine Corporation and CICAMS, China.

Recent Publications:

Forman MR, et al. *Ann NY Acad Sci* 2000;889:230–9.

Ratnasinghe DL, et al. *Alcohol Alcohol* 2000;35:355–60.

Ratnasinghe DL, et al. *Cancer Causes Control* 2000;11:219–35.

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Cancer Prevention Studies Branch
Diet and Colorectal Cancer

Keywords:
adenomatous polyps
cancer prevention
colorectal cancer
diet and cancer

Research: Our overall research goal is to understand the role of diet in the progression and prevention of colorectal cancer. Dr. Lanza is the coprincipal investigator for the Polyp Prevention Trial (PPT). The PPT was a multicenter dietary intervention trial to examine the effect of a low-fat, high-fiber, high-fruit and -vegetable intervention on recurrence of adenomatous polyps of the colorectum. The 2,070 men and women who participated in the PPT were randomized to one of two groups: an intervention group receiving extensive dietary and behavioral counseling assigned to a low-fat (20 percent of calories), high-fiber (18g/1,000kcal) and a high-fruit and -vegetable (3.5 servings/1,000kcal) eating plan, and a control group assigned to follow its usual diet. Of 1,905 who completed the study, 39.7 percent of the intervention group and 39.5 percent of the control group had at least one adenoma. The unadjusted relative risk was 1.0 (0.90 to 1.12). There was also no difference in the average number of adenomas (1.85 intervention versus 1.84 control) in each group or in the number of advanced adenomas (n=60 intervention

versus n=66 control). Even though there were no differences in adenomas between groups, there were extensive biomarker and dietary changes in the intervention group. We will continue to follow participants in a Continued Followup Study, a postintervention passive followup of PPT participants, with the overall aim of comparing the adenoma recurrence rates in the intervention and control groups at 5 years posttrial.

The extensive demographic, lifestyle, and dietary data, as well as biological specimens that included blood, pathology specimens, and normal rectal biopsies collected during the trial, allow the study of the trial participants as an observational prospective cohort. This cohort provides the opportunity to further examine important factors that predict polyp recurrence, to examine the molecular progression and early detection of this disease, and to investigate diet-gene interactions. In addition, the PPT dietary intervention provides data to study the relationship between diet and biomarkers, health and lifestyle, and quality of life factors.

To date, secondary analyses from the main trial focus on prognostic factors for adenomatous polyp recurrence including nonsteroidal anti-inflammatory drugs, physical activity, BMI, smoking, dietary intake, dietary supplements (such as folate and calcium), family history, and alcohol intake.

We are examining somatic lesions from the PPT to characterize the frequency and pattern of genetic alterations in index colorectal adenomas of PPT participants and to explore the relationships between these genetic alterations and polyp recurrence. We are also investigating hypermethylation of promoter-region CpG islands known to be methylated in colon cancer: p16, hMLH1, APC, ER-α, and PR. In addition, we are measuring genomic instability in baseline normal rectal mucosa specimens to see if they are predictors of the recurrence of rectal adenomatous polyps.

We are also beginning diet-gene interaction studies. For the 1,905 participants completing the trial, we have DNA from lymphocytes as well as extensive dietary, lifestyle, demographic, and health data. A number of polymorphisms previously associated with diet and colon cancer will be examined: NAT1, NAT2, CYP1A1, GSTM1, MTHFR, and VDR. We also plan to look at polymorphisms associated with DNA repair such as XRCC1, XPD, APE, MPG, and selenoproteins such as GPX. Since the intervention participants were asked to modify their behavior, we are planning to investigate the differences in DOPA receptor polymorphisms and dietary change.

Finally, as part of the PPT dietary intervention, we collected extensive dietary assessments (FFQ, 4-day food records, and 24 hr recalls) and lifestyle variables. We are currently using these data to examine methodological issues in dietary assessment, factors that influence dietary adherence, and biomarkers of dietary change.

Collaborating on this work are Paul Albert, Rachel Ballard-Barbash, Don Corle, Andrew Flood, Michele Forman, Aleyamma Mathew, Luke Ratnasinghe, Arthur Schatzkin, Rashmi Sinha, Joseph Tangrea, and Karen Woodson, NIH.

Recent Publications:

Schatzkin A, et al. *N Engl J Med* 2000;342:1149–55.

Schoen R, et al. *Gastroenterol* 1998;115:533–641.

McShane LM, et al. *Cancer Epidemiol Biomarkers Prev* 1998;7:605–12.

Caan B, et al. *Pub Health Nutr* 1999;2:565–9.

Clinical Trials:

Sanford Dawsey

OH95–C–N026: Early detection of esophageal cancer. A pilot study of selenomethionine and Celecoxib as chemopreventive agents for esophageal squamous dysplasia in Linxian, China

Cancer Therapeutics Branch

The Cancer Therapeutics Branch was created in July 2001 and is comprised of principal investigators located at the Warren Grant Magnuson Clinical Center and the National Naval Medical Center who conduct basic and/or clinical cancer research with particular emphasis on the following goals: (1) to develop novel therapeutic strategies for cancer that incorporate biochemical, genetic, and pharmacokinetic/pharmacodynamic principles; (2) to investigate the molecular and biochemical profiles that predict clinical response; (3) to develop directed interventions that exploit the difference between normal and tumor cells; and (4) to develop clinical trials that test molecular and pharmacologic hypotheses.

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The clinical efforts focus on devising therapies for patients with solid tumors (ovarian cancer, prostate cancer, colorectal cancer, pancreatic cancer, gastric cancer, lung cancer, renal cell cancer, melanoma, and breast cancer), and early clinical evaluation of investigational new anticancer agents.

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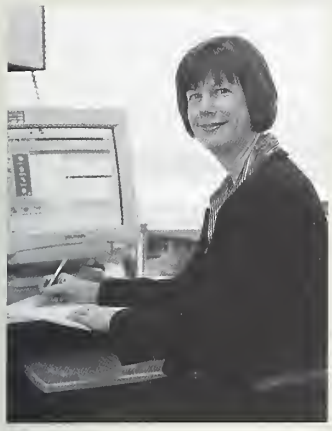

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Cancer Therapeutics Branch
Clinical Trials Research in Breast Cancer Treatment

Keyword:
breast cancer

Research: Dr. Swain has had a longstanding interest in utilizing locally advanced breast cancer as a model for the treatment of breast cancer. Inflammatory breast cancer (IBC) is a clinically and pathologically distinct form of locally advanced breast cancer associated with a rapid growth rate and high metastatic potential. IBC is highly angiogenic and the biology of this form of breast cancer is poorly understood. We are currently studying prognostic markers with a particular interest in angiogenesis markers in inflammatory breast cancer archival tissue and noninflammatory breast cancer taken from patients treated on a primary chemotherapy protocol in Tunisia. We have demonstrated a significantly higher microvessel density in inflammatory versus noninflammatory breast cancer specimens. An increased microvessel density has been shown to have prognostic significance in early stage breast cancer. Other angiogenesis markers are under investigation.

Overexpression of vascular endothelial growth factor (VEGF) has also been shown to be associated with a poorer prognosis in breast cancer. It has been demonstrated that inflammatory breast cancer overexpresses VEGF. We have designed a clinical protocol incorporating an anti-VEGF antibody (bevacizumab) with standard chemotherapeutic agents doxorubicin and docetaxel given in the neoadjuvant setting for patients with previously untreated stage IIIB/IV inflammatory breast cancer. The protocol incorporates the acquisition of tumor biopsies at predefined time points to assess tumor angiogenesis parameters including endothelial apoptosis, endothelial proliferation and tumor VEGF, and other markers and the effects of the bevacizumab alone and bevacizumab and chemotherapy on these parameters. Dynamic MRI imaging will be performed at the same time points as a radiological surrogate measure for tumor vascularity and serum vascular cell adhesion molecule levels as a soluble marker of angiogenesis. After receiving local therapy, patients will receive single agent bevacizumab to complete a total treatment duration of approximately 12 months.

Flavopiridol is a synthetic flavone that inhibits cyclin-dependent kinases. It has been shown to enhance apoptosis when administered after taxanes in human breast cancer cell lines. We are conducting a phase I/II clinical trial of flavopiridol and docetaxel in patients with locally advanced and metastatic

breast cancer. We propose that flavopiridol is synergistic with docetaxel and will increase its efficacy. Incorporated in this study is the serial examination of molecular parameters, cyclin D1, p53, bcl2, and mib1, in accessible tumor biopsies and from the buccal mucosa, as a potential surrogate tissue, to see if desired biochemical effects are achieved with this combination.

The development of target-based anticancer drugs, such as small molecule inhibitors of epidermal growth factor tyrosine kinase (EGFRTK), is becoming an attractive therapeutic strategy in the oncology field. The examination of the effects of these mechanism-based drugs at the cellular level is a logical approach to test these compounds and may in the future replace the traditional way of determining the appropriate dose of new agents. The development of reproducible assays and tools to assess the effect of a drug on targets, such as inhibition of enzyme activity, the validation of surrogate endpoints to check the desired effect of a drug, and the implementation of novel clinical trial designs that allow the testing of biologic endpoints as a primary aim, will become a challenge to both clinicians and basic scientists in the new era of targeted therapy. We are conducting a pilot study of an EGFRTK inhibitor to address these issues.

Adjuvant therapy of breast cancer has clearly provided a significant survival benefit for women in all age groups. Though screening mammography has resulted in earlier detection, still 50 percent of patients with early breast cancer present with node-positive disease. There is a significant recurrence rate in these patients, even with doxorubicin-based chemotherapy. Approaches for increasing the survival include the addition of taxanes. A multicenter trial, NSABP B30, for which Dr. Swain is the protocol chair, is a three-arm trial that will explore the efficacy of docetaxel on survival and quality of life. The three arms include a combination of docetaxel and doxorubicin versus a combination of docetaxel, doxorubicin, and cyclophosphamide, versus doxorubicin and cyclophosphamide followed sequentially by docetaxel. This trial will include 4,000 patients with node-positive breast cancer and has currently accrued 2,100 patients. A quality of life question is being asked in all patients and also specifically in premenopausal women. It has been shown in a meta-analysis of ovarian ablation that this approach increases survival to the same degree as chemotherapy. Therefore, the trial will evaluate ovarian suppression induced by chemotherapy and determine whether this suppression leads to an increased survival. The other quality of life issues relate to the toxicity of the three combinations. Other research areas with NSABP include membership in the Cardiology Advisory Panel for the adjuvant Herceptin® trial (NSABP B31) and Protocol Chair for the Iressa™ trial. Iressa is an epidermal growth factor tyrosine kinase inhibitor and will be evaluated with docetaxel in metastatic breast cancer.

Recent Publications:

McCarthy N, et al. *Oncology* 2000;14:1267–80.

Swain SM. *Semin Oncol* 2001;28:359–76.

Swain SM. *J Natl Cancer Inst* 2001;93:963–5.

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Biography: *Dr. Bates received her M.D. from the University of Arkansas School of Medicine. She completed her clinical training in internal medicine at Georgetown University in Washington, DC, and in oncology at the NCI. Dr. Bates has held positions of increasing responsibility at the NCI, receiving tenure in 1992. Her interests range from clinical studies on drug resistance to laboratory studies on drug resistance in breast cancer and renal cell cancer. Her Molecular Therapeutics Section is dedicated to finding antineoplastic agents that, alone or in combination with other anticancer agents, circumvent resistance.*

Cancer Therapeutics Branch
Reversal of Drug Resistance: Clinical and Laboratory Approaches

Keywords:
drug resistance
multidrug resistance
signaling

Research: Achieving a clinical response to chemotherapy has proven to be simple in some diseases, and almost impossible in others. Our laboratory focus has been on understanding multidrug resistance, which can be broadly defined as the simultaneous resistance to a variety of chemically unrelated chemotherapeutic agents. This multidrug resistance is present at the time of diagnosis in some tumor types, such as in renal cell carcinoma, or occurs following one or more courses of chemotherapy in more responsive diseases, such as breast cancer. As a result of this focus on mechanisms which mediate multidrug resistance, we have examined resistance mediated by membrane transporters as well as by intracellular survival mechanisms. Our goal is to identify antineoplastic agents which circumvent resistance, to use alone or in combination with other anticancer agents.

The most widely examined mechanism of this multidrug resistance is that which is due to the overexpression of P-glycoprotein, the membrane transporter that mediates active outward efflux of antineoplastic agents. We have studied the role that this transporter plays in clinical drug resistance and have pursued agents that are able to block the transport and so overcome resistance. Recently, a new multidrug resistance transporter, MXR, was cloned in our laboratory from human breast cancer and human colon cancer cells highly resistant to mitoxantrone. These cells express high levels of MXR, a half-transporter molecule. MXR confers resistance to several important clinical agents including the newly approved irinotecan and topotecan, plus doxorubicin, mitoxantrone, epirubicin, and an agent currently in clinical development, flavopiridol. Recently, we identified mutations at amino acid 482 that confer altered substrate specificity. Cells with mutation at this residue transport the anthracyclines, while cells with the wild-type sequence have a narrower substrate spectrum. Several inhibitors of MXR have been identified and studies are currently aimed at preclinical development. Identification of this transporter will lead rapidly to studies designed to measure its prevalence in human cancer, determine its clinical significance, and evaluate inhibition of resistance in the clinic.

Our second area of effort is in the development of the depsipeptide FR901228, a novel histone deacetylase inhibitor currently in clinical trials. Our interest in this agent began when we noted depsipeptide to be a substrate for P-glycoprotein-mediated drug efflux. Cells that express high levels of P-glycoprotein are thousands-fold resistant to depsipeptide; one goal is to add a P-glycoprotein inhibitor to depsipeptide for clinical development. In addition, we have pursued the mechanisms responsible for the cell cycle arrest that results from the addition of depsipeptide to sensitive cells. In a phase I trial, we have observed that depsipeptide is able to induce clinical responses in patients with cutaneous and peripheral T cell lymphoma. We have established non-Pgp-expressing depsipeptide-resistant cell lines in the laboratory with the goal of understanding the mechanism of this drug resistance.

Recent Publications:

Gamelin E, et al. *J Urol* 1999;162:217–24.

Knutsen T, et al. *Genes Chromosomes Cancer* 1999; in press.

Miyake K, et al. *Cancer Res* 1999;59:8–13.

Robey R, et al. *Blood* 1999;93:306–14.

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Biography: *Dr. Dennis received his B.A. in 1984 from the University of Virginia and his Ph.D. and M.D. degrees in 1991 and 1992, respectively, from the New York University School of Medicine as part of the Medical Scientist Training Program. He completed his internal medicine training on the Osler Medical Service at Johns Hopkins Hospital. Following his residency, he completed a fellowship in medical oncology at the Johns Hopkins Oncology Center and then joined the laboratory of Michael Kastan where he developed an interest in molecular control of apoptosis. Dr. Dennis joined the NCI in October 1998.*

Cancer Therapeutics Branch
Signal Transduction Pathways in Solid Tumor Cells

Keywords:
apoptosis
cancer
drug resistance
signal transduction

Research: The objective of our laboratory is to understand the molecular determinants of cellular survival that allow tumor cells to escape programmed cell death (apoptosis) when they are exposed to chemotherapy or irradiation. Identifying specific molecules that promote survival will provide new, attractive targets for the development of compounds that abrogate survival signals and enhance therapeutic effectiveness. In addition, we will extend in vitro studies by evaluating survival pathways in tumor specimens and designing clinical trials that rationally incorporate inhibitors of specific survival pathways with traditional forms of cancer therapy.

Cellular survival is determined by factors both within the cell and outside the cell, including the contribution of extracellular influences such as soluble growth factors and extracellular matrix molecules. Both growth factors and extracellular matrix molecules stimulate survival through activation of enzymatic pathways within the cell that involve proteins that either add phosphate to downstream substrates (kinases) or remove phosphate (phosphatases). The best described survival pathways depend on activity from kinases such as PI3K, Akt, PKC, and MAPK, which become activated when they themselves are phosphorylated. Activation can occur after binding of extracellular growth factors to their cognate receptors or, in the case of some tumor cells, activation is independent of extracellular growth factors and is constitutive.

Because lung cancer is the most lethal form of cancer, our laboratory has begun a systematic analysis of lung cancer cells to identify and characterize signaling pathways utilized by these cells to promote cellular survival and resistance to chemotherapy and radiation. We have used pharmacologic and genetic approaches to identify two signaling cascades, the PI3K/Akt and MEK/ERK pathways, that contribute to lung cancer cell survival and therapeutic resistance. In addition, we have shown that these pathways contribute to the survival and therapeutic resistance of other solid tumor cell systems such as breast cancer. Current studies in the laboratory are focused on: (1) mechanisms of regulation between the Akt and ERK pathways; (2) the identification of organ-specific, novel Akt substrates; (3) evaluation of rationally designed, Akt-specific inhibitors; and (4) measuring kinase activities in tumor tissues to establish kinase profiles that might have predictive or prognostic value for patients with cancer. Clinical trials with molecular endpoints in lung cancer are also planned.

Our collaborators in this work are Peter Blumberg, Stanley Lipkowitz, and Len Neckers, NCI; and Ken Kellar and Alan Kozikowski, Georgetown University Medical Center.

Recent Publications:

Comer KA, et al. *Oncogene* 1998;16:1299–307.

Dennis PA, et al. *Drug Resistance Updates* 1998;1;301–9.

Brognard J, et al. *Cancer Res* 2001;61:3986.

Cuello M, et al. *Cancer Res* 2001; in press.

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Biography: *Dr. Figg received his B.S. in pharmacy from Samford University and his Pharm.D. from Auburn University. Following his clinical internship at the University of Alabama at Birmingham and his fellowship training at the University of North Carolina at Chapel Hill, he joined the Clinical Pharmacology Branch of the NCI. Dr. Figg became chief of the Molecular and Clinical Pharmacology Section in 1993. More recently, the Clinical Pharmacology Research Core was formed under the Medical Oncology Clinical Research Core, which he also heads. He holds adjunct appointments at two schools of pharmacy, Virginia Commonwealth University and the University of Maryland. Dr. Figg has focused his research on utilizing pharmacological principles in anticancer drug development and evaluating novel new agents in the treatment of cancer of the prostate. He is Cochair of the NCI's Angiogenesis Working Group.*

Cancer Therapeutics Branch
Preclinical and Clinical Pharmacology and Experimental Therapeutics

Keywords:
angiogenesis
clinical trial
pharmacology
prostate cancer
receptors

Research: A successful drug development program requires a complete understanding of the clinical pharmacology of the agents being evaluated. The primary research interest of the Clinical Pharmacology Research Core (CPRC) is to use pharmacokinetic and pharmacodynamic concepts in the development of novel anticancer agents. The CPRC is directly responsible for the pharmacokinetic/pharmacodynamic analysis of numerous phase I and II clinical trials conducted within the NCI. In addition, the CPRC provides direct pharmacokinetic support for many studies performed elsewhere in the extramural community. Within the section, we utilize compartmental and noncompartmental approaches to define the disposition of agents. Also, we are often required to characterize the plasma protein-binding properties and metabolism of new agents through in vitro techniques. Furthermore, several of our clinical trials have used adaptive control with a feedback mechanism to target particular plasma concentrations.

The activity of traditional cytotoxic chemotherapies in prostate cancer has historically been disappointing. Thus, the Molecular Pharmacology Section (MPS) embarked upon an evaluation of several anticancer agents that appeared to inhibit tumor growth through novel mechanisms. We have conducted either phase I or phase II clinical trials of the following agents in patients with prostate cancer: somatuline (which inhibits insulin-like growth factor); pentosan (which inhibits angiogenesis); gallium (a heavy metal thought to inhibit bone metastasis); high-dose lovastatin (an inhibitor of isoprenylation); phenylacetate (a differentiating agent); phenylbutyrate (a differentiating agent); CAI (a signal transduction inhibitor), COL–3 (an MMP inhibitor), and decitabine (a hypomethylating agent). Although all of these agents showed promise in either laboratory animals with prostate cancer or cultured human prostate cancer cells, clinical activity in humans was not dramatic.

Currently, we are clinically evaluating thalidomide (an angiogenesis inhibitor), thalidomide plus taxotere, and ketoconazole plus alendronate (a bisphosphanate that may inhibit MMPs) in patients with hormone-refractory prostate cancer in four separate studies. We are also evaluating the activity of thalidomide in patients with androgen-dependent prostate cancer. We have recently begun phase I studies of 2ME and CC5013 (both antiangiogenic agents).

In addition, in collaboration with colleagues at the University of Leipsig and the University of Louisville, we have synthesized over 60 thalidomide analogs and have screened them for activity as angiogenesis inhibitors. To this end, we have identified seven extremely potent compounds in four different model systems and are moving those forward through clinical testing.

Originally marketed as a sedative in Europe, thalidomide was not approved in this country because of the teratogenic effects (stunted limb growth in fetuses) associated with the drug. Thalidomide is presently being used as an experimental drug in the treatment of a variety of diseases with autoimmune characteristics. Recently, in vitro data from Harvard University has suggested that thalidomide has antiangiogenic activity. We have followed up on this initial observation to determine that a metabolite of thalidomide is responsible for the antiangiogenic properties. Thalidomide's safety in nonpregnant humans has been established most recently in a study of graft-versus-host disease. Its known side effects include sedation, constipation, and sensory peripheral neuropathy (occurring in 3 percent of subjects). Because the progression of prostate cancer is highly dependent on angiogenesis, we are completing a phase II study in patients with hormone-refractory disease. Of the first 63 patients treated, 68 percent of the patients receiving 200 mg/day had some decline in PSA. This is extremely encouraging considering that we have shown that thalidomide upregulates the expression of PSA at a molecular level.

Currently, in collaboration with several pharmaceutical/biotechnology companies, we are screening for compounds that inhibit angiogenesis. Using in vitro models optimized in our laboratory, we have identified several potential agents.

Recent Publications:

Mueller E, et al. *Proc Natl Acad Sci USA* 2000;97:10990–5.

Kruger EA, et al. *Biochem Biophys Res Commun* 2000;268:183–91.

Dixon SC, et al. *Pharmacol Rev* 2001;53:73–92.

Figg WD, et al. *Clin Cancer Res* 2001;7:1888–93.

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Biography: *Dr. Fojo was born in Havana, Cuba, moved to the United States with his family in 1960, and became a U.S. citizen in 1970. He received his M.D. and Ph.D. from the University of Miami. He completed 3 years of training in internal medicine at Washington University/Barnes Hospital in St. Louis, and after a year as chief resident came to the NCI as a clinical associate in the Medicine Branch, now the Cancer Therapeutics Branch. After 3 years with Drs. Ira Pastan and Michael Gottesman, he assumed the position of senior investigator in the Cancer Therapeutics Branch.*

Cancer Therapeutics Branch
Drug Resistance: Studies of Molecular Etiology—Basic Science, Translational Research, and Clinical Studies

Keywords:
drug resistance
p53
resistance reversal
Taxol

Research: Nearly everyone agrees that drug resistance is a major impediment to effective chemotherapy. Indeed, it could be argued that our problem is not that we lack effective agents, since nearly all agents in common use can cure a variety of cancers, but instead that some cancers are more resistant. An extension of this thesis is the prediction that resistance will be an obstacle all future chemotherapeutic agents will have to overcome. To that end, efforts spent understanding drug resistance now are likely to reap long-term benefits. The available evidence suggests that resistance can be simple or complex but, more importantly, understandable. Clinical evidence suggests that it is also surmountable. High-dose chemotherapy with or without stem cell support represents the most common clinical approach to reverse drug resistance. However, it could be argued that this represents a very nonspecific approach, and that the toxicity of this approach could be lessened, and its efficacy improved, if drug resistance were better understood. The efficacy of high-dose chemotherapy in a subset of malignancies simply demonstrates that resistance is almost never absolute, an observation supported by laboratory studies that show that higher doses overcome all mechanisms of resistance. However, higher doses in patients invariably are associated with and limited by toxicity. Specific therapy designed to interfere with resistance mechanisms should help overcome this obstacle. Based on this, our laboratory efforts have concentrated on the identification of mechanisms of resistance and, equally important, understanding how resistance is acquired. In our opinion, the ultimate goal is not to reverse resistance, but to prevent it from emerging. Our emphasis is on both understanding the basic science and productively conducting translational research. Our clinical efforts emphasize drug resistance reversal trials.

In our laboratory, our efforts began with studies of multidrug resistance mediated by P-glycoprotein, which is encoded by the MDR–1 gene. Our current efforts are directed at understanding how resistance arises. In this regard, we have been able to show that breaks in chromosomes can result in the juxtaposition of drug resistance genes next to very active genes. This

phenomenon, known as gene rearrangement, results in the capture of the drug resistance gene and in its expression at very high levels. In this manner, the cancer cell can readily achieve very high levels of drug resistance. The identification of this as a mechanism of drug resistance is novel but also consistent with the use of this phenomenon by cancer cells to achieve other goals. More importantly, it provides the necessary starting point for understanding how it occurs and, eventually, how it may be prevented. We are actively pursuing this, specifically investigating the role drugs play in the phenomenon of gene rearrangement and how it might be lessened. We have evidence that giving chemotherapy drugs to normal monkeys results in significant damage to the chromosomes of their bone marrow cells, and that the extent of damage can be significantly reduced by administering drugs in different ways. If indeed there are better ways in which to administer drugs that result in less damage to chromosomes, this may prove valuable in preventing the emergence of drug resistance, reducing the likelihood of secondary cancers in cancer survivors as well as lessening the damage to the fetus in the case of a pregnant mother receiving chemotherapy. We know that drug resistance is likely to be caused by different mechanisms, but we think that understanding how it occurs and can be prevented will have wide applications.

Nevertheless, we continue to strive to identify mechanisms of drug resistance that are likely to be clinically relevant. In that regard, in collaborative studies this past year we have succeeded in identifying a new drug transporter, which evidence to date indicates is likely to be as important as P-glycoprotein in mediating resistance. In addition, we have developed additional in vitro models of drug resistance. We have extensively characterized novel, non-P-glycoprotein mechanisms of Taxol and epothilone resistance, demonstrating for the first time, at a molecular level, acquired mutations in tubulin which confer insensitivity to Taxol and the epothilones. The epothilones are new agents under development that appear very promising. The identification of these mutations provides for a greater understanding of the interactions of these drugs with tubulin, and has been the catalyst for studies to understand this at the atomic level. Molecular characterization of the phenotype has already demonstrated some common as well as unique features compared with resistance mediated by P-glycoprotein. Further characterization has identified a role for the p53 tumor suppressor protein in resistance to Taxol and also identified important interactions between p53 and the cytoskeleton. These observations provide further insight into the complex nature of drug action and drug resistance.

In addition, we have in the past and continue at present to exploit the vast resources of the NCI Anticancer Drug Screen, both as a tool to better understand mechanisms of resistance and also as a vehicle to identify new drugs. Our efforts in the past have included full characterization of the MDR–1 and MRP phenotypes and the identification of novel agents, and are currently directed at plans to further characterize and understand the role of other drug transporters.

Recent Publications:

Mickley LA, et al. *Blood* 1998;91(5):1749–56.

Knutsen T, et al. *Genes Chromosomes Cancer* 1998;23(1):44–54.

Miyake K, et al. *Cancer Res* 1999;59(1):8–13.

Robey R, et al. *Blood* 1999;93(1):306–14.

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Biography: *Dr. Grem graduated from Jefferson Medical College. Following an internal medicine residency at the University of Iowa and a medical oncology fellowship at the University of Wisconsin, she became a senior investigator in the Investigational Drug Branch, NCI, before being recruited to the former Medicine Branch in 1989. She is head of the Gastrointestinal Malignancies Section, where her research focuses on the biochemical and molecular pharmacology of antimetabolites and investigational anticancer agents in an effort to develop therapeutic strategies for patients with cancers arising in the gastrointestinal tract.*

Cancer Therapeutics Branch
Preclinical and Clinical Pharmacology of Antineoplastic Agents

Keywords:

biliary tract cancer
colorectal cancer
drug development
gastric cancer
pancreatic cancer
pharmacodynamics
pharmacokinetics
pharmacology

Research: The research mission of the Gastrointestinal Malignancies Section includes the following: (1) to characterize the cellular pharmacology and determinants of sensitivity to new antineoplastic agents, and the interaction of these agents with established drugs in an effort to develop new therapies for patients with carcinomas arising in the gastrointestinal tract; (2) to develop new analytical methods that will facilitate implementation of laboratory-based clinical studies that incorporate pharmacokinetic, pharmacodynamic, and intracellular pharmacodynamic endpoints; (3) to utilize rational clinical pharmacology principles to assist the clinical development of investigational new drugs; (4) to characterize the clinical pharmacokinetic profile of chemotherapeutic agents and determine if any pharmacodynamic relationships exist with efficacy or toxicity; (5) to study the relationship between biochemical and molecular targets with response to therapy; and (6) to develop therapeutic strategies that selectively target cancer cells with unique biochemical and/or molecular phenotypes.

My laboratory research efforts primarily support the pharmacologic, biochemical and molecular endpoints of our clinical studies. Additional basic laboratory studies include evaluation of drug combinations as a prelude to testing in clinical trials, determining the cellular pharmacology of investigational anticancer drugs prior to clinical evaluation, and studying the cellular determinants of sensitivity to promising investigational new

drugs. Such preclinical studies are used to elucidate the optimal schedule of administration and mechanism of drug action and drug interactions, and this information is used to design clinical trials. Our goals are to develop new agents and drug combinations for patients with gastrointestinal malignancies. Our clinical research is conducted in the Gastrointestinal Tumor Clinic, NCI–Navy Medical Oncology Program at the National Naval Medical Center. Our focus has been on biochemical modulation of 5-fluorouracil (5–FU), combinations of 5–FU with other active agents including oxaliplatin, irinotecan, and gemcitabine, and evaluation of investigational new drugs such as 17-(allylamino)-geldanamycin. Laboratory components of the clinical trials include pharmacologic studies (to measure the drug levels achieved), pharmacodynamic studies (to correlate the drug levels with clinical toxicity), intracellular pharmacodynamic studies (to determine the effect of the agent on its intracellular target), and intratumoral expression of pertinent molecular and biochemical targets of the therapeutic regimen. New analytic techniques often need to be developed to permit measurement of the pharmacokinetics and intracellular pharmacodynamics of the anticancer agents. Analytic techniques used in the laboratory include high performance liquid chromatography with either UV, photodiode array, fluorescence, or mass spectral detection; gas chromatography with mass spectral detection; quantitative real-time PCR; flow cytometry; DNA and protein electrophoresis; and pulsed-field gel electrophoresis.

Recent Publications:

Takimoto CH, et al. *J Clin Oncol* 2000;18:659–67.

Grem JL, et al. *J Clin Oncol* 2000;3952–63.

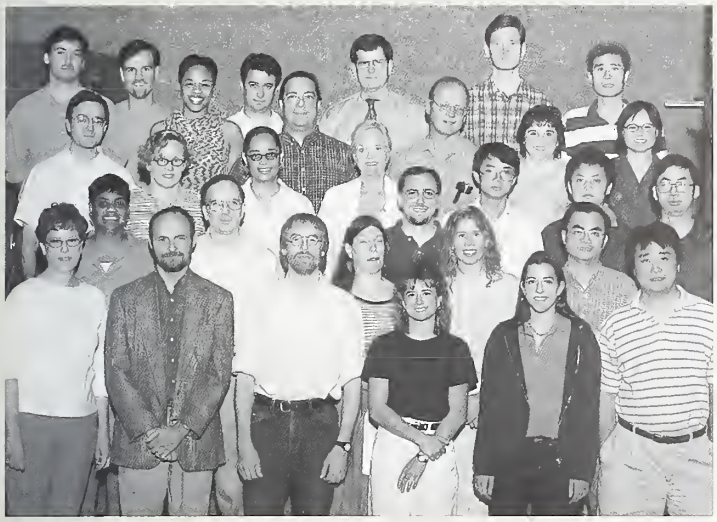
Grem JL, et al. *Cancer Chemother Pharmacol* 2001;47:117–25.

Grem JL, et al. *Clin Cancer Res* 2001;7:999–1009.

Clinical Trials:

Susan E. Bates	**92–C–0268:** A phase I study of infusional chemotherapy with the P-glycoprotein antagonist PSC833
	94–C–0119: A phase I study of infusional paclitaxel with the P-glycoprotein antagonist PSC833
	97–C–0074: A phase I/II study of continuous intravenous infusion of PSC833 and vinblastine in patients with metastatic renal cell cancer
	97–C–0135: A phase I trial of a 4-hr infusion of depsipeptide (NSC 630176) given on days 1 and 5 of a 21-day cycle in patients with refractory neoplasms
	99–C–0121: A phase II trial of trastuzumab (recombinant humanized anti-p185HER2 monoclonal antibody) and paclitaxel in patients with HER–2 overexpressing breast and ovarian tumors who have relapsed or progressive disease following conventional cytotoxic chemotherapy regimens for metastatic cancer
	00–C–0078: Collection of blood, bone marrow, tumor, or tissue samples from patients with cancer
Antonio Tito Fojo	**93–C–0200:** A study of combination chemotherapy and surgical resection in the treatment of adrenocortical carcinoma: continuous infusion doxorubicin, vincristine and etoposide with daily mitotane before and after surgical resection
	00–C–0044: A clinical trial of the P-glycoprotein antagonist XR7576 in combination with Vinorelbine in patients with cancer: analysis of the interaction between XR7576 and Vinorelbine
Jean L. Grem	**98–C–0143:** A phase I study of weekly gemcitabine in combination with infusional 5–fluorouracil and oral calcium leucovorin in adult cancer patients
	99–C–0054: A phase I and pharmacologic study of 17-(allylamino)-17-demethoxygeldanamycin (AAG, NSC 330507) in adult patients with solid tumors
	99–C–0117: A pilot study of Oxaliplatin in combination with capecitabine in adult cancer patients
	01–C–0082: A phase I and pharmacologic trial of sequential irinotecan as a 24-hr IV infusion, leucovorin and fluorouracil as a 48-hr IV infusion in adult cancer patients
	01–C–0172: A phase I/II trial of epirubicin, carboplatin, and capecitabine in adult cancer patients

Cell and Cancer Biology Branch



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The laboratories within the Cell and Cancer Biology Branch conduct investigations into the molecular mechanisms of cellular transformation, tumorigenesis, and metastasis with the goal of applying this knowledge towards prevention of and intervention in human carcinogenesis. Major areas of research include: (1) the definition of cellular biochemical pathways regulating normal growth and development which, when deregulated, contribute to transformation; (2) the identification of targets useful for the early detection and diagnosis of cancer; and (3) the development of molecularly targeted therapeutic modalities for intervention. Our investigations utilize a multidisciplinary approach including animal models as well as human cancers with an emphasis on lung, prostate, breast, and colon cancers. The basic scientific findings and mechanistic concepts developed in the Cell and Cancer Biology Branch contribute to the development and design of clinical trials within the Center for Cancer Research. The branch has established and operates a microinjection and laser scanning confocal microscopy core facility. Microinjection is an important tool that allows introduction of DNA or protein directly into cells. This technique may be used to observe transient phenotypic changes following specific protein activators or inhibitors. Scanning laser (confocal) microscopy uses lasers of various wavelengths to excite fluorescent dyes in a stained sample. The laser light can penetrate the entire sample, producing optical sections of tissue samples or cells at the highest resolution. This capability of the confocal microscope has proven to be extremely useful for many different applications, including 3D reconstruction of cells, measurements of changes in cell volume, and colocalization of proteins in a cell.

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Biography: *Dr. Kelly received her Ph.D. from the University of California, Irvine. She completed her postdoctoral training in the laboratory of Philip Leder, Harvard Medical School, and she has maintained an independent research program at the NCI since 1984. Dr. Kelly's interests have focused on the genetic regulation of cell growth and immune function.*

Cell and Cancer Biology Branch
Signal Transduction in Cancer and Immune Cell Activation

Keywords:
cell proliferation
cell signaling
cytoskeleton
inflammation
metastasis

Research: Our laboratory conducts investigations into signaling pathways that direct processes of growth and differentiation with a view toward their involvement in transformation. In particular, a main area of focus has been signaling pathways mediated by early response genes. Such pathways represent the genetic response to growth and differentiation signals, and their dysregulation may underlie some aspects of oncogenesis. G proteins act as molecular switches to regulate a variety of significant downstream signaling events. Signaling pathways coupled to G protein activation are being studied, including a ras family member, Ral, a novel small G protein, Gem, and a G protein-coupled receptor, CD97. Thus, there is conceptual and technical cross fertilization between each of the projects being investigated.

Signaling pathways that mediate transformation, differentiation, and metastasis: ERK and Ral. We have investigated the signaling pathways initiated by Ras activation that are necessary and sufficient for metastasis and invasion. We determined that ERK and RalGEF pathways contribute distinct properties to metastasis. We discovered that activation of the RalGEF pathway leads to highly invasive and aggressive metastasis formation by 3T3 cells. This discovery forms the basis for further investigations. Because Ras-independent as well as Ras-dependent pathways of RalGEF activation exist, Ral targets may play a frequent role in the determination of an invasive phenotype. The future aims of this project are to identify biological targets of the RalGEF-initiated pathway, particularly with respect to invasion, and to determine whether activation of the RalGEF pathway occurs in human cancers.

Functional analysis of the Gem GTP binding protein. Gem is a small GTP binding protein belonging to a unique structural family within the Ras superfamily. We have determined that Gem can play a role in the cytoskeletal rearrangement and/or morphological differentiation of neurons. Interestingly, Gem expression in neuroblastomas correlates with ganglionic differentiation, a positive prognostic indicator. Relevant to a biochemical mechanism of Gem function is the observation that Gem binds Rho kinase (ROK) and appears to inhibit its activity. ROK is an important Rho effector mediating changes in cytoskeletal structure. The identification of a putative

Gem effector and an associated function will allow us to pursue structure/function analyses addressing the biochemical mechanism and regulation of Gem activity. The physiological role for Gem and a related protein, Rad, is being examined in mouse null strains.

Functional analysis of CD97, a G protein-coupled receptor that contains adhesion motifs. CD97 is a member of a novel family that appears to couple diverse adhesion properties to a G protein-coupled signaling mechanism. CD97 is expressed at highest levels within activated lymphocytes and cells important for innate immune responses. CD97-bearing cells are highly concentrated at inflammatory sites. We are addressing the biological role of CD97 and a related family member, F4/80, by assaying responses of the acquired and innate immune system in mice null for these loci.

Recent Publications:

Leone A, et al. *Oncogene* 2001;20:3217–25.

Gray J, et al. *J Immunol* 1996;157:5438–47.

Avantaggiati ML, et al. *Cell* 1997;89:1175–84.

Ellinger-Ziegelbauer H, et al. *Mol Cell Biol* 1999;19:3857–68.

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Biography: *Dr. Birrer received his M.D. and Ph.D. from the Albert Einstein College of Medicine where he participated in the NIH-funded Medical Scientist Training Program. He completed his clinical training in internal medicine at the Massachusetts General Hospital and in oncology at the NCI. Dr. Birrer became an investigator in 1988 and a senior investigator in 1990, and chief of the Molecular Mechanisms Section in 1991.*

Cell and Cancer Biology Branch
Early Activation Events in Human Epithelial Cancers

Keywords:
carcinogenesis
gynecologic cancers
oncogenes
ovarian cancer
transcription factors

Research: Epithelial carcinogenesis is a complex, multistage process involving the biologic processes of tumor initiation and promotion, and results in mutational events in dominant and recessive oncogenes. The major research goals of the Molecular Mechanisms Section are directed at elucidating and characterizing the molecular mechanisms involved in the early events of the carcinogenic process and utilizing this information to design rational intervention approaches.

To study tumor initiation events, our group has focused on gynecologic cancers. We have characterized endometrial, cervical, and ovarian specimens which span the histologic spectrum from benign to malignant for mutations in known genes including ras, p53, Rb, cyclin E, p27, p16, c-myc, Her-2/neu,

EGFr, and FHIT. For endometrial cancers, we have analyzed curettage specimens: activated ras genes are found in the atypical hyperplasias (14 percent) and endometrial carcinomas (5 percent); p53 mutations are found in approximately 15 percent of atypical hyperplasia and carcinomas. For cervical cancers, the newly described FHIT gene appears to be abnormally expressed in a high percentage of cancer cell lines in a high proportion of cervical cancers and preneoplastic lesions. Evaluation of ovary tumors revealed that activated ras genes are found in benign (10 percent) and "LMP" tumors (30 percent), while they are not found in ovarian carcinomas (<5 percent). In addition, mutations in the tumor suppressor gene p53 occur in ovarian carcinomas (48 percent) but are not present in LMP tumors. This suggested that these tumors are discrete biologic entities.

We have extended these results by examining large numbers of specimens of early and advanced ovarian cancer from a prospective trial (GOG#95, #111, respectively) for mutations in the p53 gene, abnormal HER/2neu EGF cyclin E, and p27. This study has demonstrated that overexpression of cyclin E is an important poor prognostic factor for advanced ovarian cancer. Finally, to identify potential new markers of ovarian cancer and genes important in its pathogenesis, a malignant ovarian epithelium is being compared to its benign counterpart. Our laboratory is applying three approaches including differential display, representational display analysis, and microarrays. Genes which are differentially expressed will be isolated, cloned, and characterized for their role in the development of ovarian cancer.

Tumor promotion is a critical yet reversible step in the multistage process of epithelial carcinogenesis. It is clear that tumor promotion involves growth factors and cellular processes such as differentiation and proliferation. The signals these factors generate from the cell membrane are then mediated in turn by transcription factors.

The AP–1 complex has been specifically implicated in mediating the biologic effects of the tumor promoters "phorbol esters," a wide variety of signals from the cell membrane, and a number of cytoplasmic and nuclear oncogenes. A major component of this complex is the c-jun oncogene. We performed a structure/function analysis of the c-jun oncogene several years ago and established the transactivation, DNA binding, and dimerization domains as critical regions for its biochemical and biologic activities such as fibroblast transformation and cellular differentiation. We have helped identify and characterize the N terminal phosphorylation sites and their role in ras-dependent c-jun transactivation and transformation. Our most recent efforts are aimed at exploring the biologic activity of c-jun in relevant human epithelial cancers such as breast and ovarian cancers. We have overexpressed c-jun in the breast cancer cell line MCF–7. This expression has rendered these cells tumorigenic, more invasive, and hormone independent.

Based on our understanding of the structure/function relationships of c-jun, we have created and tested a series of dominant-negative mutants of c-jun which are able to inhibit the biochemical functions of this oncogene. A trans-activation mutant with a deletion of the N terminal amino acids 2–122 has been shown to inhibit AP–1 transactivation and c-jun transformation. In addition, this mutant has been shown to inhibit cellular transformation by

a wide range of oncogenes including c-fos, c-raf, ras, mos, and myc. Further, stable expression of this mutant protein in mouse epidermal cells can block phorbol ester-induced tumor promotion, and high level expression of this protein can inhibit signal transduction from a wide variety of growth factors. The mechanism of action of this mutant has been demonstrated to be heterodimerization with and neutralization of other AP–1 complex components such as c-fos.

These mutant peptides should be useful as molecular tools to dissect signaling and regulatory pathways and potentially as clinical agents in treating human cancers. Future efforts are aimed at refining the potency, specificity, and delivery of these mutants. We are creating smaller c-jun mutants with higher affinities for dimerization and testing them as peptides in specific human tumor systems such as breast and lung cancers. Further, we have packaged one of our dominant-negative mutants into an adenovirus vector and are testing its biologic function(s) in cell culture systems. Lastly, in vivo testing using model systems such as transgenic technology and other rodent model systems and, ultimately, human clinical trials, will be performed.

In addition, utilizing subtractive cDNA libraries and cDNA microassays, we are identifying downstream c-jun target genes that mediate the biologic effects of the c-jun family proteins.

Recent Publications:

Sabichi AL, et al. *J Natl Cancer Inst* 1998;90:597–605.

Hendricks DT, et al. *Cancer Res* 1997;57:2112–5.

Verheij M, et al. *Nature* 1996;380:75–9.

Smith LM, et al. *Oncogene* 1999; in press.

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Biography: *Dr. Clark received his Ph.D. from the University of Manchester's Department of Oncology at the Paterson Institute for Cancer Research in 1989. He underwent postdoctoral training at the University of Southern California, Los Angeles, at the Burnham Institute, San Diego, and at the Department of Pharmacology at the University of North Carolina at Chapel Hill. After establishing an independent research program at Chapel Hill, Dr. Clark was recruited to the NCI in 1998. He has been working with Ras oncogenes for over 10 years and has a particular interest in the ability of specific small molecules to antagonize Ras oncogene action.*

Cell and Cancer Biology Branch
Ras and Ras-Related Proteins

Keywords:
oncogene
ras
signal transduction
tumor suppressor

Research: Mutant *ras* oncogenes show a close association with the development of many human cancers. Ras proteins are the prototypes for a large family of related proteins, many of which may also play a key role in tumor development. This laboratory seeks to understand the role of Ras and Ras-related proteins in the development of cancer at a molecular level.

Ras proteins activated by mutation promote a broad and sometimes contradictory array of biological responses. These include the ability of cells to grow in the absence of growth factors, resistance to differentiation, loss of contact inhibition, enhanced motility, invasiveness, and resistance to cell death by apoptosis. These are all characteristics that promote tumor growth and survival. However, under certain cellular conditions, activated Ras proteins may actually induce cell death via terminal differentiation, senescence, or apoptosis. These diverse responses to Ras can occur because Ras can bind to a diverse array of effector proteins through which it mediates its action.

We have examined the mechanism by which Ras promotes tumorigenic transformation by studying the mode of interaction of the Ras protein with its effectors Raf-1 and p120 GAP. We have found that both p120 GAP and Raf-1 form intramolecular binding contacts that are modulated by Ras during activation. This has allowed us to design small molecule inhibitors of Raf-1 and p120 GAP that block their ability to modulate signal transduction pathways.

We are also investigating the role of Ras effectors that may induce some of the growth inhibitory effects of Ras including RASSF1 and Nore1/Maxp1. The novel Ras effector Nore1/Maxp1 is a potent inhibitor of cell growth and appears to form the connection between Ras and the CREB pathway. Structure/function studies suggest that Ras may mediate the activation of this protein in a manner similar to that of Raf-1.

Ras proteins are processed by an unusual type of lipid, farnesyl. Without this processing, Ras proteins cannot function. This processing has served as the target for drug development and farnesyl transferase inhibitors (FTIs) are now in clinical trials. However, Ras is not the only target of these drugs, as a small subset of Ras-related proteins are also processed by farnesyl. We have examined the role of two novel farnesylated Ras-related proteins in tumorigenesis and the response of cells to FTIs.

We have found that Rho7 is a farnesylation-dependent inhibitor of cell growth. The processing can be blocked by FTI. Rho7 may be part of the machinery which downregulates growth stimulatory signals, because the Rho7 promoter is strongly activated by Ras. We have also examined the role of a novel Ras-related protein that we have cloned and call Rig. Rig is also farnesylated and is a potent inhibitor of cell growth. It is normally only expressed in the brain and heart; however, protein expression is frequently lost in neural tumors.

Few studies have been documented which investigate the ability of FTIs to cooperate with other anticancer drugs. We have found that FTI and tamoxifen, an antiestrogen widely used in the treatment of breast cancer, synergize to inhibit the growth of breast tumor models. Moreover, this inhibition is dependent upon the antiestrogenic properties of tamoxifen. We intend to extend these studies to other drug combinations with FTI.

We have collaborated with Michel Bernier and Debbie Morrison, NIH; Sharon Campbell and Channing Der, University of North Carolina at Chapel Hill; Albert Deisseroth, Yale University; Lawrence Quilliam, Indiana University; John Raymond, Medical University of South Carolina; Manuel Serrano, Universidad Autonoma Cantoblanco, Madrid, Spain; and Ian Whitehead, University of New Jersey.

Recent Publications:

Clark G, et al. *J Biol Chem* 1997;272:20990–3.

Ellis C, et al. *Cell Signal* 2000;12:425–34.

Drugan JK, et al. *J Biol Chem* 2000;275:35021–7.

Vos M, et al. *J Biol Chem* 2000;275:35669–72.

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Biography: *Dr. Cuttitta received his Ph.D. from the University of Maryland in 1980 with majors in immunology and biochemistry. He completed his postdoctoral fellowship training at the Navy Medical Oncology Branch of the NCI (1981 to 1986) and was appointed to a joint staff position as part of the NCI/Uniformed Services University of the Health Sciences Comparative Agreement (1986 to 1991). Dr. Cuttitta served as the deputy branch chief from 1992 to 1995 and as acting branch chief from 1995 to 1996 of the Biomarkers and Prevention Research Branch. He is currently a tenured principal investigator in the Cell and Cancer Biology Branch.*

Cell and Cancer Biology Branch
Identification of Autocrine/Paracrine Growth Regulators in Human Cancers

Keywords:
carcinogenesis
cell proliferation
diabetes
embryogenesis
inflammation
wound repair

Research: The main focus of this project is to define the potential autocrine/paracrine mechanisms regulating the growth of human tumors and utilize these as rational targets for early detection and intervention of malignant disease. We have developed several investigative strategies to achieve this goal. Our initial effort was accomplished by acclimating tumor cell lines to grow in a protein-free/peptide-free medium (R_0), thereby forcing the cell to express maximum survival capabilities in a nutrient-poor environment. Under these conditions we have been able to establish long-term growth (1 to 6 years) from a variety of epithelial cancer lines including those of lung, breast, colon, ovary, pancreas, prostate, and neuroblastoma/glioblastoma lineage. Biochemical analysis of the conditioned media from R_0 cells revealed the presence of protein/peptides such as gastrin-releasing peptide (GRP), insulin-like growth factors (IGF–I/IGF–II), transforming growth factor-α (TGFα), adrenomedullin (AM), and transferrin (Tf), which are established mitogens of tumor growth. Since R_0 base medium contains no exogenous protein/peptides, the only source of such products was for the R_0-adapted cells themselves. These conditions may mirror steps in carcinogenesis, which involve clonal expansion of the transformed cell. Thus by identifying the growth factors and their respective receptor systems expressed during R_0 adaptation of tumor cell lines, we have been able to generate appropriate molecular and immunological reagents to evaluate their involvement during normal to malignant conversion in pathological specimens. In addition, since disruption of ligand/receptor binding results in growth cessation, these factors make potential targets to intervene in the carcinogenesis pathway by blocking the promotion event.

During the past several years we have focused our research on the peptide hormone adrenomedullin and evaluated its role in carcinogenesis, embryogenesis, wound repair, inflammation, and diabetes. AM was initially isolated and characterized from a human pheochromocytoma (adrenal tumor) shown to activate platelet adenylyl cyclase and induce hypotension when injected intravenously into experimental animals. We have found that

AM and its respective receptor, AM–R, are highly expressed in a variety of human tumor cell lines (cancers of the lung, breast, colon, brain, ovary, prostate, and skin). This peptide was shown to mediate autocrine proliferation of the tumor cell (in vitro/in vivo), and its action could be blocked by an anti-AM monoclonal antibody in a dose-dependent manner, causing growth cessation. Similarly, we have shown, using both molecular and immunohistochemical techniques, that AM is upregulated during embryogenesis and that the peptide plays an important role in the implantation process. At the site of the ectoplacental cone (area of attachment where fetal trophoblasts invade maternal tissue, forming the decidual interface and thereby mimicking events associated with tumor metastasis), AM and AM–R were found to be highly expressed. Very recently, several laboratories have shown that homozygous knockouts of the AM gene result in a lethal deletion for embryogenesis. To circumvent the dilemma, we have begun construction on inducible knockout mice using a CRE/Lox technology with the intention of targeting the lung (CC10 promoter) for our initial study. If successful with this project, we will be able to study AM influence on lung development and, if not a lethal deletion for the adult mouse, determine what effect AM plays in pulmonary carcinogenesis.

We have also shown that AM functions as an antimicrobial peptide causing the lysis of both bacterial and fungal pathogens. Endotoxin-activated macrophages are the main source of AM expression in the immune response pathway, and plasma AM levels rise dramatically during sepsis. Very early in our investigation of AM, we demonstrated that AM is produced in the F cell of the pancreatic islet (cell that makes pancreatic peptide, PP) and that AM blocks glucose-induced insulin release from the beta cell. In collaboration with the United States Department of Agriculture (USDA), we have found that intraperitoneal injections of our anti-AM monoclonal antibody could lower the blood glucose levels of genetically engineered diabetic/obese rats (SKR/corpulent) which approached concentrations of normal controls. Additionally, with the USDA group, we have shown that endotoxin can enhance AM expression in the pancreatic islet and alter glucose metabolism in experimental cattle with superimposed parasitic infections, causing an augmentation in this phenomenon. Finally, in collaboration with the Uniformed Services University of the Health Sciences (USUHS), we have shown that AM expression in wound repair is elevated at sites of neovascularization and that topical application of this peptide can dampen drug-induced suppression of wound repair in dexamethasone-treated rats.

Our collective studies have identified potential targets for the early detection and intervention of malignant disease and offer a rational approach in the investigative avenues to define new biological markers of neoplasm expression.

Among our collaborators are Sam Bhathena and Ted Elsasser, U.S. Department of Agriculture; Nadya Tarasova and Lino Tessarollo, Advanced Bioscience Laboratories, Inc.; and Stefanie Vogel, Uniformed Services University of the Health Sciences.

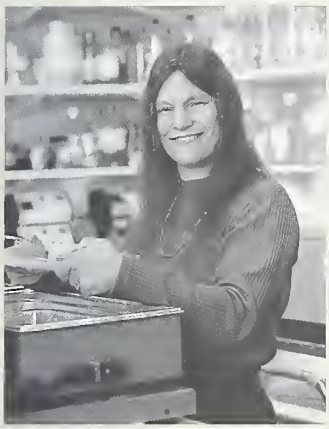
Recent Publications:

Garayoa M, et al. *Mol Endocrinol* 2000;14:848–62.

Montuenga LM, et al. *J Neuroendocrinol* 2000;12:607–17.

Pío R, et al. *Peptides* 2000;21:1859–63.

Pío R, et al. *J Biol Chem* 2001;276:12292–300.



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Biography: *Dr. Jakowlew received her Ph.D. from Rutgers University with research conducted at the Roche Institute of Molecular Biology. After a postdoctoral fellowship with Dr. Pierre Chambon at the Laboratorie de Genetique Moleculaire des Eukaryotes, she joined the NCI as a staff fellow. She received tenure as a senior investigator in the Biomarkers and Prevention Research Branch of the NCI. Dr. Jakowlew's research interests include the role of polypeptide growth factors and their receptors in epithelial cell growth, proliferation, and cancer.*

Cell and Cancer Biology Branch
Transforming Growth Factor-ß in Lung Cancer and Carcinogenesis

Keywords:
cell proliferation
gene expression
gene regulation
lung cancer
lung carcinogenesis
signal transduction
tumor suppressor

Research: Several different polypeptide growth factors have been identified in the lung, including transforming growth factor-β (TGFβ). TGFβ is a multifunctional polypeptide that is the prototypical member of a large family of cytokines that regulate many aspects of cellular function, including proliferation, differentiation, adhesion, and migration. TGFβ inhibits the proliferation of many epithelial cells through interaction with its receptors, the type 1 and type 2 receptors (TGFβ RI and RII), by activating their serine/threonine kinase activities. Loss of growth inhibition by TGFβ is thought to contribute to the development of many types of tumors. Restoring the ability of a lung cancer cell to be inhibited by TGFβ and/or its target genes may decrease lung cancer cell proliferation.

Expression of the proteins and mRNAs for TGFβ1, TGFβ RI, and TGFβ RII was examined in normal human lung, well-characterized nonsmall cell lung cancer (NSCLC) cell lines, and primary NSCLC specimens. Immunohistochemical staining for TGFβ1, TGFβ RI, and TGFβ RII using specific antibodies in normal human lung showed expression of the three proteins in the epithelium of normal bronchi and bronchioles, as well as in alveoli. Expression of the mRNAs for TGFβ1, TGFβ RI, and TGFβ RII was detected at different levels using Northern blot hybridization in five NSCLC cell lines, with an inverse relationship occurring between the levels of TGFβ RI and TGFβ RII mRNAs in some of these cell lines. Western blot analysis showed detectable, but reduced, TGFβ RI and TGFβ RII proteins in three NSCLC cell lines. A panel of 45 formalinfixed and paraffin-embedded human NSCLC

specimens showed positive immunostaining for TGFβ1, TGFβ RI, and TGFβ RII, with reduced TGFβ RII in some tumors. In situ hybridization studies conducted with specific riboprobes for TGFβ1, TGFβ RI, and TGFβ RII showed corresponding localization of expression of the mRNAs in the specimens that showed positive immunostaining for these proteins. To investigate the roles of TGFβ1, TGFβ RI, and TGFβ RII in chemically induced mouse lung tumorigenesis, we examined the expression of their proteins and mRNAs in two mouse model systems. Expression of TGFβ1, TGFβ RI, and TGFβ RII mRNAs and proteins in adenomas was detected at levels comparable with that of bronchioles in C57BL/6 mice and their littermates heterozygous for deletion of the TGFβ1 gene treated with diethylnitrosamine after 9 months. Interestingly, decreased immunostaining and hybridization for TGFβ RII protein and mRNA was detected in some adenomas of A/J mice treated with benzo(α)pyrene. These findings show that reduced levels of expression of TGFβ RII occur in some, but not all, human and mouse lung tumors. This suggests that different mechanisms of action, some of which may involve the TGFβ signaling pathway, may contribute to the progression of lung tumorigenesis.

To examine their roles in mouse lung chemically induced tumorigenesis, expression of the proteins and mRNAs for TGFβs 1, 2, and 3 and TGFβ RI and RII was examined in A/J mice treated with the carcinogen urethane to induce lung adenomas using immunohistochemical and in situ hybridization analyses. Immunostaining for the TGFβ ligands and receptors was detected in the epithelium of the bronchioles of untreated and treated A/J mice at similar levels. Immunostaining for the TGFβ ligands and receptors was also detected in adenomas by 2 months. While immunostaining for TGFβ1, β2, β3 and TGFβ RI in adenomas was detected at levels comparable with bronchioles, immunostaining for TGFβ RII was less intense in adenomas compared to bronchioles. Decreased immunostaining for TGFβ RII in adenomas persisted for at least 8 months following exposure to urethane whereas immunostaining for TGFβs 1, 2, and 3 and TGFβ RI persisted at levels comparable to those in normal bronchioles. In situ hybridization studies conducted using TGFβ receptor riboprobes show, compared with bronchioles, a corresponding reduction in expression of TGFβ RII mRNA, but not of TGFβ RI mRNA, in adenomas. Expression of TGFβ RII mRNA was also examined in nontumorigenic and tumorigenic mouse lung cells; in these cells, expression of TGFβ RII mRNA was lower in the tumorigenic cells derived from urethane-induced lung tumors. These findings suggest that a decrease in expression of TGFβ RII may contribute to autonomous cell growth and may play an important role in mouse lung carcinogenesis induced by urethane.

To determine more conclusively whether loss of TGFβ affects cell proliferation and predisposes to tumorigenesis, mice in which one of the TGFβ1 alleles was deleted were compared with wild-type (WT) mice. Mice heterozygous (HT) for deletion of the TGFβ1 gene expressed only 10 to 30 percent of WT TGFβ1 protein levels. Although grossly normal, these HT mice have a subtly altered proliferative phenotype, with increased cell turnover in the liver and lung. Treatment of these mice with the carcinogen diethyl-nitrosamine resulted in enhanced tumorigenesis in the lung when compared with WT littermates. However, lung tumors in the HT mice did not lose the

remaining TGFβ1 allele. These findings indicate that the TGFβ1 ligand is a new form of tumor suppressor that shows true haploid insufficiency in its ability to protect against tumorigenesis.

To elucidate the role of TGFβ1 and TGFβ1 RII as tumor suppressor genes in lung carcinogenesis, we mated C57BL/6 TGFβ1 HT mice with A/J mice to produce AJBL6 TGFβ1 HT progeny and their WT littermates. Immunohistochemical staining, in situ hybridization, and Northern blot analyses showed lower staining and hybridization for TGFβ1 protein and mRNA, respectively, in the lungs of normal HT mice compared to WT mice. Competitive reverse transcription-polymerase chain reaction (CRT–PCR) amplification showed the level of TGFβ1 mRNA in the lungs of HT mice to be four-fold lower than in WT lungs. When challenged with ethyl carbamate, lung adenomas were detected in 55 percent of HT mice by 4 months, while in only 25 percent of WT littermates at this time. While all HT mice had adenomas by 6 months, it was not until 10 months before all WT mice had adenomas. After 12 months, the average number of adenomas was five-fold higher in HT lungs compared to WT lungs. Most dramatic was the appearance of lung carcinomas in HT mice 8 months before they were visible in WT mice. Thus, the AJBL6 TGFβ1 HT mouse provides an excellent model system to examine carcinogen-induced lung tumorigenesis by increasing progressive lesion incidence and multiplicity relative to their WT littermates. Immunohistochemical staining showed expression of the TGFβ RI at moderate to strong levels in lung adenomas and carcinomas in HT and WT mice. In contrast, while weak immunostaining for TGFβ RII was detected in 67 percent of HT carcinomas at 12 months, only 22 percent of WT carcinomas showed weak staining for this protein. Individual lung carcinomas showing reduced TGFβ RII expression and adjacent normal bronchioles were excised from HT lungs using laser capture microdissection, and CRT–PCR amplification of the extracted RNA showed 12-fold less TGFβ RII mRNA in these carcinomas compared to bronchioles. Decreasing TGFβ RII mRNA levels occurred with increasing tumorigenesis in lung hyperplasias, adenomas, and carcinomas, with carcinomas having four- and seven-fold lower levels of TGFβ RII mRNA than adenomas and hyperplasias, respectively. These data show enhanced ethyl carbamate-induced lung tumorigenesis in AJBL6 HT mice compared to WT mice, suggesting that both TGFβ1 alleles are necessary for tumor suppressor activity. Reduction of TGFβ RII mRNA expression in progressive stages of lung tumorigenesis in HT mice suggests that loss of TGFβ RII may play an important role in the promotion of lung carcinogenesis in mice with reduced TGFβ1 gene dosage upon challenge with carcinogen.

To identify known and novel target genes of TGFβ1 that are involved in TGFβ1-mediated responses, the TGFβ1- responsive epithelial NSCLC cell line NCI–H727 was used. Comparative cDNA expression patterns between cells treated with TGFβ1 or vehicle were generated by differential mRNA display. One 496-bp fragment, differentially increased three-fold by TGFβ1 and hybridizing to a 2.7-kb mRNA species in NCI–H727 cells by Northern analysis, revealed no significant match to any known gene sequence. The mRNA transcript of this novel gene that we named "Differentially Expressed Nucleolar TGFβ1 Target" (DENTT) is expressed in several normal human tissues, with highest expression in the brain. Human brain cDNA library

screening and 5′ RACE yielded full-length DENTT cDNA containing an 1899-bp open reading frame encoding a predicted 633-amino acid protein with four potential nuclear localization signals (NLS) and 2 coiled coil regions. DENTT contains a conserved 191-residue domain that shows significant identity to, and defines, the TSPY/TSPY-like/SET/NAP–1 (TTSN) superfamily. Enhanced green fluorescent protein (EGFP)-tagged full-length DENTT transfected into COS–7 cells showed nucleolar and cytoplasmic localization. Transfection of EGFP-tagged DENTT NLS deletion constructs lacking the bipartite NLS–1 were excluded from the nucleolus. While NLS–1 is necessary for nucleolar localization of DENTT, it is not sufficient for sole nucleolar localization. Our findings show that DENTT mRNA induction by TGFβ1 correlates with induction of TGFβ1 mRNA, induction of extracellular matrix gene expression, and inhibition of colony formation in soft agarose in TGFβ1-responsive NSCLC cells when exposed to TGFβ1. TGFβ1 does not induce DENTT mRNA expression in TGFβ1-nonresponsive NSCLC cells. These findings suggest that this novel TGFβ1 target gene has distinct domains for direction to different subnuclear locations.

Collaborating with us are Moy-Fong Chen, McGill University, Canada; Alfredo Martinez and Lalage Wakefield, NIH; Luis Montuenga, University of Navarra, Spain; and Liang You, University of California.

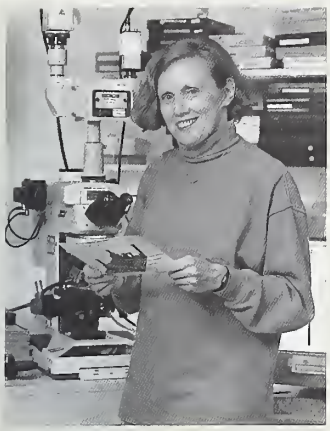
Recent Publications:

Chen M–F, et al. *Pediatr Res* 1999;46:61–70.

Kang Y, et al. *Mol Carcinog* 2000;29:112–26.

Kang Y, et al. *Exp Lung Res* 2000;26:685–707.

Ozbun L, et al. *Genomics* 2001;73:179–93.



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Biography: *Dr. Linnoila received her M.D. from the University of Helsinki, Finland. After postdoctoral work at the National Institute of Environmental Health Sciences in North Carolina and residency training in anatomic pathology at the NCI, she joined the NCI–Navy Medical Oncology Branch. Dr. Linnoila was staff pathologist there until 1992, when she accepted her current position as chief of the Experimental Pathology Section. Her scientific interests and expertise are in lung carcinogenesis, differentiation, and neuroendocrine tumors.*

Cell and Cancer Biology Branch
Molecular Pathology of Lung Carcinogenesis and Differentiation

Keywords:
Clara cell protein
differentiation
image analysis
lung cancer
neuroendocrine

Research: The major research objectives of the Experimental Pathology Section are directed toward understanding the molecular pathology of transformation, differentiation, and early neoplasia in the lung. We study the molecular basis of these events in their actual anatomic context featuring topographic genotyping through imaging coupled with interactive morphometric analysis, confocal microscopy, laser capture, microdissection, and molecular analysis. We utilize material obtained through clinical studies from patients with and without lung cancer as well as several experimental models including transgenic animals. Our current efforts focus on the potential tumor suppressor role of Clara cell-specific protein (CC10) in lung carcinogenesis and the family of achaete-scute basic helix-loop-helix transcription factors, exemplified by hASH1, in pulmonary neuroendocrine (NE) differentiation and tumorigenesis.

Several years ago we made the observation that the proportion of lung adenocarcinomas was increasing while the data on the precursor lesions was scanty. CC10 is a major product of the metabolically active airway cells that are progenitor cells for the nonneoplastic and neoplastic epithelium, including many adenocarcinomas. Our data from humans and animal models demonstrate that CC10 expression is markedly downregulated during early carcinogenesis. To address whether CC10 expression is compatible with the neoplastic phenotype, CC10 cDNA was overexpressed in cancer cells and immortalized normal bronchial epithelial cells. Enhanced expression in a nonsmall cell cancer cell line resulted in decreased clonogenic survival, decreased invasiveness, and decreased metalloproteinase expression. These data suggest that downregulation of CC10 contributes to neoplastic progression. We are further evaluating the potential tumor suppressor role of CC10 by investigating CC10 knockout animals during experimental lung carcinogenesis.

In our second approach, our laboratory used clinical lung cancer resection specimens to screen for precursors for adenocarcinoma. Systematic review of the three lung compartments revealed that in the alveolar region of the lung,

marked changes in CC10 expression patterns were associated with metaplasia and atypia, such as bronchialization of alveoli and atypical alveolar hyperplasia. These areas also demonstrated a number of molecular abnormalities including loss of heterozygosity of 3p, a site of putative tumor suppressor gene, increased c-myc expression, and p53 abnormalities, suggesting that these abnormalities may in fact be early neoplastic changes.

We have previously shown that early changes and field cancerization in the lung are also characterized by alterations in the neuroendocrine (NE) differentiation. A minority of normal bronchial epithelial cells and many lung cancers, especially small cell lung cancer (SCLC), which is the most lethal form of human lung cancer, exhibit a NE phenotype that may reflect a common precursor cell population. The family of achaete-scute basic helix-loop-helix transcription factors, exemplified by hASH1, plays a pivotal role in the neurosensory development in *Drosophila* and vertebrates. We have shown hASH1 is selectively expressed in normal pulmonary NE cells and their precursors as well as in various lung cancers with NE features. A disruption of the gene in mice caused a complete lack of pulmonary NE cells. Depletion of hASH1 from lung cancer cells by antisense oligonucleotides resulted in a significant decrease in NE markers. To determine whether hASH1 overexpression is sufficient to drive NE differentiation in heterologous airway epithelial cells or is associated with aberrant growth, we established a transgenic mouse system where hASH1 was expressed under CC10 promoter. Overexpression of hASH1 alone led to hyperplasia of airway epithelium with metaplasia. When coupled with SV40 Large T antigen, greatly enhanced epithelial growth resulted in massive tumors with NE differentiation. Thus, a homolog of *Drosophila* neural fate determination genes appears necessary for progression of lung epithelial cells through a NE differentiation pathway and carcinogenesis that is characteristic for approximately one-third of human lung cancers.

The significance of our project is that the results will provide a rational basis for early detection and intervention in human lung carcinogenesis by identifying specific markers and models for multistep epithelial carcinogenesis.

We have collaborated with Douglas Ball and Stephen Baylin, Johns Hopkins University; Francesco DeMayo, Baylor College of Medicine; Bruce Johnson, Harvard University; Anil Mukherjee, Seth Steinberg, and Sandra Swain, NIH; and Hanspeter Witschi, University of California at Davis.

Recent Publications:

Jensen-Taubman SM, et al. *Int J Cancer* 1998;75:489–96.

Szabo E, et al. *Cell Growth Differ* 1998;9:475–85.

Linnoila RI, et al. *Cancer Res* 2000;60:4005–9.

Linnoila RI, et al. *Exp Lung Res* 2000;26:595–615.

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Biography: *Dr. Liu received both his bachelor's and master's degrees in biochemistry from Peking University, People's Republic of China. He completed his Ph.D. training in Dr. Larry Schwartz's laboratory at the University of Massachusetts in 1995 and carried out his postdoctoral training in the laboratory of Dr. Michael Karin at the University of California, San Diego. He joined the former Department of Cancer and Cell Biology, Medicine Branch, DCS, in 1998. His research interests have focused on the molecular mechanisms of apoptosis.*

Cell and Cancer Biology Branch
Molecular Mechanisms of TNF Signaling and Apoptosis

Keywords:
apoptosis
kinases (IKK/JNK)
signal transduction
tumor necrosis factor

Research: The research goal of our laboratory is to understand the molecular mechanism of tumor necrosis factor (TNF) signaling and the regulation of apoptosis.

TNF Signal Transduction

TNF is a proinflammatory cytokine that plays a critical role in diverse cellular events. Upon TNF treatment, cells could undergo proliferation, differentiation, and apoptosis. In a previous study, we found that the activation of transcription factors NFκB and AP–1 as well as the induction of apoptosis are mediated by three distinct pathways in response to TNF. While the adaptor molecule FADD mediates apoptosis, the death domain kinase RIP and the TNF receptor-associated factor TRAF2 are essential for NFκB and AP–1 activation. Because it is unknown how RIP and TRAF2 regulate the NFκB and AP–1 activation, we are investigating the mechanism by which RIP and TRAF2 activate their downstream kinases—namely, IKK and JNK1—with the combination of biochemical and cellular approaches.

Regulation of Apoptosis

Apoptosis (programmed cell death) is a common phenomenon during development and occurs to eliminate harmful or unwanted cells from the organism. Apoptosis is the crucial process for organisms to keep their cellular homeostasis. Deregulation of apoptosis is involved in many diseases; for instance, inefficient apoptosis has been found in many different cancers. Since all cells have the genetic machinery required to commit suicide, the ability to selectively regulate this process has profound implications for treating disease. Because more and more evidence indicates that irregular cell growth often leads to apoptosis, we believe that in addition to promoting growth signals, inactivation of apoptosis is essential for normal cells to become tumor cells. This can be achieved by either increasing a signal that actively blocks apoptosis or generating a defective mutation in the cell death machinery. Identification of these apoptosis-inactivating targets in different cancers will greatly enrich our knowledge about tumorigenesis and help to develop new cancer therapies.

One of our research interests is to identify the genes that protect cancer cells from apoptosis and, upon understanding the mechanisms of their actions, to develop new cancer therapies. To do so, we use TNF-mediated apoptosis as a model system. It is known that the activation of NFκB protects cells from apoptosis induced by TNF and many chemotherapeutic agents. While we continue to study the regulation of TNF signal transduction, we also like to understand the mechanism of the transcription factor NFκB-mediated antiapoptotic effect. Using several different approaches including microarray, we have identified some candidate genes which may protect cells from apoptosis. Currently we are further testing their antiapoptotic effect. Because NFκB activation protects cells against apoptosis and also is essential for the development of several types of cancer, the identification of the antiapoptotic genes activated by NFκB will provide new targets for developing new cancer therapies. Inhibition of the functions of these antiapoptotic genes may result in apoptosis of cancer cells and lead to cure of the disease.

Recent Publications:

Lin Y, et al. *Genes Dev* 1999;13:2514–26.

Lewis J, et al. *J Biol Chem* 2000;14:10519–26.

Devin A. *Immunity* 2000;12:419–29.

Lin Y, et al. *Mol Cell Biol* 2000;20:6638–45.

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Biography: *Dr. Mulshine completed cancer research training at the NCI and has been on the faculty since 1983. His research involves translating basic science into meaningful tools for cancer prevention, especially lung cancer. He has been awarded a number of United States patents and has worked with industry through Collaborative Research and Development Agreements to move those clinical management tools into broader clinical application. His research productivity has been recognized with awards from the Department of Commerce, the Public Health Service, the NIH, and the NCI.*

Cell and Cancer Biology Branch
Developing Integrated Early Epithelial Cancer Detection and Intervention Based on Tumor Biology

Keywords:
chemoprevention
cyclo-oxygenase
early cancer detection
lung carcinogenesis
retinoids

Research: Our focus is to understand key steps in carcinogenesis while developing new prevention tools. A goal is to identify early (i.e., airway-confined) lung cancer since that may lead to improved survival for carcinogens-exposed individuals. Airway injury due to chronic tobacco exposure was previously termed "field cancerization," but the diagnostic tools to routinely allow meaningful assessment of bronchial epithelial injury are just becoming available. Our primary effort has been on developing an

effective diagnostic approach for early lung cancer. As outlined in a series of joint NCI and Hopkins patents, we have demonstrated that in most cases lung cancer can be detected at least a year earlier (than with x-ray) using an immunocytochemical assay to characterize the overexpression of the protein, heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNP A2/B1). In four prospective cohorts with at least preliminary analysis, the use of sputum-based immunocytochemical detection has been associated with an accuracy of lung cancer detection of 70 to 90 percent. We developed the diagnostic reagents, evaluated the biological basis for this detection approach, and are now assembling the requisite data to submit our sputum-early lung cancer detection test for FDA approval. In addition, we are elucidating the reason why the overexpression of hnRNP A2/B1 is so consistently informative about lung cancer status. We have shown that this molecule has an interesting pattern of expression at the protein and mRNA level during critical phases of mammalian lung cancer development. This behavior suggests that this molecule is an oncofetal antigen, similar to the known status of difucosylated Lewis X, which is the other marker shown to detect early lung cancer. We have recently shown that the areas of hnRNP A2/B1 overexpression in lung tissue are also significantly associated with a higher frequency of genetic injury. Recently published work suggests that hnRNPA2/B1 defines populations of cells that are caught up in field carcinogenesis.

For aerodigestive cancers, innovative work with 13 cis-retinoic acid chemoprevention by M.D. Anderson investigators suggests a benefit in reducing the frequency of tobacco-induced second cancers, especially from the lung. We have shown that serum albumin binds to retinoic acid and greatly reduces the ability of the drug to control the growth of lung cancer cells. Changing the route of administration to directly deliver retinoid to the early cancer cell in the airway, such as through the use of aerosols, results in a high first-pass drug exposure and minimizes the problems of albumin binding. With other NIH investigators, we are working with the pharmaceutical industry under a CRADA mechanism with Battelle to develop effective approaches for airway delivery of drugs that control early lung cancer growth. Preclinical work with aerosolized retinoid delivery in mice that were exposed to tobacco-related carcinogens has been published that supported the utility of this approach. Initial protocol evaluation in humans with these inhaled agents will be starting this year at NIH. High efficiency delivery technologies developed for this work will also be used to evaluate other promising drugs for lung cancer. We have already begun a proof-of-concept prevention trial evaluating a direct drug delivery strategy for preinvasive oropharyngeal carcinogenesis. Under a Clinical Trials Agreement with Procter and Gamble, we are evaluating if an aspirin-like drug delivered as an oral rinse can arrest oral precancer without incurring dangerous side effects. The 57-person randomized phase IIB trial will soon complete testing the ability of direct drug delivery strategies to improve outcomes with prevention of a premalignant condition associated with tobacco-induced cancers.

To significantly reduce lung cancer mortality will require the integrated and simultaneous development of both early diagnostic and early intervention capabilities. In the intramural program, we are attempting to catalyze this process. Using approved mechanisms for technology transfer, we have

systematically recruited areas of strength in biotechnology to complement our areas of research strength for the rapid development of public health tools for cancer prevention. Improving lung cancer mortality will have a significant benefit for an overall cancer outcome and provide a model for similar approaches in other major epithelial cancers.

Recent Publications:

Avis I, et al. *FASEB J* 2001; in press.

Man Y–G, et al. *Am J Respir Cell Mol Biol* 2000;23:636–45.

Hong SH, et al. *FASEB J* 2000;14:1499–507.

Wang D, et al. *Clin Cancer Res* 2000;36:3636–45.

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Biography: *Dr. Neckers received his Ph.D. from the University of Connecticut, completed postdoctoral training at the NIH, and joined the NCI in 1981. He became Chief of the Tumor Cell Biology Section, Medicine Branch, in 1988. An early proponent of translational research, Dr. Neckers pioneered development of oligonucleotide-based therapeutic strategies. Recently, Dr. Neckers has been investigating the role of chaperone proteins in signal transduction. His identification of benzoquinone ansamycins as specific antagonists of the chaperone HSP90 uncovered the importance of this protein for the growth and survival of cancer cells and led directly to the first phase I clinical trial of an HSP90 antagonist as an anticancer agent. Dr. Neckers holds several patents and has been the recipient of both an NIH Merit Award and an NIH Inventor's Award.*

Cell and Cancer Biology Branch
Molecularly Targeted Therapeutic Approaches to Modulated Cancer Cell Growth and Survival

Keywords:

antisense
apoptosis
cancer
cell signaling
chemotherapeutics
drug development
heat shock proteins
HIF
hypoxia
immunostimulatory
oligodeoxynucleotides
p53 gene
protein kinase
signal transduction

Research: The goal of this laboratory is to identify mechanism-based, molecularly targeted therapeutic approaches to modulate cancer cell growth and survival. An important facet of this goal is identification of novel intracellular targets for specific pharmacologic intervention. Our research currently encompasses two areas: (1) understanding the biologic activity of chaperone proteins, particularly HSP90, in mediating signal transduction, and (2) examining the utility of oligonucleotides as anticancer agents.

Benzoquinone ansamycin antibiotics were isolated in the late 1970s and classified as tyrosine kinase inhibitors. However, we observed that these drugs did not function as classic tyrosine kinase inhibitors and we investigated their true mechanism of action. Our discovery in 1993 that the HSP90 family of chaperone proteins was the predominant intracellular target of benzoquinone ansamycins led to a series of ongoing, interrelated studies in which we are (1) delineating the drug-protein interaction, (2) characterizing the sequelae of this interaction, and (3) identifying the components of the

signal transduction machinery that are the secondary targets of this interaction. In addition, we are collaborating with the Developmental Therapeutics Program, NCI, on clinical development of benzoquinone ansamycins and we are currently participating in the phase I testing of such a drug in cancer patients.

This research emphasizes the critical role of the HSP90 chaperone family in diverse signal transduction pathways and identifies the HSP90 family as novel targets for anticancer drug development. Although others have proposed, based on in vitro renaturation experiments, that HSP90 may act as a generally nonspecific chaperone, our findings support a different hypothesis; namely, that HSP90 function in vivo is highly specific and is regulated by interaction with unique accessory proteins. In addition, we are the first to describe the importance of HSP90 in the function of mutated p53 and the importance of the HSP90 homolog, GRP94, in processing, trafficking, and stability of the proto-oncogenic receptor tyrosine kinase p185c-erbB2.

We have identified the ansamycin binding site on HSP90/GRP94 as a unique region in the amino terminal portion of the molecule. By studying many chemical derivatives of the basic benzoquinone ansamycin structure, we determined that biologic activity requires high affinity binding to HSP90/GRP94. We showed that this drug class disrupts HSP90/GRP94 interaction with several signal transduction proteins, resulting in destabilization and improper intracellular localization of the signal transducers. We have determined that, in addition to p185c-erbB2 and mutated p53, pp60v-src and c-raf-1 proteins require HSP90 interaction for function. Protein destabilization triggered by benzoquinone ansamycins is mediated by ubiquitination followed by proteasome-dependent proteolysis. In the case of p53, drug treatment inactivates and destabilizes the mutated protein without affecting function of wild-type p53. Thus, cellular activity of multiple oncogene/proto-oncogene products depends on a common mechanism, making the HSP90 chaperone family an exciting new molecular target for anticancer drug development. Our current studies include: (1) identification and characterization of other HSP90 client proteins; (2) identification and characterization of other small molecule antagonists of HSP90 that might be better suited for clinical use; and (3) investigation of the role of HSP90 and other chaperone proteins in nuclear/cytoplasmic transport, using the nuclear import/export of the transcription factors p53 and HIF as model systems.

Our longstanding interest in the development of antisense technology dates to our early observation that antisense oligodeoxynucleotides targeting c-myc mRNA blocked mitogenic activation of normal lymphocytes. Because we believe that site-restricted administration of oligodeoxynucleotides may reduce systemic toxicity and ease delivery, we developed site-restricted in vivo model systems to test the antitumor efficacy of these compounds. These model systems have included direct introduction of oligodeoxynucleotides to the central nervous system of rodents and use of oligodeoxynucleotides as ex vivo tumor cell purging agents in bone marrow transplantation models. As an outgrowth of investigating the mechanism of cellular oligodeoxynucleotide uptake, we identified electroporation as an alternative technique for ex vivo (and perhaps in vivo) delivery of oligodeoxynucleotides.

Despite obvious utility, certain oligodeoxynucleotides exhibit nonantisense-mediated effects due to sequence and chemical composition. We believe that it is important to recognize and understand these phenomena if the promise of oligodeoxynucleotide-based therapeutics is to be fully realized. In fact, certain "nonantisense" effects of oligodeoxynucleotides may prove beneficial if properly utilized. Thus, we have characterized effects of oligodeoxynucleotides not mediated by direct hybridization to complementary mRNA, including the potent antitumor activity of oligodeoxynucleotides containing motifs which mimic bacterial DNA and stimulate the host immune system.

Among our collaborators are Shiro Akinaga, Kyowa Hakko Kogyo Co., Ltd.; Yair Argon and M. Celeste Simon, University of Chicago; Frank Cuttitta, Zheng-gang Liu, Edward Sausville, and Jane Trepel, NIH; Randall Johnson, University of California, San Diego; Arthur Krieg, University of Iowa; Christopher Nichitta, Duke University; Moshe Oren and Yosef Yarden, Weizmann Institute; and David Toft, Mayo Clinic.

Recent Publications:

Schulte TW, et al. *Mol Endocrinol* 1999;13:1435–48.

Soga S, et al. *Cancer Res* 1999;59:2931–8.

An WG, et al. *Nature* 1998;392:405–8.

Blagosklonny MV, et al. *J Biol Chem* 1998;273:11995–8.

Clinical Trials:

Kathleen Kelly

CPB334: A phase I study of the combination of CAI and paclitaxel in adult patients with refractory cancers or lymphoma: This clinical trial has shown safety of combining pulse CAI with 3 weekly paclitaxel and is now testing daily CAI with 3 weekly paclitaxel

MB349: A multi-institutional phase II study of cyclophosphamide, paclitaxel, cisplatin with G–CSF for patients with newly diagnosed advanced stage ovarian cancer: Molecular evidence of the supra-additive effects of the combination of cisplatin, cyclophosphamide, and paclitaxel has led to this phase II study for the treatment of newly diagnosed epithelial ovarian cancer. Molecular diagnostic questions related to ovarian cancer progression are posed in the phase II study and are underway

MB392: A phase II time-to-progression study of orally administered CAI to patients with persistent epithelial ovarian cancer: This protocol follows our evaluation of CAI and asks whether the agent might provide disease stabilization to patients with advanced ovarian cancer. Translational endpoints include assessment of a CAI resistance-associated gene and markers of angiogenesis.

MB415L: A pilot study of proteomic evaluation of epithelial ovarian cancer patients in first clinical remission. Development of a protein fingerprint profile associated with relapse: This protocol enrolls women in first clinical response to treatment for ovarian cancer and follows them serially until relapse is diagnosed. Advanced proteomic technologies are being applied to serum samples to identify proteomic patterns that are predictive of recurrent disease. This is in concert with similar work ongoing in study sets of sera from newly diagnosed women.

Collaborative Clinical Trials:

Co–PI GOG–175: A randomized phase III trial of IV carboplatin (AUC 6) and paclitaxel 175 mg/m2 q 21 days x 3 courses plus low dose paclitaxel 40 mg/m2/wk versus IV carboplatin (AUC 6) and paclitaxel 175mg/m2 q 21 days x 3 courses plus observation in patients with early stage ovarian carcinoma.

Scientific PI GOG–0170C: A phase II trial of ZD1839 (Iressa™) (NSC#715055) in the treatment of persistent or recurrent epithelial ovarian or primary peritoneal carcinoma.

James L. Mulshine

98–C–0118: A randomized, double blind, placebo-controlled phase IIB trial of ketorolac mouth rinse evaluating the effect of cyclo-oxygenase inhibition on oropharyngeal leukoplakia

91–C–0014: A phase II trial of leuprolide plus flutamide plus suramin in untreated D2 prostate carcinoma

95–C–0056: A phase II trial of 72-hr continuous IV infusion of 9-amino-camptothecin with G–CSF support in patients with advanced ovarian cancer previously treated with paclitaxel and cisplatin

95–C–0178: A randomized phase II study of oral thalidomide in patients with hormone refractory prostate cancer

Clinical Trials (continued):

James L. Mulshine

98–C–0015: A phase I study of oral COL–3, a matrix metalloproteinase inhibitor, in patients with refractory metastatic cancer

98–C–0162: A phase II study of MGI–114 in patients with recurrent or persistent epithelial ovarian cancer

Dermatology Branch

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The Dermatology Branch conducts both clinical and basic research studying the etiology, diagnosis, and treatment of inflammatory and malignant diseases involving the skin and the host's response to these diseases. The basic research involves biochemical as well as biological studies of skin and is subdivided into seven separate, though frequently interacting, areas. More specifically, the research areas of interest include the role of skin as an immunological organ, the role of accessory cell molecules in the generation of cellular immune responses, and the role of adhesion molecules in inherited and acquired blistering diseases as well as their role in other inflammatory and neoplastic diseases. Other studies include the use of skin and, in particular, keratinocytes in gene therapy; the virologic and immunologic mechanisms involved in the pathogenesis of a variety of HIV-associated skin diseases; and DNA repair in normal human cells and in cells from patients with xeroderma pigmentosum and Alzheimer's disease. In addition, the branch conducts clinical studies, both diagnostic and therapeutic, in a broad range of diseases. It is also responsible for patient consultations throughout the Clinical Center.

The Dermatology Branch has a long tradition of being a fellowship training center for individuals who have become outstanding scientists and leaders in investigative dermatology in the United States and abroad. During their fellowship, our trainees have won many national awards as outstanding young investigators.

Dermatology Branch Staff

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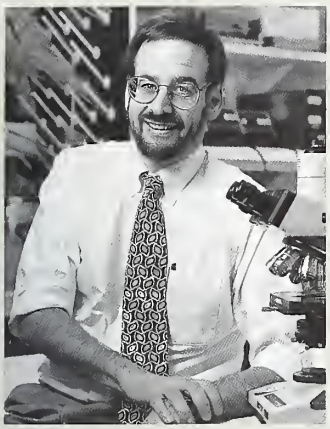

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Biography: *Dr. Udey majored in chemistry at the University of Wisconsin–Madison and received his M.D. and Ph.D. degrees from Washington University. He completed a medical internship and dermatology residency at Barnes Hospital and was a faculty member in dermatology at Washington University prior to coming to the NIH. Dr. Udey is a senior investigator and an editor of several journals. His research focuses on elucidating important aspects of epidermal Langerhans cell and dendritic cell biology.*

Dermatology Branch
Langerhans Cell Function in Health and Disease

Keywords:
dendritic cells
Langerhans cells
Leishmania
protein transduction
tumor vaccines

Research: Langerhans cells belong to a family of cells that exhibits similar dendritic morphologies and functional activities. These immature epidermal dendritic cells are responsible for the initiation and propagation of immune responses directed towards antigens encountered in skin. Because they can ingest and process complex antigens, Langerhans cells are well situated to function as sentinels at interfaces between organism and environment. After antigen capture, Langerhans cells migrate from skin to regional lymph nodes where, as mature dendritic cells, they initiate and shape primary immune responses in naive T cells. Thus, Langerhans cells (and other dendritic cells) represent cellular bridges between innate and adaptive immune responses.

Two major initiatives are ongoing in the laboratory. The first involves identification and characterization of gene products that are preferentially expressed by dendritic cells to gain insight into specialized functions of these potent accessory cells. We have utilized array technology and representational difference analysis to identify genes that are expressed by activated but not resting dendritic cells, or genes that are expressed by dendritic cells but not closely related cells such as macrophages. These kinds of studies are possible only because we previously defined culture conditions that allow expansion of Langerhans cell-like cells from mouse skin in vitro.

After an initial screening period, we have focused on three genes of interest. One gene was chosen because it encodes a signal transduction molecule that regulates cytokine production in dendritic cells and other lymphoid cells and is predicted to influence the ability of dendritic cells to shape T cell responses. Another gene was chosen because the primary sequence of its protein product suggests that it plays an important role in recognition and acquisition of particulate antigen by dendritic cells for presentation to cytotoxic lymphocytes. This protein is anticipated to play an important role in the initiation of antitumor immunity. The third gene was chosen because it encodes the mouse homolog of a previously described human protein that is expressed only in Langerhans cells and related cells and that is thought to

function as a receptor for antigen uptake. Studies of this latter gene will focus on elucidation of function of the protein and definition of upstream gene regulatory regions that will facilitate future studies in transgenic mice.

The second major initiative is an attempt to exploit the ability of HIV TAT protein transduction domain (PTD)-containing proteins to translocate across cell membranes to modify dendritic cell immunogenicity. This pretranslational project is designed to develop methodology that may facilitate the exploitation of the antigen-presenting power of dendritic cells for patient benefit. We predicted that PTD-containing recombinant proteins would be internalized and presented by dendritic cells to helper T cells as expected for exogenous antigens. We also predicted that PTD-containing proteins would selectively accumulate in the cytoplasm of dendritic cells and be metabolized for presentation to cytotoxic lymphocytes. We have demonstrated that PTD antigen-treated dendritic cells are potent stimulators of both helper and cytotoxic lymphocytes, that they prime antigen-specific cytotoxic lymphocyte responses in vivo, and that they efficiently vaccinate against tumors expressing model tumor antigens. Future studies will determine the general applicability of this approach, attempt to improve its efficacy and/or practicality, and assess the impact of this strategy on other serious diseases (e.g., chronic infections such as cutaneous leishmaniasis).

Our collaborators are David Sacks, NIH; and Esther von Stebut, Johannes Gutenberg University, Germany.

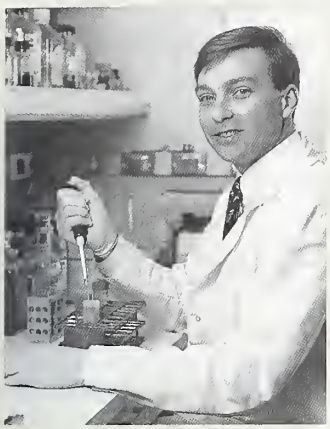
Recent Publications:

von Stebut E, et al. *J Exp Med* 1998;188:1547–52.

Jakob T, et al. *J Invest Dermatol* 1999;112:102–8x.

Walker P, et al. *Proc Natl Acad Sci* 1999;96:6970–5.

von Stebut E, et al. *Eur J Immuol* 2000;30:3498–506.



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Biography: *Dr. Blauvelt received his M.D. from Michigan State University in 1988. After an internal medicine internship at Henry Ford Hospital, he trained in clinical dermatology at the University of Miami until 1992, then obtained 4 years of immunology and virology research training in the Dermatology Branch, NCI, and in the Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases. He was honored by the American Academy of Dermatology with a Young Investigator in Dermatology Award in 1996 and by the U.S. Public Health Service with an Outstanding Service Medal in 1999.*

Dermatology Branch
Pathogenesis of Virologic and Immunologic Cutaneous Diseases

Keywords:
AIDS
cancer
dendritic cells
herpesviruses
HHV–8/KSHV
HIV
Kaposi's sarcoma
Langerhans cells
microbicides
psoriasis

Research: Our focus is the detailed investigation of mechanisms involved in sexual transmission of HIV. Langerhans cells (LC) are skin and mucosal dendritic cells that are the first cells infected by HIV following sexual exposure to virus. Using LC obtained from skin and vaginal mucosa, as well as LC-like dendritic cells derived from blood, we have determined that these cells interact with HIV through two distinct pathways: infection of LC is dependent on CD4 and HIV coreceptors, whereas capture of HIV virions on the cell surface of these cells (with subsequent transfer to cocultured T cells) occurs independently of CD4 and HIV coreceptors. Recently, we have also studied expression, function, and cytokine regulation of the HIV coreceptors CCR5 and CXCR4 on LC, adding insight into several aspects of AIDS pathogenesis. Current studies include testing of potential topical microbicides designed to block sexual transmission of HIV and determining mechanisms underlying HIV-induced dysfunction of dendritic cells. This research is designed to enhance understanding of both early and late immunologic events that occur during HIV infection.

Kaposi's sarcoma (KS) is associated with KS-associated herpesvirus (KSHV) infection, but specific cellular and molecular interactions between KSHV and cells derived from skin are poorly understood. Recently, we have developed a system to rapidly and quantitatively identify lytically infected single cells, which will aid in the detailed characterization and phenotyping of these cells in patients with KS. Additional KS pathogenesis studies are focused on elucidating the growth-promoting effects of latently expressed viral proteins within KS tumors. These studies should lead to better understanding of KS pathogenesis and to improved treatments for this disease.

Clinically, a protocol using recombinant human interleukin IL–10 for moderate-to-severe psoriasis has recently been completed, and a new protocol using micellar paclitaxel for severe psoriasis has been initiated. Psoriasis, a type 1 cytokine-dominated inflammatory skin disease, can be

particularly difficult to control and novel therapies are needed. Laboratory studies conducted in conjunction with these clinical trials are conducted to better understand the mechanisms of drug action.

Collaborators are Stephen Chanock, Mark Connors, Hiroaki Mitsuya, Gene Shearer, and Robert Yarchoan, NIH; Michael Lederman and Peter Zimmerman, Case Western Reserve University; and Jan Orenstein, George Washington University.

Recent Publications:

Zoeteweij JP, et al. *J Virol* 1999;73:5894–902.

Kawamura T, et al. *J Exp Med* 2000;192:1491–500.

Kawamura T, et al. *Eur J Immunol* 2001;31:360–8.

Zoeteweij JP, et al. *Blood* 2001;97:2374–80.

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Biography: *Dr. Hwang received his M.D. from Harvard Medical School and his Ph.D. in biochemistry from the University of Basel in Switzerland. He completed a medical internship at the Brigham and Women's Hospital and a dermatology residency at the University of California at San Francisco (UCSF). He continued there as a Howard Hughes Physician Fellow in the laboratory of Dr. Steven D. Rosen, studying the role of L-selectin in signaling the activation of T lymphocytes as part of normal recirculation in peripheral lymph nodes. He served as clinical instructor and associate physician in the Department of Dermatology at UCSF prior to coming to the NIH in 1997. Dr. Hwang was honored by the American Academy of Dermatology with a Young Investigator in Dermatology Award in 1996 and became a tenure track investigator in the Dermatology Branch in 1997.*

Dermatology Branch
Trafficking of Immune Cells Into and Out of the Skin

Keywords:

chemokine
chemokine receptor
dendritic cells
lymphocytes
mast cells
SDF–1
skin
trafficking

Research: Dr. Hwang's work focuses on the biology of immune cell trafficking to and from skin in inflammatory and neoplastic processes with a particular emphasis on the roles of chemoattractant cytokines (also known as chemokines), chemokine receptors, and adhesion molecules. Initial studies in his laboratory have addressed the question of how antigen-presenting cells or dendritic cells (DC) of skin leave the skin after being triggered by inflammatory signals or antigens. He has found that a chemokine named secondary lymphoid tissue chemokine (SLC) and its receptor, CCR7, play significant roles in this process by recruiting activated DC into afferent lymphatic vessels. The emigration process appears to be regulated by altering the expression of CCR7 such that it is strongly upregulated by inflammatory conditions in which DCs leave the skin. By understanding the mechanisms

through which DCs leave skin and enter lymph nodes, scientists may develop strategies for implementing more effective dendritic cell vaccines and therapies.

Dr. Hwang has also discovered that DCs express chemokines as they become activated. In particular, his laboratory has detailed the expression of a novel membrane-bound chemokine called fractalkine on maturing DCs. This molecule is strongly upregulated by maturing DCs and is still associated with DCs in peripheral secondary lymphoid tissues such as tonsil. Interestingly, a subset of T cells expresses the fractalkine receptor, and thus it is possible that fractalkine mediates the cell-cell binding/communication between DCs and T cells. Investigations with fractalkine receptor knockout mice are under way to test this hypothesis.

Dr. Hwang has recently investigated the role of endothelial cell-produced chemokines in the adhesion of memory T cells to inflamed blood vessels in skin. Using a dynamic parallel plate flow chamber system, his laboratory has shown the CC chemokine, liver and activation-regulated chemokine (LARC), plays a critical role in the firm attachment of CCR6-positive human memory T cells to activated dermal endothelial cells.

The Hwang Laboratory uses a wide variety of cell biology and molecular biology techniques including transgenic expression of chemokines under skin-specific promoters, fluorescence microscopy, gene expression analysis using glass microarrays, and RT–PCR. Recently, Dr. Hwang has implemented the use of a parallel plate flow chamber assay to simulate blood flow and interactions of leukocytes with endothelial adhesion molecules.

Collaborators in this work are Wright Caughman and Shubha Naik, Emory University; and Jiliang Gao, Alasdair Gilfilan, Dean Metcalfe, and Philip Murphy, NIH.

Recent Publications:

Papadopoulos E, et al. *Eur J Immunol* 1999;29:2551–9.

Saeki H, et al. *J Immunol* 1999;162:2472–5.

Fitzhugh DJ, et al. *J Immunol* 2000;165:6677–81.

Saeki H, et al. *Eur J Immunol* 2000;30:2808–14.

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Biography: *Dr. Katz received his M.D. from Tulane University Medical School in 1966. He trained in dermatology at the University of Miami between 1967 and 1970. He then earned a Ph.D. in immunology from the University of London in 1974. He has been a member of many scientific advisory and editorial boards and currently serves as director of the National Institute of Arthritis and Musculoskeletal and Skin Diseases.*

Dermatology Branch
Immunopathologic Mechanisms Involved in Inflammatory and Neoplastic Skin Diseases

Keywords:
cytokines
immune response
keratinocytes
Langerhans cells

Research: Our research is focused on the skin immune system—how it functions in the generation and perpetuation of skin and systemic immune responses and how its constituents, keratinocytes and Langerhans cells, function in normal immune surveillance and in inflammatory and infectious diseases. Allergic contact dermatitis is used as the paradigm for immunological, inflammatory, infectious, and neoplastic diseases of the skin. Specific aims include the following:

- **Study of the cytokine response profile during both the sensitization and elicitation phases of allergic contact sensitivity.** We have studied the very earliest events (during sensitization) that occur in allergic contact dermatitis. Within 24 hrs after exposure of epidermis to haptens, there is "activation" of Langerhans cells, as demonstrated by their expressing greatly increased amounts of class II MHC on their surfaces and becoming much more potent antigen-presenting cells. There is an almost immediate upregulation of IL–1β mRNA from Langerhans cells after hapten application and later an upregulation of IL–10 mRNA by keratinocytes. We are also studying the effects of UV radiation on the expression of cytokine mRNA and have found that even small amounts of UVB enhance expression of IL–10 and IL–12 but inhibit IL–15, which is constitutively seen in human skin. These cytokines exert functional effects on the skin, both in vivo and in vitro.

We have more recently focused our studies on understanding the relative contributions of various cell types in cellular infiltrates during the elicitation phase of contact sensitivity. Using semiquantitative RT–PCR and functional studies, we found that IL–4 downregulates contact sensitivity reactions. These studies have been validated using anti-IL–4 mAb, and using mice with genetic disruptions in CD28–B7 signaling.

- **The role of dendritic cells in intracutaneous DNA sensitization.** We have had a long-term interest in the role of Langerhans cells in the generation and perpetuation of immunological reactions. We have extended our studies to the utilization of Langerhans cells for DNA vaccination, i.e., for the generation of protective immunity following injection of protein-encoding DNA. The mechanisms by which immunization occurs when naked DNA is injected are not known, and the role of dendritic cells in the process is being

studied. We have demonstrated that intracutaneous injection of plasmid DNA leads to association of nominal protein antigen with cutaneous dendritic cells that can induce primary and secondary humoral and cellular responses. The focus of current studies is to identify whether we can utilize this approach in vitro using dendritic cells cultivated from blood and skin.

- **Study of the role of epidermal components in the maintenance of tolerance to protein and self antigens.** We have developed a model system whereby keratinocytes of transgenic mice produce ovalbumin under the control of a K14 promoter. The skin of these mice is used as a target for T cells that are obtained from OT–I and OT–II mice that are transgenic for a TCR recognizing either ovalbumin peptides in association with class I MHC or class II MHC molecules, respectively. We have also crossed the K14-ovalbumin transgenic mice with the OT–I mice and have found that the T cells with a TCR that recognizes ovalbumin peptides is depleted. The focus of our current studies is to identify the mechanisms for this deletion and to try to modulate these immunological responses.

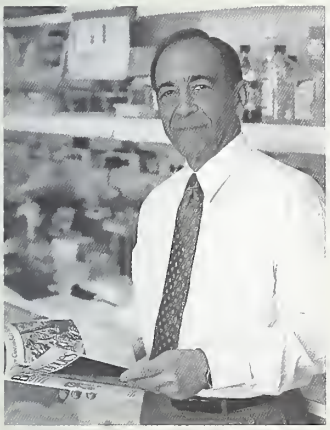
Recent Publications:

Bouloc A, et al. *Eur J Immunol* 1999;29:446–54.

Asada H, et al. *J Virol* 1999;73:4019–28.

Yamada N, et al. *J Immunol* 1999;63:5331–7.

Shabaki A, et al. *Eur J Immunol* 2001; in press.



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Biography: *Dr. Robbins received his A.B. from Harvard College in 1956 and his M.D. in 1960 from the College of Physicians and Surgeons, Columbia University. He interned and had his residency training in medicine at the Mount Sinai Hospital, New York City. Since 1965 he has been a senior investigator/medical officer (research) in the Dermatology Branch, NCI. Dr. Robbins has performed clinical and DNA-repair studies on patients having conditions such as xeroderma pigmentosum, Cockayne syndrome, and Alzheimer's disease.*

Dermatology Branch
Defective DNA Repair: Relationship to Cancer and Neurodegeneration

Keywords:
DNA repair
ionizing radiation
skin cancer
ultraviolet radiation

Research: Dr. Robbins' studies are designed to elucidate the role of DNA repair processes in carcinogenesis and in neurodegeneration. We have studied primarily xeroderma pigmentosum (XP), an inherited disease in which cells are unable to conduct nucleotide excision repair (NER) of damaged DNA. People with this condition are at a very high risk of developing sunlight-induced skin cancer, and some also experience degeneration of the nervous system. Cancer arises in these patients because

their cells cannot repair the DNA lesions induced by UV radiation in sunlight. We have proposed that death of nerve cells in XP results from accumulation of unrepaired neuronal DNA damaged not by UV-radiation, which cannot reach the nervous system, but by free radicals which are plentiful in rapidly metabolizing neurons. While UV radiation-induced bulky DNA lesions have been synthesized and/or well-characterized, the same is not true of free-radical-induced bulky lesions. Accordingly, we set out to synthesize such lesions in order to study in cell extracts and in living cells their repair, their effects on DNA replication, and their effects on transcription.

Several years ago we identified by gas chromatography-mass spectroscopy the formation of 8,5′-cyclo-2′deoxyadenosine (cyclo-dA) in human cells exposed to ionizing radiation. Cyclo-dA is a principal type of bulky damage in DNA exposed to oxidative free radicals. To study its repair, we synthesized a cyclo-dA phosphoramidite whose structure was confirmed by nuclear-magnetic-resonance and mass-spectral analysis. Cyclo-dA was then incorporated into DNA oligonucleotides.

Mammalian cell extracts were assayed for the excision nuclease of NER and for DNA glycosylase activity, the initial step in base excision repair (BER). In the BER assay, extracts of the wild-type Chinese hamster ovary (CHO) cell line AA8 released oligonucleotides 25–30 bases in length from the 140-mer duplex substrate containing the cyclo-dA. Extracts from two NER-deficient cell lines, UV135 and UV20 (derived from the AA8 line and defective in the rodent XP–G and ERCC–1 proteins, respectively), were unable to carry out excision. However, when extracts from the NER-deficient lines were mixed, excision activity was restored, indicating that the excision activity towards the cyclo-dA substrate was due to NER. In the glycosylase assay, adult rat brain extracts did not cleave oligonucleotides containing cyclo-dA but cleaved those containing known DNA glycosylase substrates (e.g., 8-hydroxy-2′-deoxyguanosine), suggesting that cyclo-dA is a substrate for NER but not BER.

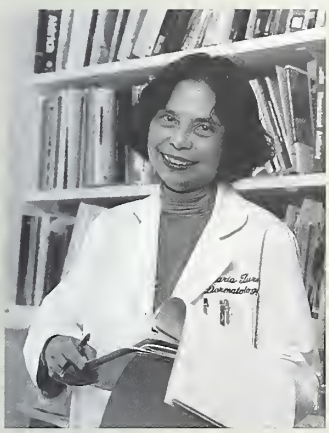
We placed cyclo-dA into the transcribed strand of a luciferase reporter gene contained in a nonreplicating plasmid and transfected it into the CHO cells. Because it was difficult to obtain large amounts of these constructs, we developed a host cell reactivation assay to measure luciferase expression in cultures with as few as 3,000 cells transfected with less than 0.1 ng of plasmid. The cyclo-dA-containing plasmid was repaired well by the normal AA8 cells but was poorly repaired by the UV20 and UV135 cells. A single cyclo-dA lesion resulted in more than a 90 percent reduction of luciferase expression in these NER-deficient cells, indicating that this lesion is a significant block to RNA polymerase II. If cyclo-dA occurs in nerve cells, its lack of repair could prevent transcription of vital genes. Thus, it is possible that lack of repair of cyclo-dA may lead to death of neurons not only in diseases with known defects in NER, such as XP and Cockayne syndrome, but also in other neurodegenerations with suspected defective NER, including Alzheimer's and Parkinson's diseases. We intend to study the repair of cyclo-dA and of other free-radical-induced DNA lesions in cells from patients with these and other neurodegenerations.

Our collaborators are Eric Ackerman and Alex Spoonde, Pacific Northwest National Laboratory; David Berry and Dean Wise, Berry and Associates; Philip Brooks and Robert Tarone, NIH; Roger Brumback, University of Oklahoma; and Hugh Mackie and Robert Somers, Glen Research Corporation.

Recent Publications:

Moriwaki S–I, et al. *J Invest Dermatol* 1996;107:647–53.

Parshad R, et al. *Proc Natl Acad Sci USA* 1996;93:5146–50.



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Biography: *Dr. Turner is a board-certified dermatologist who came to the NIH 10 years ago, having spent the previous 13 years as professor of dermatology at George Washington University Medical School. She received her M.D. from the University of the Philippines and trained in dermatology at Yale University. Her major interests are the characterization, pathogenesis, and treatment of the cutaneous manifestations of systemic diseases, and teaching. Dr. Turner is the recipient of the NIH Clinical Center Teacher of the Year award in 1995.*

Dermatology Branch
Consult Service

Keywords:

adverse drug reactions
Birt-Hogg-Dube syndrome
cutaneous T cell lymphoma
dermatitis in immunodeficiencies
psoriasis

Research: Activities in Dr. Turner's laboratory can be grouped into three major categories: (1) **Service**—responsibility for the organization and delivery of the clinical services/consultations offered by our branch as well as providing institutional memory as clinical fellows rotate every 4 to 6 months. (2) **Education**—supervision of the clinical fellow on consult rotation while the fellow sees consults and makes arrangements for biweekly clinical grand rounds. Grand rounds, attended by 70 to 80 physicians including residents from all the local training programs as well as community dermatologists, are a resource for second opinions on problem cases and serve as a conduit for an active interchange between our branch and the community. The type of cases seen by the Consult Service and the one-on-one interchange between fellow and attending make for an intense learning experience. The American Board of Dermatology credits us for 1 year of training. (3) **Research**—activities in this sphere consist of formal protocols in which Dr. Turner is either the principal investigator or a coinvestigator. With Dr. Blauvelt, she is a co-mentor for the branch's clinical research fellow (clinical trials) and supervises the write-up of selected cases from grand rounds for publication in a special section of the *Journal of the American Academy of Dermatology*. Current projects include continuing studies on the feasibility of using photographic images for the assessment of dermatologic disease burden in conjunction with NHANES, the efficacy of paclitaxel for the treatment of moderate to severe

psoriasis, the efficacy of yttrium-tagged anti-Tac in the treatment of HTLV–1-associated cutaneous T cell lymphoma, characterization of cutaneous T cell lymphoma, characterization of the dermatitis associated with Job's syndrome, the association of fibrofolliculomas with familial renal cancer (Birt-Hogg-Dube syndrome), cutaneous manifestations of alkaptonuria, and the genetics of keloids.

Collaborating with us are Andrew Blauvelt, William Gahl, Steven Holland, Sam Hwang, W. Marston Linehan, Constantine Stratakis, Thomas Waldmann, and Berton Zbar, NIH; and Alexa Kimball, Stanford.

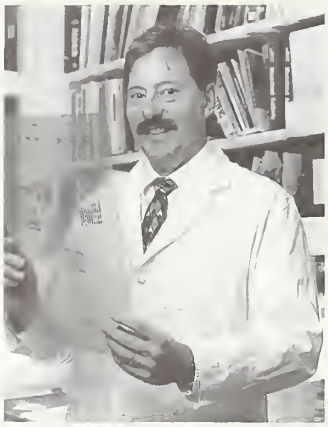
Recent Publications:

Kimball AB, et al. *Arthritis Rheum* 2000;43:1866–73.

Egan CA, et al. *J Am Acad Dermatol* 2001;44:282–4.

Ghate JV, et al. *Arch Dermatol* 2001;137:471–4.

Stratakis CA, et al. *J Med Genet* 2001;38:338–43.



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Biography: *Dr. Vogel received his M.D. from Rush Medical College in Chicago in 1981. His training includes an internal medicine residency at the Barnes Hospital of the Washington University Medical Center, St. Louis, and a fellowship at the NCI. Following a 3-year faculty position at the Holland Laboratories of the American Red Cross, Rockville, MD, Dr. Vogel joined the Dermatology Branch in the NCI where he has focused on skin gene therapy, specifically targeting keratinocytes.*

Dermatology Branch
Genetic Manipulations of Keratinocyte Gene Expression

Keywords:
animal models
gene therapy
lentiviral vectors
retroviral vectors
skin cancer
stem cells

Research: The first research objective of this laboratory is to develop methods for stable introduction and expression of genes into keratinocytes for gene therapy purposes. Keratinocyte gene therapy could be used to treat genetic skin diseases caused by a genetic defect of an endogenous keratinocyte protein, and could also be used to express needed protein products such as growth factors, cytokines, or enzymes for systemic delivery.

Two general approaches can be used for targeting genes to the epidermis: the in vivo approach and the ex vivo approach. The in vivo approach directly introduces the gene into epidermis, while the ex vivo approach is one in which we first isolate keratinocytes, insert the desired gene while in tissue culture, and graft the genetically modified keratinocytes back onto the donor. Previously, we developed a novel approach for directly introducing and transiently expressing genes in epidermis simply by injecting naked plasmid DNA into the dermis of skin. In a series of studies, we characterized the

uptake and expression of plasmid DNA in human, pig, and mouse skin and demonstrated that biological response modifiers such as cytokine genes could be expressed in the epidermis and achieve an expected biological effect, such as the recruitment of neutrophils. Although skin gene expression with this direct approach was transient, we found it to be very effective for DNA vaccination. We next demonstrated that DNA vaccination with plasmids expressing *Leishmania* proteins can provide protection against *Leishmania* infection in susceptible mice due, in part, to the presence of immunostimulatory DNA sequences (nonmethylated CpG dinucleotides flanked by two 5′ purine nucleosides and two 3′ pyridine nucleosides) on the plasmid DNA. Furthermore, oligodeoxynucleotides containing these immunostimulatory sequences were found to be effective adjuvants when combined with *Leishmania* protein antigens and were able to protect susceptible mice from infection. The adjuvant effect of these CpG-containing oligodeoxynucleotides could be explained by their activation of the dendritic antigen-presenting cells in the skin (e.g., Langerhans cells).

In order to overcome the problem of transient keratinocyte gene expression, we are currently developing an ex vivo approach to achieve long-term expression by linking expression of the desired gene (bicistronically) to a selectable marker gene (the multidrug resistance or MDR gene) and then topically selecting for keratinocytes that express the MDR gene in vivo. A significant advantage of this approach is that it does not depend on direct keratinocyte stem cell identification or selective keratinocyte stem cell targeting. With topical application of colchicine, only keratinocytes that contain the MDR gene will survive and repopulate the epidermis and this, by definition, will only be possible if a significant percentage of keratinocyte stem cells contain and express the MDR gene. In skin organ culture studies, we have demonstrated the feasibility of this approach by showing that MDR expression and colchicine selection do not interfere with keratinocyte proliferation, differentiation, or stratification and, most importantly, by showing that colchicine treatment increases the percentage of keratinocytes expressing MDR. In order to demonstrate feasibility in vivo, artificial human skin or skin equivalents prepared with MDR-transduced keratinocytes have been grafted onto immunocompromised mice and topically selected with colchicine. Compared to control mice, MDR expression can be maintained in significant percentages of cells for long periods of time.

We are also evaluating whether lentiviral vectors are able to introduce desired genes into keratinocyte stem cells more efficiently than retroviral vectors. Lentiviral vectors are superior in transducing and integrating into nondividing cells as compared to retroviral vectors and may be superior for introducing genes into slowly cycling keratinocyte stem cells. Animal models with artificial skin grafts constructed with lentiviral transduced keratinocytes are currently being assessed for duration and level of gene expression. Prolonged in vivo expression is probably the best indicator of keratinocyte stem cell transduction since specific markers of keratinocyte stem cells are not yet available. Finally, we are attempting to distinguish keratinocyte stem cells from other basal keratinocytes by identifying unique markers so that the keratinocyte stem cells can be purified or enriched to further enhance gene introduction. We have identified slowly dividing keratinocyte stem cells based on their ability to retain a nucleotide label and can isolate these labeled keratinocyte stem cells using laser capture microdissection and flow

cytometry cell sorting. The patterns of gene expression in keratinocyte stem cells can therefore now be compared to other basal layer keratinocytes on both the mRNA (using cDNA microarrays) and protein levels. Genes that are uniquely expressed in keratinocyte stem cells in situ may provide a means to identify and purify keratinocyte stem cells for gene therapy purposes, and the differential expression of genes in keratinocyte stem cells and basal keratinocytes should, in turn, be informative about stem cell biology.

Collaborating with us are Cynthia Dunbar and Mark Udey, NIH; Lorne Taichman, State University of New York; and Kim Yancey, Medical College of Wisconsin.

Recent Publications:

Walker PS. *Proc Natl Acad Sci USA* 1999;96:6970–5.

Pfutzner W. *Hum Gene Ther* 1999;10:2811–21.

Hildesheim J. *J Biol Chem* 1999;274:26399–406.

Hildesheim J. *J Cell Sci* 2001;114:1913–23.

Clinical Trials:

Andrew Blauvelt

76–C–0293: Induction of suction blisters in patients with urticaria, blistering diseases, inflammatory dermatoses and neoplastic disorders, and in normal volunteers.

93–C–0050: Induction of suction blisters in HIV-infected patients.

96–C–0097: The acquisition of blood and skin samples from normal volunteers to support research activities on dermatologic diseases

99–C–0027: A randomized double-blind placebo-controlled trial using recombinant human interleukin 10 for moderate-to-severe psoriasis

00–C–0211: A pilot open-label single-dose study using intravenous micellar paclitaxel for patients with severe psoriasis

99–C–0027: A randomized double-blind placebo-controlled study using recombinant human interleukin 10 for moderate-to-severe psoriasis

Jonathan Vogel

01–H–0120: The origin of keratinocytes and keratinocyte stem cells following allogeneic hematopoietic stem cell transplantation

Experimental Immunology Branch

The Experimental Immunology Branch consists of 11 research laboratories, a flow cytometry facility, and a digital microscopy facility that perform investigations in basic immunobiology with particular emphasis on: (1) lymphocyte recognition, differentiation, and regulation; (2) cell biology of immune responses; (3) signal transduction; (4) structure, regulation, and function of genes involved in immune responses; (5) lymphocyte effector functions; (6) developmental biology; (7) transplantation and tumor immunology; and (8) cellular regulatory mechanisms.

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Niti Puri	Postdoctoral Fellow

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Flow Cytometry Facility

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Experimental Immunology Branch
Lymphocyte Development

Keywords:
development
lineage commitment
signaling
T cell receptor
thymus

Research: Development of all αβ T cells takes place in the thymus and proceeds via an ordered sequence of developmental steps that is best described by changing expression patterns of surface CD4/CD8 coreceptor molecules. Each differentiation step is signaled by components of the T cell antigen receptor (TCR) complex. The differentiation of immature CD4+8+ (double positive, DP) thymocytes into mature T cells is the key event in T cell development as it gives rise to functional T cells and determines the mature T cell repertoire. DP thymocytes expressing TCR with appropriate specificities are "positively selected" to further differentiate into mature T cells, while DP thymocytes expressing potentially autoreactive TCR are "negatively selected" and removed. The end result is a mature T cell repertoire that is reactive against foreign antigens but tolerant of self components. It is one of the paradoxes of thymic selection that both positive and negative selection are based on TCR specificity for intrathymic MHC/peptide complexes. Adding to the complexity of thymic selection is that TCR specificity additionally determines lineage direction, with TCR-signaled DP thymocytes differentiating into either CD4+ T helper cells or CD8+ T cytotoxic cells. Elucidating the cellular interactions and molecular signals that regulate these intrathymic events has been the major focus of our research program.

One of our major recent efforts has focused on identifying the molecular mechanisms by which a developing DP thymocyte determines its appropriate cell fate. In this regard we have developed an experimental in vitro model of positive selection that led us to a number of startling observations and discoveries that flatly contradicted accepted paradigms. We have discovered that DP thymocytes initially terminate CD8 gene transcription, even when differentiating into mature CD8+ T cells, so that signaled DP thymocytes transcriptionally become CD4+8– intermediate cells regardless of their TCR specificity. It is at this CD4+8– intermediate stage of differentiation that the decision to become either a CD4+ helper or CD8+ cytolytic cell is made. The decision to become a CD8+ T cell requires IL–7 that permits thymocytes to extinguish CD4 gene transcription and reinitiate CD8 transcription, novel

molecular events we have referred to as "coreceptor reversal." We have synthesized our experimental results into a new model of thymocyte differentiation and lineage determination called kinetic signaling that applies not only to DP thymocytes, but also to bipotential cells in multiple biological systems.

Recent Publications:

Bosselut R, et al. *Immunity* 2001;14:483–94.

Brugnera E, et al. *Immunity* 2000;13:59–71.

Bosselut R, et al. *Immunity* 2000;12:409–18.

Cibotti R, et al. *Mol Cell Biol* 2000;20:3852–9.

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Experimental Immunology Branch
T Cell Population Homeostasis/T Cell Regulation

Keywords:
bone marrow
immunoregulation
T cells
transgenic mouse

Research: The generation and maintenance of T cell populations is a critical consideration in the regulation of transplantation responses. Initial studies of T cell population regeneration in T cell-depleted states suggested that, in certain circumstances, reconstituting T cells were derived from mature T cells rather than from early hematopoietic precursors or stem cells. Distinguishing between these possibilities is of central importance to considerations of the biology of mismatched marrow transplantation, mechanisms of tolerance induction and maintenance, and the development of either vaccine strategies or general strategies for enhancing T cell recovery from diseases or therapies associated with depletion of T cells. Subsequent studies in murine models demonstrated that in circumstances of limited thymic function, the expansion of mature T cells in fact contributes substantially to T cell reconstitution. Cells arising by such expansion are predominantly of memory phenotype while naive T cells are largely generated by the thymic pathway. This information has been applied to studies of T cell generation in young patients with depleted peripheral T cell populations. An age dependence of T cell generation by the thymus was found such that even young adults were severely compromised in terms of an ability to generate T cell populations in the

setting of extensive depletion of mature T cell populations. These studies demonstrated the validity of using naive versus memory T cell phenotype to study T cell generation in patient populations, and so provided a new approach to identify pathways of T cell generation in humans. In contrast to these studies in children, studies in adults have shown that CD4+ T cell regeneration initially proceeds primarily by way of mature T cell expansion. Parallel studies in murine models have shown that such expansion is antigen driven, prone to skewing, and cytokine modulated. Two cytokines were identified as active in modifying CD4+ T cell reconstitution—namely, IL–2 and IL–7. The former acts through upregulation of thymic-dependent generation of T cells; the latter is the only cytokine identified in these studies which upregulates T cell regeneration by peripheral expansion of mature T cells in the absence of a functional thymus. Investigations have also been carried out to identify cytokines that may negatively regulate T cell population maintenance. A transgenic mouse expressing on T cells a dominant-negative receptor for TGFβ has been found to have ongoing expansion of CD8+ T cells, indicating a critical negative regulatory role for this cytokine in the homeostasis of CD8+ T cells. Unlike CD4+ T cells, subsets of CD8+ T cells have been identified that appear to be generated directly from marrow precursors through extrathymic pathways. These studies have served to increase information about the regulation of T cell-mediated transplantation responses and T cell generation, and create a foundation and rationale for new therapies in the treatment of cancer.

Recent Publications:

Mackall C, et al. *N Engl J Med* 1995;332:143–9.

Mackall C, et al. *Blood* 1997;89:3700–7.

Hakim F, et al. *Blood* 1997;90:3789–98.

Lucas PJ, et al. *J Exp Med* 2000;191:1187–96.

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Experimental Immunology Branch
Cytotoxic Lymphocyte Mechanisms and Apoptosis

Keywords:
apoptosis
caspase
cytotoxicity
lymphocyte
proteases

Research: This laboratory has had a long-term objective of defining the molecular mechanism of cell death induced by cytotoxic lymphocytes and has recently broadened this to include other cell death stimuli. Cytotoxic lymphocytes have been shown to be potent immunological mechanisms for the highly specific destruction of virus-infected and foreign cells, and most current immunological approaches to cancer therapy envision stimulating the differentiation of cytotoxic T lymphocytes with the ability to recognize tumor cells selectively. A basic understanding of endogenous cell-death pathways may be critical to designing better therapeutic agents for cancer as well as enhancing tumor-specific immune responses. We have been studying the basic mechanism by which cytotoxic lymphocytes kill their target cells in vitro and have developed the now well-accepted granule exocytosis model for the lethal mechanism. The model proposes that cytotoxic lymphocytes rapidly secrete preformed mediators in response to target binding, utilizing a modified form of the regulated secretory process found in many cell types. However, the details of how proteins secreted by the killer lymphocyte cause target cell death remain to be elucidated. While we initially focused on the pore-forming properties of the lymphocyte cytolysin/perforin molecule, we have found that the secreted serine proteases known as granzymes enter the target cell cytoplasm where they trigger death.

Our current objective is to understand the molecular pathway of this death. We have shown that target cell nuclei are not involved, and we have recently considered the hypothesis that granzymes activate the caspase proteases that are part of a general apoptotic death pathway operative in all cells. Two different types of specific caspase inhibitors block apoptotic nuclear damage via the CTL granule exocytosis pathway but do not detectably block target lysis. In contrast, these inhibitors block the rapid target lysis induced by the CTL FasL/Fas pathway in the same target cells. We have examined the caspase dependence of a number of prelytic cytoplasmic indicators of apoptotic damage induced in target cells by the granule exocytosis pathway. We have found that three such types of

damage are caspase independent for the granule exocytosis pathway but caspase dependent for the FasL/Fas pathway: phosphatidyl serine exposure on the outer membrane, mitochondrial potential drop, and plasma membrane blebbing. The enhanced rate of plasma membrane endocytosis, another readout of target damage, was found to be caspase dependent. These results lead us to propose that granzymes introduced into the target cell cytoplasm by the granule exocytosis pathway do activate caspases, which in turn damage nuclei and enhance the rate of membrane endocytosis. However, granzymes also act independently of caspases to trigger lysis and other types of cytoplasmic damage, and this pathway leads to target cell death. We speculate that granzymes directly activate a downstream death pathway that can also be activated by caspases in response to other death stimuli.

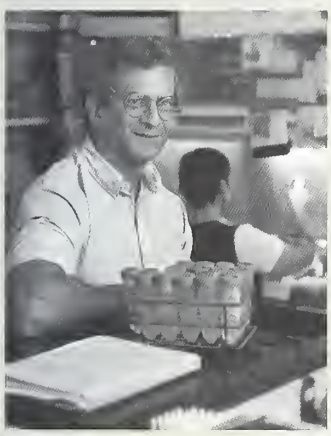
Recent Publications:

Haddad E, et al. *J Cell Biol* 2001;152:835–41.

Henkart PA, et al. *Semin Immunol* 1997;9:135–44.

Sarin A, et al. *J Immunol* 1998;62:2810–6.

Komoriya A, et al. *J Exp Med* 2000;191:1819–28.



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Experimental Immunology Branch
Cellular and Molecular Regulation of Immune Response

Keywords:

aging
animal model
B lymphocytes
CD4+ and CD8+ T lymphocytes
cell proliferation
chromosomal translocations
immune function
T cells
telomere

Research:

Regulation of Lymphocyte Replicative Capacity: Telomere Length and Telomerase Expression in the Immune System

A major interest of this lab is the study of mechanisms determining replicative capacity of T and B lymphocytes. This work has analyzed the role of telomerase and telomere length regulation. It had previously been reported that malignant tumors and germline cells, but not normal somatic cells, express telomerase, and it was proposed that this accounts for selective immortality of germline and cancer cells. On this background, studies tested the hypotheses that telomere length differs as a reflection of replicative history and that T and B cells express telomerase as a mechanism for extending replicative capacity. Recent findings are:

- **Telomerase activity is highly regulated during activation and development of T and B lymphocytes, and this regulation is mediated by both transcriptional and posttranslational mechanisms.** We have shown that both components of telomerase, RNA template (TER) and catalytic reverse transcriptase (TERT), are regulated at the level of steady state RNA during T and B cell development and activation. In addition, we demonstrated that telomerase activity is regulated by novel posttranslational mechanisms that may be related to TERT phosphorylation and nuclear translocation.

- **Telomere length in normal somatic cells is genetically regulated.** We have shown that differences in telomere length between mouse species are regulated by a single recessive locus. These findings demonstrated that increases in telomere length can occur during normal differentiation.

- **A recombinase-associated gene (Rag)-dependent mechanism mediates TCR αδ translocation in thymic lymphomagenesis in mice deficient for the ataxia telangiectasia mutated (ATM) gene.** ATM plays a role in repair responses to double-strand DNA breaks and influences telomere maintenance. We have characterized the role of ATM in Rag-dependent and Rag-independent recombination in tumorigenesis and have shown a role for ATM in Ig class switch recombination.

Current efforts will extend analysis of the relationship between telomerase activity and control of telomere length, and the relationship of these variables to replicative capacity. Systems have been established that will use transgenic and knock-out mice to elucidate the role of telomerase and telomere length maintenance in somatic cell function, with specific focus on lymphocyte biology.

The Role of Costimulatory Receptors in Lymphocyte Activation

A second area of interest is the study of costimulatory molecules. Findings include:

- **CD28 costimulation by B7–1 (CD80) and B7–2 (CD86) plays a substantial role in homeostasis of CD4 and CD8 T cell subsets.** Using mice genetically engineered to be either overexpressing or deficient in B7, we have shown that modulation of B7 expression results in profound alterations in the homeostasis of peripheral CD4 and CD8 cells.

- **Both CD40/CD40L-dependent and -independent pathways mediate negative selection of self-reactive T cells.** The function of CD40/CD40L and B7/CD28 costimulatory interactions in negative selection was studied using mice deficient in one or more of these molecules. We demonstrated that a CD40/CD40L-dependent pathway mediates negative selection of CD4 and CD8 thymocytes and that this pathway is independent of B7 and CD28. A second path of negative selection is CD40/CD40L-independent and acts late in differentiation of CD4 but not CD8 single positive T cells. Current studies are directed at further analyzing the influence of costimulatory molecules on T cell selection and tolerance.

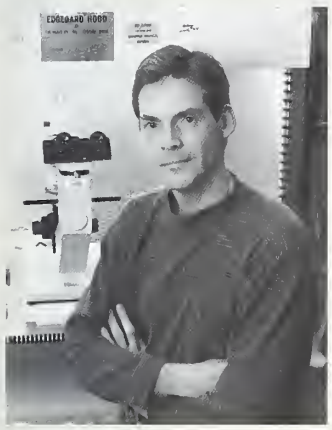
Recent Publications:

Hathcock K, et al. *Adv Immunol* 1996;62:131–66.

Weng NP, et al. *Immunity* 1998;9:151–7.

Yu X, et al. *J Immunol* 2000;164:3543–53.

Petiniot LK, et al. *Proc Natl Acad Sci USA* 2000;97:6664–9.



Immunology Branch in 1991.

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Experimental Immunology Branch
Genetic Control of Vertebrate Embryonic Development

Keywords:
development
gastrulation
left/right asymmetry
nodal
SUMO
ubiquitin

Research: Our research program is focused on basic mechanisms underlying the ordered growth, differentiation, and patterning of the mammalian embryo. Our experimental approaches involve mutagenesis and functional screens to identify and isolate genes that are essential for normal embryonic development of the mouse.

One of our main efforts has been the analysis of the nodal gene, which we identified some years ago on the basis of an insertional mutation. Nodal is a member of the transforming growth factor (TGF)β superfamily of secreted signaling molecules. Our recent studies have shown that nodal signals through Smad2 and Smad3, intracellular transducers also used by TGFβ and activin. Nodal signaling uses activin type 2 and type 1 receptors, but differs from activin signaling in requiring the function of extracellular proteins of the EGF–CFC family. Through the analysis of both a null and a hypomorphic nodal mutant, we have shown that nodal plays a major role in gastrulation, when the three germ layers form and the primary body axes are established. Nodal signaling prior to gastrulation is essential for the movement of specific extraembryonic cells to a position adjacent to the future head region. These extraembryonic cells provide the initial neural inducing signal to embryonic cells that will become the forebrain. Nodal signaling then is required to establish the node, notochord, and prechordal plate cells, which migrate to the head region, replacing the original extraembryonic cells, and provide a second neural inducing signal. Nodal is expressed around the node as it forms. This expression becomes progressively asymmetric, stronger on the left side. At the same time, nodal expression turns on in the left lateral plate mesoderm. We have found that this asymmetric pattern is essential for the

subsequent development of organs such as the heart and lungs that are asymmetric in structure or position within the body. Our ongoing studies on nodal signaling, which take advantage of the availability of recombinant nodal proteins, include the identification of nodal target genes in the embryo.

As a second approach to isolate developmentally important molecules we identified three novel proteins that interact with Nedd-4, a developmentally regulated E3 ubiquitin ligase, the component of the ubiquitin/proteasome pathway conferring specificity. Nedd-4 binding protein (N4BP)–1 is monoubiquitylated by Nedd-4, whereas N4BP–2 is polyubiquitylated. N4BP–1 appears to be a new component of promyelocytic leukemia (PML) bodies, subnuclear structures implicated in leukemia. The number and size of PML bodies is significantly increased in N4BP–1 transfected cells, indicating that N4BP–1 can regulate PML body assembly. Like other proteins in PML bodies, N4BP–1 may be modified by SUMO, a small ubiquitin-like protein that is conjugated to proteins in a process similar to ubiquitylation. Mutation of the consensus SUMOylation sites in N4BP–1 eliminates monoubiquitylation of N4BP–1, suggesting that these sites mediate monoubiquitylation as well. We are now testing the hypothesis that there is a competition between SUMOylation and monoubiquitylation of N4BP–1, with the SUMOylated form participating in PML body formation and the monoubiquitylated form kept in the cytosol or outside of PML bodies in the nucleus. Our eventual goal is to understand the role of N4BP–1 in embryonic development by generating gain of function and loss of function alleles.

Quite serendipitously, analysis of a second retroviral insertional mutation causing prenatal lethality has allowed us to identify the gene encoding SUMO-specific protease 1 (SENP1) as a strong candidate for the mutated gene. SENP1 is one of a family of enzymes responsible for removing SUMO moieties from proteins. The provirus lies in the first intron of the SENP1 gene, and SENP1 expression levels are reduced by approximately 50 percent in retroviral insertion homozygotes. Consistent with reduced levels of this deSUMOylating enzyme, there is a dramatic increase in the levels of SUMO modified RanGAP1 in homozygous mutant embryos. Homozygous embryos die around midgestation due to umbilical and placental defects, indicating an essential role for Ran-mediated nuclear transport in extraembryonic development. The complete elucidation of the SENP1 mutant phenotype will provide important new insights into the developmental functions of SUMO/Sentrin modification.

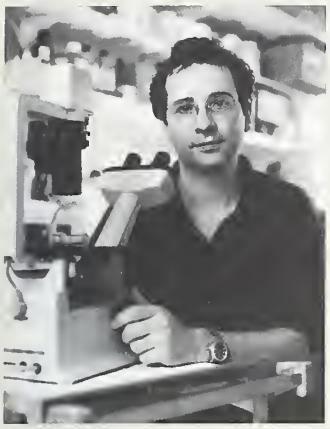
Recent Publications:

Izraeli S, et al. *Nature* 1999;399:691–4.

Lowe LA, et al. *Genesis* 2000;26:118–20.

Kumar A, et al. *J Biol Chem* 2001;276:656–61.

Lowe LA, et al. *Development* 2001;128:1831–43.



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Experimental Immunology Branch
Mechanisms of DNA Damage Detection and Repair in Lymphocytes

Keywords:
cancer
DNA repair
senescence
V(D)J recombination

Research: The focus of this group is to understand the mechanisms by which cells monitor and repair DNA double-strand breaks (DSBs). DSBs are generated spontaneously by reactive byproducts of oxygen metabolism during DNA replication and exposure to ionizing radiation. DSBs also serve to initiate a number of recombination events such as meiotic recombination and antigen receptor gene rearrangements in lymphocytes. While our research has addressed processes that are utilized by all cell types, we are particularly interested in how immune cells have adapted DNA damage detection and repair systems for their unique requirements.

Chromosomal DNA is continually broken and repaired during lymphocyte development in order to diversify the germline repertoire. Genes encoding variable regions of antigen receptors are assembled in immature B and T cells by a site-specific recombination reaction referred to as V(D)J recombination. During an immune response, mature B cells undergo further alterations of functionally rearranged immunoglobulin genes by class-switch recombination and somatic mutation, both of which involve DNA DSB intermediates. The fidelity of DNA breakage and rejoining events during these chromosomal rearrangements is critical for maintaining genomic stability as oncogenic translocations may result from aberrations in physiological recombination.

Mammalian cells have evolved several DNA repair mechanisms to counteract the deleterious effects of DSBs, including homologous recombination and nonhomologous end joining. Recently, our group has shown that components of the nonhomologous end-joining pathway, which repair breaks during V(D)J and class-switch recombination, are caretaker genes that maintain the integrity of the genome. Mice lacking both a nonhomologous end-joining protein (Ku80 or Ku70) and the p53 tumor suppressor protein develop pro-B cell lymphomas at an early age that result from a specific set of chromosomal translocations and gene amplifications involving IgH and c-myc, reminiscent of Burkitt's lymphoma. We are currently studying the mechanisms by which oncogenic translocations are generated in the absence of nonhomologous end joining. We are also

characterizing proteins that mediate DNA damage detection and signaling functions during V(D)J and class-switch recombination using molecular approaches including targeted gene inactivation and transgenic mice. Overall, the goal of these experiments is to elucidate the mechanisms by which DNA damage detection, signaling, and repair are integrated during lymphocyte development and activation.

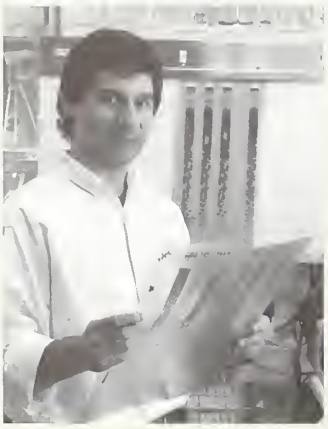
Recent Publications:

Ouyang H, et al. *J Exp Med* 1997;186:921–9.

Nussenzweig A, et al. *Proc Natl Acad Sci USA* 1997;94:13588–93.

Casellas R, et al. *Embo J* 1998;17:2404–11.

Difilippantonio M, et al. *Nature* 2000;404:510–4.



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Experimental Immunology Branch
Protein Trafficking in Lymphocytes

Keywords:
antigen presentation
exocytosis
membrane traffic
protein transport

Research: The main interest of our lab is in understanding the molecular mechanisms controlling protein traffic in lymphocytes and other cells of the hemopoietic lineage. We have two distinct areas of research in our group: one is in characterizing the proteins that regulate secretory granule fusion with the plasma membrane of cytotoxic lymphocytes, and the other is in understanding the role of MHC class II trafficking in the activation of antigen-specific T lymphocytes.

Regulation of Granule Exocytosis in Lymphocytes

The orderly transport of proteins within the secretory pathway of eukaryotic cells is mediated by the recognition of donor membrane-derived vesicles with distinct target organelles. The specificity of this interaction is thought to be mediated in part by the specific interaction of membrane proteins called SNAREs. There are SNARE proteins present on vesicles called v-SNAREs as well as SNARE proteins present on target membranes called t-SNAREs. Each class of SNARE proteins contains numerous isoforms that are present on distinct intracellular compartments, adding support to the idea that the specific interactions of distinct SNAREs are important for transport fidelity. It has been proposed that the formation of a v-SNARE/t-SNARE complex is important for the eventual fusion of opposing membranes. For example,

fusion of vesicles with the plasma membrane results in the insertion of integral membrane proteins at the cell surface and secretion of soluble proteins into the extracellular medium.

In our investigation of intracellular trafficking of proteins in lymphoid cells, we have used the yeast two-hybrid system to identify novel SNAREs. Using the plasma membrane SNARE protein syntaxin 4 as the "bait," we have isolated a ubiquitously expressed t-SNARE protein termed "SNAP–23." Like its neuron-specific homolog SNAP–25, SNAP–23 is palmitoylated and is able to bind to a number of syntaxin isoforms in vitro and in vivo. Recent data from our laboratory suggests that a significant fraction of the total pool of SNAP–23 present in cells is in fact complexed with various syntaxin family members to form functional SNAP–23/syntaxin t-SNARE complexes. A major focus of our research is to understand the regulation of SNARE complex assembly in living cells.

SNARE proteins do not contain signal sequences and are posttranslationally translocated to membranes. Using a combination of pulse-chase radiolabeling and subcellular fractionation techniques, we have found that the SNAP–23 can associate with syntaxin in the cytosol prior to translocation of the t-SNARE complex to intracellular membranes. Thus syntaxin seems to serve two distinct functions: (1) serving as a "molecular chaperone" for SNAP–23, and (2) forming the t-SNARE complex that, together with SNAP–23, can interact with specific v-SNAREs to form ternary SNARE fusion complexes.

Since t-SNARE complexes seem to be essential for many fusion steps, we are interested in understanding how the formation of these complexes is regulated in living cells. We have recently identified a novel serine/threonine kinase, termed "SNAK," that phosphorylates SNAP–23 and in so doing enhances the kinetics and efficiency of t-SNARE complex formation in vivo. Future goals include the identification of the SNAP–23 phosphorylation site(s) in order to better understand the role of SNAP–23 phosphorylation in t-SNARE function.

Regulation of T Lymphocyte Activation by Antigen-Presenting Cells
Antigen-specific T cells can be activated by engagement of T cell receptors with remarkably small numbers of MHC class II peptide complexes (about 300/cell). In our attempt to understand the mechanisms leading to T cell activation by antigen-presenting cells, we set out to examine whether class II molecules on the surface of APCs were associated with raft microdomains, reasoning that by analogy with T cell signaling molecules, association with lipid rafts could serve to concentrate class II molecules in subdomains of the cell surface. Using subcellular fractionation studies, we showed that approximately half of the surface pool of class II molecules partitioned in detergent-insoluble raft microdomains. Aggregation of surface I–E (class II) molecules dramatically alters the distribution of plasma membrane lipid rafts, adding additional support to this finding. We went on to show that agents that disrupted class II association with lipid rafts profoundly inhibited the ability of APCs to stimulate antigen-specific T cells. Based on these findings, we proposed a model in which concentration of class II molecules in lipid rafts increased the local density of class II- eptide complexes necessary for

T cell activation. At low antigen dose, the partitioning of class II peptide complexes into lipid rafts is necessary to achieve a class II density sufficient to activate T cells, while at high antigen dose the class II peptide complex density is sufficiently high to activate T cells even without concentration in microdomains. While this model can explain how very small amounts of class II peptide complexes are capable of activating T cells, it is a static model (since the experiments were performed using fixed APCs). Therefore, our future studies are aimed at extending this model toward understanding the molecular events in live cells in dynamic APC–T cell interactions.

Recent Publications:

Anderson HA, et al. *J Immunol* 1999;163:5435–43.

Vogel K, et al. *J Biol Chem* 2000;275:2959–65.

Anderson HA, et al. *Nat Immun* 2000;1:156–62.

Vaidyanathan VV, et al. *J Biol Chem* 2001; in press.

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Biography: *Dr. Segal received his Ph.D. from Johns Hopkins University in 1966 and was a postdoctoral fellow at the Weizmann Institute of Science in Israel from 1966 to 1968. Upon his return, he studied antibody structure as a staff fellow in the Laboratory of Molecular Biology of the National Institute of Arthritis and Metabolic Diseases and then joined the NCI's Immunology Branch in 1974 to pursue an interest in immune effector functions. Dr. Segal pioneered in and holds a major patent in the field of redirected cellular cytotoxicity. He has been an organizer of several International Conferences on Bispecific Antibodies and a frequent reviewer for leading immunological and biochemical journals. He received the 1997 Centeon Award for Innovative Breakthroughs in Immunology.*

Experimental Immunology Branch
Targeted Cellular Cytotoxicity

Keywords:

adhesion molecules
cancer immunotherapy
cell-mediated immunity
immunosuppression
innate immunity
TLR

Research: Over the past several years, the objective of our laboratory has been to identify cell surface receptors that trigger immune effector responses, to determine how these receptors deliver signals and to find ways of manipulating these molecules for scientific and therapeutic applications. In particular, we pioneered the use of bispecific antibodies (bsAbs) to redirect a variety of effector cells against tumors, both in vitro and in experimental animals. Originally we focused mainly on T cells and Fc receptors, but more recently we have moved into the area of innate immunity. Innate effector cells have the capacity to recognize and react immediately to pathogens in the absence of T cells or antibody. Using redirected lysis, we and others showed that adhesion molecules such as CD44, CD69, and CD38 have the ability to trigger lysis on NK cells,

neutrophils, and monocytes, the primary mediators of innate immunity. These adhesion molecules gain triggering function after activation by cytokines, and most likely represent events secondary to the direct recognition of pathogenic substances. Recently, mammalian homologs to insect and plant pathogen recognition receptors, known in mammals as toll-like receptors (TLRs), have been identified. Two of these, TLR2 and TLR4, were cloned and shown to trigger inflammatory responses to LPS in humans and mice. Six TLRs have been found so far and we are following their expression in normal human cells; to date we have found message for several of the TLRs in monocytes, dendritic cells, NK cells, T cells, and PMN. Monocytes express the highest levels of TLR message, and these levels decrease during differentiation of monocytes to dendritic cells. A mAb to TLR1 stains all monocytes strongly, but gives lower level staining on dendritic cells. Cotransfection of TLR1 with TLR2 in 293 cells potentiates the response of TLR2 to some forms of LPS, but inhibits the response in other systems. Our first priority is to determine the expression patterns of TLRs and then to study TLR signaling in immunocytes and in cells from peripheral tissues. Toward this end we have cloned TLR1 and 5, and are in the process of cloning (or obtaining from others) the other TLRs. Our intention is to raise antibodies to each of the TLRs and to use them to study protein expression and to perform cross-linking studies. Current data indicate that at least some TLRs induce inflammatory responses; thus an understanding of TLR triggering could lead to the development of novel pro- or anti-inflammatory agents.

In studying the activation conditions required to generate redirected antitumor activity in mice, we found that subcutaneous mammary tumors could profoundly suppress cytotoxic T cell function and antibody production, thus reducing the effectiveness of any immunotherapeutic approach. We also showed that tumor growth resulted in a selective loss in STAT5 protein expression in B and T cell compartments, which could lead to unresponsiveness to several cytokines including IL–2. To further our understanding of the immune suppression, we have, in collaboration with Dr. Vincenzo Bronte, used an in vitro system to study immunosuppression. Bronte's group originally found that immunosuppression was induced by a suppressor type macrophage/dendritic cell that invades the lymphoid tissue at later stages of tumor growth, and he has immortalized these cells. We have found that these cells strongly block T cell proliferation in response to stimulation by peptide and alloantigen, and by Con A. Moreover, inhibition of proliferation is due to the induction of a nonresponsiveness to IL–2. Earlier events, such as upregulation of CD69 and CD25 as well as induction of IL–2 secretion are not impaired. The nonresponsive state is dependent on nitric oxide production. We have shown that IFN-γ, produced by splenocytes, induces the production of nitric oxide in the suppressor cells, and that antibody to IFN-γ and an inhibitor of iNOS (inducible nitric oxide synthase) both block the induction of the nonresponsive state. Nonresponsiveness is not accompanied by a loss in STAT5 (A and B) expression—that occurs later—but there is a marked reduction in tyrosine phosphorylation of STAT5. Early results have not yet detected a change in JAK3 expression or phosphorylation. These results suggest that the induction of iNOS in macrophage-related cells plays a

pivotal role in determining whether the cells will stimulate or suppress immune responses, and we intend to study iNOS and its product in macrophages that have been subjected to various differentiation signals.

Our collaborators include Vincenzo Bronte, University of Padova, Italy, and Steven Dower, University of Sheffield, England.

Recent Publications:

Segal DM, et al. *Curr Opin Immunol* 1999;11:558–62.

Sconocchia G, et al. *Blood* 1999;94:3864–71.

Griffin MD, et al. *J Immunol* 2000;164:4433–42.

Visintin A, et al. *J Immunol* 2001;166:249–55.

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Biography: *Mrs. Sharrow joined the Experimental Immunology Branch as a chemist after receiving a B.Sc. in biochemistry from Michigan State University. In 1973, she established the first immunology-oriented flow cytometry laboratory on the East Coast. A member of the American Association of Immunologists, Mrs. Sharrow's primary research interests have been in the areas of thymocyte development and major histocompatibility antigen expression.*

Experimental Immunology Branch
Flow Cytometry in Immunobiology

Keywords:
antibodies (monoclonal)
flow cytometry
fluorescence
immunofluorescence
immunology

Research: Flow cytometry (FCM) is a unique experimental technology which provides rapid, quantitative, multiparametric, single cell analysis and separation. The mission of the Flow Cytometry Laboratory of the Experimental Immunology Branch (EIB) encompasses two related programs. First, basic research support is provided to members of the EIB and, on a limited basis, to investigators elsewhere at the NIH. The laboratory staff also participates in developments of flow cytometry applications and resources for use in immunological research. Resource development in the lab includes advances in instrumentation, reagents, methodology, and computer hardware/software. Since 1973, the laboratory has trained over 250 investigators in the principles and practice of flow cytometry. More than 50 of these investigators have established flow cytometry laboratories worldwide. Instrumentation includes two customized three-laser (two tunable argon or krypton and rhodamine 6G argon-pumped dye laser) flow cytometers with electronic cell separation capabilities that are operated by staff of the Flow Cytometry Laboratory in support of multiple research projects. These investigations involve quantitative, single cell analyses of parameters associated with cells freshly

prepared from different species/tissues as well as a wide spectrum of in vitro cultured cells. Cell-associated molecules are measured with a variety of probes, most often fluorochrome-labeled monoclonal antibodies. The laboratory specializes in multicolor immunofluorescence analysis (up to six colors), rare event analysis, and cell separation.

Currently supported projects include the following areas of study: (1) in vivo and in vitro analyses of intracellular signaling via cell surface and intracellular molecules; (2) analyses of cellular defects in animals with genetic or induced immune dysfunction; (3) investigations of T and B cell ontogeny and differentiation; (4) studies of the mechanisms of T cell repertoire generation; (5) analyses of expression of cell surface molecules such as adhesion molecules, receptors, and transplantation antigens; and (6) separation and analyses of genetically engineered cells for studies of intracellular processes.

The laboratory also maintains three single-laser, user-operated flow cytometers for use by members of the EIB. In addition, staff provide training and consultation in flow cytometry techniques, protocol design, reagent selection, and data analysis and presentation. Customized flow cytometry data analysis and data archiving software is developed and maintained, including automated cluster analysis software for statistical classification of quantitative multiparametric data and n-plot graphics display of multiparameter data on either subpopulations or individual cells. Network access is provided such that investigators may access and analyze data remotely from their individual laboratories. Current instrumentation development includes additional high-speed cell sorting capabilities and crossbeam electronic fluorescence compensations.

Collaborators include members of this branch and other principal investigators throughout the Center for Cancer Research.

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Biography: *Dr. Shaw received his B.A. from Harvard College and his M.D. from Harvard Medical School. After his pediatric residency at the University of Minnesota, he came to the NIH where he has conducted basic research in human immunology. Among other scientific and editorial advisory boards, he serves on the Scientific Council of the International Workshops on Human Leukocyte Differentiation Antigens. He has a longstanding interest in the use of computers to facilitate biological research and information sharing. Among his awards is the Public Health Service Exceptional Capabilities Promotion.*

Experimental Immunology Branch
Mechanisms of Human Cellular Immune Responses

Research: Historically, our laboratory has made fundamental contributions to the understanding of human T lymphocyte adhesion, migration, activation, and differentiation. Our current emphasis is on molecular understanding of T cell responses to acute stimulation by chemokines and by T cell receptor-mediated signals; areas of particular emphasis are cytoskeletal reorganization and the phosphorylation cascades involved in transducing these signals.

Lymphocyte cytoskeleton is the framework that orchestrates and controls many aspects of lymphocyte signaling, as well as adhesion and migration. We are investigating the organization of lymphocyte cytoskeleton and its reorganization during acute lymphocyte responses. Our recent contributions in this area include the following: We have discovered a cage-like organization of vimentin intermediate filaments in resting T cells and demonstrate that this system is a major contributor to lymphocyte resistance to deformation, previously ascribed to other filament systems. We have also discovered that plectin, best known in epithelial cells, is expressed in lymphocytes and that this huge protein is a key contributor to their intermediate filament organization. Vimentin, plectin, and fodrin are physically interconnected in lymphocytes; this "VPF" assembly spans all the way from the nucleus to the plasma membrane and condenses into the uropod within 1 min of chemokine stimulation. We have been elucidating central roles played by serine/threonine phosphorylation cascades in cytoskeletal reorganization. Understanding individual phosphorylation/dephosphorylation events is a powerful way to dissect this complex process, as illustrated by our studies of moesin phospho-Thr-558 (pT558), one of five phosphorylation sites we have identified in the chemokine response. We have demonstrated that moesin is acutely dephosphorylated early in chemokine stimulation, resulting in resorption of microvilli and other key structural changes in cytoskeletal organization.

Serine/threonine phosphorylation cascades are critical both to cytoskeletal reorganization and to antigen-specific stimulation of T cells. Mechanism-based understanding of these processes depends on understanding the kinases involved. The protein kinase C family of serine/threonine kinases has been repeatedly implicated in lymphocyte adhesion and cytoskeletal reorganization, but there is little precise understanding of mechanisms. We have undertaken a systematic structure/function analysis of PKCθ. Our approach emphasizes careful analysis of evolutionary sequence conservation and mutagenesis-based hypothesis testing. We have identified three sites of regulatory phosphorylation on PKCθ and others are currently under investigation. Informative panels of mutant constructs are being constructed and their functional capacities are being analyzed for diverse functions including in vitro catalytic activity and its regulation by phospholipids, intracellular phosphorylation and localization, regulation of signal transduction via NFκB activation, and phosphorylation of cytoskeletal elements. These results are elucidating structure/function relationships for PKC and challenging the conventional wisdom on this important kinase. We are extending these approaches to other structurally related kinases in order to understand more broadly the structural basis for regulation of serine/threonine phosphorylation during lymphocyte signal transduction.

To efficiently conduct these studies, we have developed resources to serve both ourselves and others. We are developing a novel computer-based approach in which information about biological systems is stored in an unusually simple and coherent way. Utilization of this software has been indispensable in synthesizing data on the many elements (genes, transcripts, proteins, domains, phosphorylation sites, drugs, knock-out mice, references, etc.) that are pertinent to our ongoing studies. We are also developing computational tools for protein sequence and structure analysis to facilitate analysis of kinases and their substrate specificity.

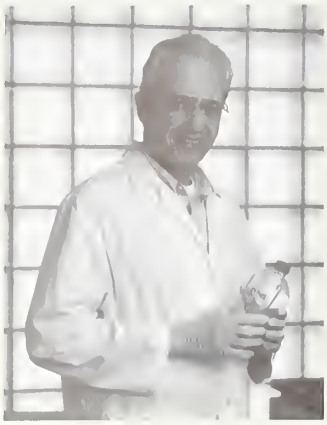
Recent Publications:

Brown MJ, et al. *J Immunol* 2001;166:6640–6.

Brown MJ, et al. *J Immunol* 2001; in press.

Liu Y, et al. *J Leukoc Biol* 2001;69:831–40.

Liu Y, et al. *Trends Immunol* 2001;22:227–9.



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Biography: *Gene Shearer received his Ph.D. from the University of Tennessee in 1967. He obtained postdoctoral training at Roswell Park Memorial Institute, Buffalo, NY, and the Weizmann Institute of Science, Rehovoth, Israel. He joined the NCI in 1972, where he has been a senior investigator in the Experimental Immunology Branch for 29 years. His research interests focus on analyses of protective mechanisms against HIV infection and immune dysregulation seen in AIDS and autoimmune disease. His NIH honors include an NIH Director's Award, a Technology Transfer Award, five SES Service Awards, and a cash award for 25 years of service on various NCI animal committees. He was appointed to the Senior Biomedical Research Service in 1998.*

Experimental Immunology Branch
Immune Dysregulation in HIV/AIDS and Autoimmune Disease, and Natural Immunity Against HIV Infection

Keywords:
acute and chronic immune activation
autoimmunity
cytokines
HIV/AIDS
immune dysregulation
innate immunity

Research: Immune dysregulation is a characteristic of several etiologically-distinct diseases, including cancer, autoimmune conditions, and HIV infection that progresses to AIDS (HIV/AIDS). Some of these diseases such as HIV/AIDS go through an acute phase that shifts to persistent chronic immune activation, in which cellular immunity (CI) and a Th1-like cytokine profile is dominant. The acute phase resulting from HIV infection is associated with protective cellular immunity (CI) and a dominant Th1-like cytokine profile. This contrasts with the chronic phase that is associated with humoral immunity (HI) and Th2-like cytokines, loss of CI, and AIDS progression. Using intracellular techniques for simultaneously detecting HIV infection, cytokine production, and leukocyte phenotyping, this laboratory is investigating the mechanism(s) responsible for this shift. In addition, the laboratory is utilizing a murine model to investigate some basic aspects of these changes. A model of parental T cell-induced graft-versus-host disease (GVHD) in F1 mice was developed that spontaneously shifts from acute GVHD (reflected by CI against MHC alloantigens) to chronic GVHD (characterized by autoantibodies and a lupus-like autoimmune condition). During the acute-to-chronic GVHD shift, a unique population of CD8+CD86+ T cells was detected that also appears in humans during AIDS progression. Current studies are determining whether this cell population is associated with the acute-to-chronic shift in both diseases.

The laboratory is also studying antigen-induced murine experimental autoimmune encephalomyelitis (EAE), which is an animal model of multiple sclerosis (MS). In contrast to the dogma that the relapsing and remitting phases and EAE and MS are regulated by Th1 and Th2 cytokines, respectively, the laboratory recently found that Th1 cytokines are associated with relapse, but that remission is associated with increased expression of

indoleamine 2–3, dioxygenase (IDO), an enzyme that catabolizes tryptophan, resulting in the death of T lymphocytes. Thus, Th1 and IDO appear to be more important in EAE regulation than does Th1/Th2. The laboratory is currently testing whether the same type of regulation also applies to MS, and whether IDO is involved in other examples of immune regulation.

Immune protection against HIV infection is a high priority of AIDS research. A recent development in protection against HIV is the realization that soluble factors produced by leukocytes can block HIV entry or replication. The laboratory has discovered soluble factors that are induced by either infection with influenza virus (FLU) or stimulation with allogeneic leukocytes (ALLO). These factors inhibit HIV replication prior to reverse transcription, and are none of the β-chemokines or other HIV inhibitory factors. Part of the FLU- but not of ALLO-generated HIV inhibitory activity is due to IFN-α. The ALLO- but not the FLU-generated HIV inhibitory activity is under the control of an HLA–A-linked gene. Both factors can be generated by fetal cord leukocytes, suggesting a role for innate immunity. Studies are in progress to: (1) identify the HIV inhibitory factors generated by FLU and ALLO stimulation; (2) determine the mechanism(s) of HIV inhibition; (3) determine whether these factors inhibit other viruses; and (4) elucidate the HLA–A association with the regulation of ALLO-stimulated factor activity.

Collaborating in our work are Mary Carrington and Ligia Pinto, NIH; Mario Clerici, University of Milano, Italy; Matthew Dolan, USAF Medical Center, Lackland AFB, TX; Eric Hoffmann and Hans Spiegel, Children's National Medical Center, Washington, DC; and Alan Landay, Rush-St. Luke's Presbyterian Medical Center, Chicago.

Recent Publications:

Tschetter J, et al. *J Immunol* 2000;165:5987–94.

Pinto LA, et al. *J Virol* 2000;74:4505–11.

Grene E, et al. *J Infect Dis* 2001;183:409–16.

Chougnet C, et al. *J Immunol* 2001;166:3210–7.

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Biography: *Dr. Singer is chief of the Molecular Regulation Section of the Experimental Immunology Branch and director of the Division of Cancer Biology, NCI. After receiving her B.S. from the Massachusetts Institute of Technology and her Ph.D. from Columbia University, Dr. Singer was a postdoctoral fellow in the Laboratory of Biochemistry, NCI, and a senior investigator in the Immunology Branch, NCI. She serves on a number of scientific and advisory boards, is a member of the American Association of Immunologists, and has served as a senior science officer at the Howard Hughes Medical Institute. Dr. Singer has received a number of awards, including the NIH Director's Award. Her research interests are in the areas of regulation of gene expression and molecular immunology.*

Experimental Immunology Branch
Molecular Mechanisms Regulating MHC Gene Expression

Keywords:
autoimmunity
regulation
transcription

Research: Major histocompatibility complex (MHC) class I molecules are ubiquitously expressed cell surface molecules which function as receptors for intracellularly derived peptide antigens, displaying them for inspection by cells of the immune system. Whereas self-peptides complexed with class I elicit no response, peptides derived from viruses or other intracellular pathogens trigger specific immune responses. Given the critical role that MHC class I molecules play in immune surveillance, and the multiplicity of viral mechanisms that have evolved to reduce class I expression and avert immune recognition, it is important to understand how MHC class I genes are regulated.

Regulation of MHC class I gene expression is primarily transcriptional and governed by two distinct mechanisms: homeostatic and dynamic. Among tissues, class I expression varies markedly, ranging from high expression in cells and tissues of the immune system to very low levels in kidney, liver, and endocrine tissues. Homeostatic mechanisms establish tissue-specific setpoints. Within an individual cell type, class I expression fluctuates from the setpoint in response to external signals. For example, cytokines such as interferons increase class I transcription, whereas hormones such as TSH reduce it.

The central hypothesis underlying the studies in the laboratory is that this regulatory network ensures a proper balance between immune surveillance and the maintenance of self-tolerance. Thus, the need to enhance immune surveillance by maximizing class I expression is balanced by the need to reduce the risk of autoreactivity by minimizing class I expression. We hypothesize that these two opposing forces have determined the array of factors that regulate expression of class I genes. Further, we postulate that this optimal balance differs among different tissues and among cells of the same tissue under different physiological conditions. Failure to regulate class I expression appropriately would result in dysregulation of the immune system. For example, viruses capable of repressing class I expression could

permit the accumulation of a resistant reservoir of infected cells. Conversely, overexpression of class I could lead to activation of autoreactive cells and the generation of autoimmune disease.

A major research focus of the laboratory continues to be the precise characterization of this regulatory network. Our studies of class I transgenic mice have shown that normal patterns of expression are established by a DNA segment containing 1 kB of upstream regulatory sequences and extending 1 kB downstream of the coding sequences. Within the upstream 1 kB segment, we have identified a series of regulatory elements that contribute to both homeostatic and dynamic regulation. Recently, most of our efforts have focused on the regulation of expression mediated by elements contained within the 1 kB extended promoter region of the class I gene PD1. Within the 1 kB, the core promoter spans a region between approximately –30 bp and +12 bp; upstream tissue-specific and hormone-specific domains have been identified. The core promoter is a complex region, required to integrate signals emanating from the upstream homeostatic and dynamic regulatory domains. Our studies are designed to provide a basis to achieve a complete mechanistic understanding of how homeostatic and dynamically modulated transcription factors regulate class I gene expression.

Among our findings are that:

- class I core promoter activity depends upon the basal transcription factor TAFII250 and its acetyl transferase (AT);
- the dependence of the class I core promoter on TAFII250 is governed by upstream regulatory elements;
- the class I promoter is activated by CIITA and repressed by ICER, both of which function through an upstream CRE element, which is within the hormone-specific regulatory domain;
- CIITA has AT activity, which is modulated by the downstream GTP-binding domain; this AT activity is necessary for CIITA-mediated activation;
- CIITA can functionally replace TAFII250 in supporting class I expression. In contrast, the b/HLH transcription factor USF, which activates class I expression via an upstream E-box, requires TAFII250;
- HIV Tat represses class I transcription by binding to the AT domain of TAFII250 and inhibiting its AT activity; and
- susceptibility to autoimmune disease is determined by class I expression in the nonlymphoid periphery.

Collaborators on this research include John Brady, Leonard Kohn, and Paul Roche, NIH; and Edna Mozes, Weizmann Institute, Israel.

Recent Publications:

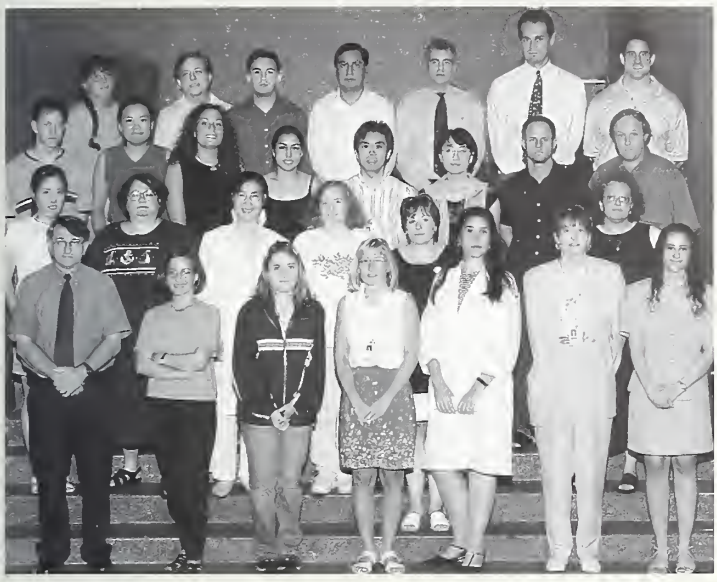
Carroll I, et al. *Mol Immunol* 1998;35:1171–8.

Howcroft TK, et al. *Mol Cell Biol* 1999;19(7):4788–97.

Weissman J, et al. *J Biol Chem* 2000;275:10160–7.

Kirshner S, et al. *Mol Endocrinol* 2000;14:82–98.

Experimental Transplantation and Immunology Branch



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The Experimental Transplantation and Immunology Branch carries out research in the biology of marrow transplantation as a treatment for cancer with emphasis on cellular hematopoiesis, T cell regeneration, T cell response to antigen, antigen presentation, cytokine regulation of the immune response, and tumor biology. Findings from these basic investigations proceed to preclinical studies with the intent of developing information necessary for clinical application. New clinical approaches and strategies for the treatment of cancer are executed with a specific focus, both on manipulation of genes and antigens and on hematopoietic precursor, antigen-presenting, and T cell populations and their associated growth factors and cytokines, for therapeutic gain in bone marrow transplantation. These endeavors are constructed to maximize strengths present in the community at large by vigorous interactions and collaborations.

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Biography: *Dr. Gress received his M.D. from Baylor College of Medicine and his internal medicine residency and oncology fellowship training at the Johns Hopkins Hospital and the NCI. His research interests have been in the area of transplantation immunology with emphasis on the regulation of allogeneic responses and the mechanisms by which peripheral lymphocyte populations are generated and maintained. Dr. Gress is also chief of the Transplantation Immunology Section, Experimental Immunology Branch.*

Experimental Transplantation and Immunology Branch
Transplantation Therapy

Keywords:
bone marrow transplantation
breast cancer
immunoreconstitution
T cells

Research: The primary goals of the Transplantation Therapy Section are to generate new understandings in transplantation biology and to develop new transplantation therapies based on those basic science investigations. The section concentrates on efforts in four complementary areas:

- In the area of cellular hematopoiesis, investigations are carried out to characterize negative regulation; the clinical aim is to generate strategies for establishing effective hematopoiesis of progenitor cells manipulated in vivo or in vitro (including purging). These studies have delineated the regulatory role of IGF–II in CD34+ cell proliferation and have begun to characterize the role of chemokines as negative regulators of hematopoiesis.

- In the area of T cell regeneration, the goal of studies is to characterize mechanisms of T cell reconstitution; clinically, the aim is to maximize T cell regeneration for therapeutic gain. We have found that efficient T cell regeneration is dependent on thymic activity, which varies inversely with age so that compromise exists even in young adults. In older adults, expansion of postthymic T cells accounts for the majority of CD4+ T cell reconstitution, but such T cell populations then tend to diminish with time. This latter observation has prompted our work to identify mechanisms by which expanded T cell populations are lost.

- With respect to cytokine regulation of T cells, the goal is to characterize the role of cytokine-defined T cell subsets (Th1/Th2 and Tc1/Tc2 cells) in transplantation responses in vivo; the clinical aim is to generate controlled, directed T cell antitumor responses in vivo. We have found that Th1/Tc1 cells maximally mediate graft-versus-leukemia (GVL) effects, that Th2 cells regulate GVL and graft-versus-host disease (GVHD) activity, and that Tc1 and Tc2 T cells mediate GVL with reduced ability to mediate GVHD. The possibility that Tc1 and Tc2 populations may be of special therapeutic utility in marrow transplantation is being pursued in current studies.

- With respect to studies in antigen-presenting cell biology, the purpose is to characterize the regulation of dendritic cell generation, identify precursor populations, and generate strategies for effective presentation of antigen in vivo; the clinical aim is to generate strategies for stimulating T cell responses

in immunologically compromised patients by manipulation of antigen-presenting cells generated from peripheral blood cells.

Current and planned clinical studies follow themes developed within the preceding projects—namely, the augmentation of T cell regeneration/responses. Current clinical studies focus on treatment of residual disease in patients with breast cancer and exploit the understanding that postchemotherapy T cell regeneration in adults involves primarily extrathymic expansion, requires mature T cell progenitors, is antigen driven, is prone to skewing, and is cytokine regulated. Such studies also seek to characterize further the regeneration of T cells (especially CD8+ cells) in patients with cancer.

Recent Publications:

Fowler D, et al. *Blood* 1998;91:4045–50.

Hakim F, et al. *Blood* 1997;90:3789–98.

Mackall C, et al. *J Immunol* 1996;156:4609–16.

Mackall C, et al. *N Engl J Med* 1995;332:143–9.

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Biography: *Dr. Bishop received his M.D. from the University of Illinois in 1985. He completed clinical training in internal medicine at Northwestern University in 1988 and a fellowship in hematology and oncology at Loyola University Medical Center in 1991. He was an assistant professor of medicine at the University of Kentucky Medical Center from 1991 to 1992 and an associate professor of medicine at the University of Nebraska Medical Center from 1992 to 1999, where he served as director of the Leukemia and Allogeneic Stem Cell Transplantation Programs. Dr. Bishop joined the Medicine Branch in March 1999 to serve as the clinical head of the Stem Cell Transplantation Program.*

Experimental Transplantation and Immunology Branch
Clinical Transplantation Therapy

Keywords:
breast cancer
lymphoma
transplantation

Research: The primary goal of the Clinical Transplantation Therapy Program is to develop and conduct novel clinical trials in allogeneic and autologous stem cell transplantation. These trials are being performed in a programmatic fashion with other members of the Experimental Transplantation and Immunology Branch, as well as with collaborators within the NCI and the NIH. Particular areas of interest within the program include the therapeutic use of T cells to enhance engraftment in the setting of nonmyeloablative preparative regimens and to abrogate graft-versus-host disease and T cell reconstitution. In addition, the program is looking specifically at the use of tumor vaccines in both allogeneic and autologous transplants, with primary focus on B cell malignancies and metastatic breast cancer.

Recent Publications:

Pavletic ZS, et al. *Ann Oncol* 1998;9:1023–6.

Pavletic ZS, et al. *Bone Marrow Transplant* 1998;21:33–41.

Bishop MR, et al. *J Hematother* 1997;6:441–6.

Bishop MR, et al. *J Clin Oncol* 1997;15:1601–7.

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Biography: *Upon completion of medical school and residency at Wayne State University, Dr. Fowler completed medical oncology training at the NCI in 1991. After a research fellowship in the NCI Experimental Immunology Branch, he returned to the NCI's former Medicine Branch in 1995. In 1996, he received the Translational Research Award from the Leukemia Society of America. Dr. Fowler was recently selected as a tenure track investigator at the NIH, where he continues to develop new approaches to modulation of the immune system after autologous and allogeneic transplantation.*

Experimental Transplantation and Immunology Branch
Autologous and Allogeneic T Cell Strategies for the Treatment of Hematologic Malignancy and Breast Cancer

Keywords:
animal models
bone marrow transplantation
breast cancer
cancer immunotherapy
cellular immunity
clinical trial
cytokines
Fas/FasL
immunoreconstitution
leukemia
Th1/Th2

Research: Donor immune T cells mediate both beneficial and detrimental effects in the allogeneic stem cell transplantation (SCT) setting. Such T cells prevent marrow graft rejection and mediate a graft-versus-leukemia (GVL) effect, but also generate graft-versus-host disease (GVHD). Our primary focus has been to identify T cell functional subsets that prevent graft rejection and mediate a GVL effect without severe GVHD. In murine models, we have demonstrated that donor CD4+, Th2 cells (defined by their secretion of the type II cytokines IL–4, IL–5, and IL–10) prevent the generation of GVHD. We have also shown that donor CD8+, Tc2 cells (defined by their potent cytolytic function and secretion of the type II cytokines) can mediate a GVL effect with minimal ongoing GVHD. Furthermore, we have found that such Tc2 cells can prevent marrow rejection by a mechanism that is independent of GVHD. In addition to marrow rejection and GVHD, another obstacle to allogeneic SCT has been the transplant-related toxicity associated with traditional myeloablative preparative regimens. To address this problem, we developed a murine model of allograft rejection that utilized fludarabine-based chemotherapy preparative regimens. We have now defined regimens that result in host immunoablation without myeloablation, and allow for the engraftment of fully-MHC disparate marrow without contribution from GVHD effects. In sum, these murine experiments provide the rationale for an NCI clinical trial (99–C–0143) in allogeneic stem cell transplantation. In this protocol, patients with lymphoid or hematologic malignancy who have an HLA-matched donor will undergo allogeneic stem cell transplantation after receiving a novel fludarabine-based chemotherapy regimen. Although this

regimen may generate antitumor responses in patients with refractory lymphoid malignancy, the primary objective of the chemotherapy will be to induce host immunoablation for the purpose of preventing allograft rejection. Following stem cell transplantation, we will evaluate whether donor Th2 cells, generated by in vitro culture, can prevent the development of GVHD. If successful, this approach would reduce the main barriers to allogeneic SCT (graft rejection, preparative regimen-related toxicity, and GVHD), and provide the basis for allogeneic transplantation trials in patients lacking an HLA-matched donor.

In addition to these efforts in allogeneic transplantation, we are also developing autologous immune T cell therapies, particularly in the setting of chronic lymphocytic leukemia (CLL). Our major emphasis in CLL has been to evaluate whether modulation of the fas apoptosis pathway might represent a new therapeutic approach to this incurable disease. We have recently shown that freshly isolated CLL cells have greatly reduced fas receptor expression and that exposure of such CLL cells to the type I cytokines, such as IL–12, upregulates CLL cell fas expression and sensitivity to lysis by fas ligand. In marked contrast, we have shown that the type II cytokine IL–4 reduces CLL cell fas expression, and induces a post-fas signaling defect. Because CLL patients overexpress IL–4, it is likely that a shift from type I to type II cytokines contributes to the underutilization of the fas pathway in CLL cells. Translational research efforts are under way to develop approaches to modulate the fas pathway in CLL patients. As a step in this direction, we are developing a clinical trial to evaluate whether type I cytokine administration can modulate the fas pathway in CLL cells in vivo. We have also developed in vitro T cell culture methodologies which generate autologous T cells that are purged of contaminating CLL cells. Importantly, we have demonstrated that such in vitro expanded T cells can mediate fas-based lysis of autologous CLL cells. These results provide the basis for clinical trials evaluating the antitumor effects of autologous fasL-expressing T cells and for studies evaluating the ability of autologous T cells to reconstitute immunity after fludarabine-based chemotherapy regimens.

Among our collaborators are Michael Bishop, Charles Carter, Ronald Gress, Susan Leitman, E.J. Read, and Wyndham Wilson, NIH; and Carl June, University of Pennsylvania Cancer Center.

Recent Publications:

Williams J, et al. *Br J Haematol* 1999;107(1):99.

Fowler DH, et al. *Blood* 1998;91(11):4045.

Halverson D, et al. *Blood* 1997;90(5):2089.

Fowler DH, et al. *J Immunol* 1996;157:4811.

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Biography: *Dr. Hickstein received his B.A. from the University of Nebraska–Lincoln in 1972, his M.D. from the University of Nebraska Medical Center in 1978, and completed training in internal medicine at the University of Michigan in 1982 and in hematology at the University of Washington in 1985.*

Experimental Transplantation and Immunology Branch
Molecular Oncology and Gene Transfer

Research: The research in our laboratory focuses on gene transfer into hematopoietic stem cells with the ultimate goal of applying the techniques developed to the transfer of genes into the hematopoietic stem cells of individuals with leukemia and lymphoma. Our current studies have focused upon gene transfer into the peripheral blood stem cells on individuals with the genetic hematologic disease leukocyte adhesion deficiency or LAD. LAD provides an excellent model for assessing the efficacy of gene transfer into hematopoietic stem cells in that: (1) the disease is due to defects in a single gene, the leukocyte integrin CD18 subunit; (2) since the CD18 molecule is a membrane receptor, flow cytometry can be used to assess the efficacy of gene transfer; (3) very low levels of expression of CD18 will likely reverse the disease phenotype of several bacterial infections; and (4) the presence of a canine model of LAD provides the opportunity to test strategies for gene replacement in an appropriate, large-animal model prior to their application in humans with the disease. We recently conducted a human clinical trial of retroviral-mediated gene transfer in LAD, in which we demonstrated that short-term correction of the defect can be achieved. We have now initiated studies in canine LAD that are expected to form the basis for future clinical trials of gene transfer of CD18 in LAD. These studies are designed to achieve long-term gene correction of hematopoietic stem cells.

Recent Publications:

Bauer TR Jr, et al. *Blood* 1998;91:1520–6.

Kijas JMH, et al. *Genomics* 1999;61:101–7.

Bauer TR, et al. *Curr Opin Mol Ther* 2000;2:383–8.

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Biography: *Dr. Khleif earned his M.D. from the University of Jordan in Amman in 1986. He completed his internal medicine residency at the Medical College of Ohio in 1990 and joined the NCI as a medical oncology fellow. Since 1993, he has been on the staff of the Experimental Transplantation and Immunology Branch. Currently he is a senior investigator heading the Cancer Vaccine Clinic in the branch and also holds an adjunct academic appointment with the Medicine Department of the Uniformed Services University of the Health Sciences, Bethesda, MD. Dr. Khleif serves on many local, national, and international committees and as a cancer vaccine expert for a number of national organizations.*

Experimental Transplantation and Immunology Branch
Cancer Vaccine Development

Keywords:
breast cancer
cancer immunotherapy
cervical cancer
cervical dysplasia
clinical trials
colon cancer
dendritic cells
immunotherapy
kidney cancer
ovarian cancer
p53
pancreatic cancer
Ras
target antigens
tumor immunity
vaccine
VHL

Research: Dr. Khleif's research involves integrated translational basic laboratory research and clinical trials in cancer vaccine. His laboratory's emphasis is on the preclinical identification of potential new vaccine targets, the development of improved and more effective methods for vaccine delivery, the further understanding of the mechanism of immune response in vaccinated patients, and the incorporation of these findings into clinical trial development. His research has a special emphasis on viral and cellular oncogene antigenic potentials.

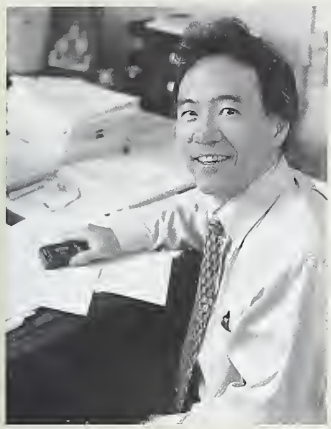
Collaborating on this work are Jay Berzofsky, Michael Hamilton, Lance Liotta, and Seth Steinberg, NIH; Augusto Ochoa, Louisiana State University; Walter Urba, Earl Chils Research Institute; and Theressa Whitside, University of Pittsburgh, PA.

Recent Publications:

Khleif SN, et al. The role of vaccines in cancer prevention. In *Cancer Chemoprevention.* Bergan R, editor. Norwel, MA: Kluwer Academic Publishers, 2001.

Simon RM, et al. *J Clin Oncol* 2001;19:1848–54.

Khleif SN, et al. Preclinical drug development. In *Principles of Clinical Pharmacology.* Atkinson AJ, editor. Academic Press: 2001.



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Biography: *Dr. Kwak received his M.D. (accelerated 6-year B.S.–M.D. honors program) and Ph.D. in tumor cell biology from Northwestern University Medical School. He completed clinical training in internal medicine and medical oncology at Stanford University. Originally recruited to the Biological Response Modifiers Program of the NCI on the Frederick campus in 1992, Dr. Kwak assumed his current position in 1996. He has a broad range of scientific and clinical interests spanning tumor immunology, cancer vaccines, adoptive T cell therapy, and management of lymphomas and myelomas. He serves as cochair of the DCS Cancer Vaccine Working Group (nonmelanoma).*

Experimental Transplantation and Immunology Branch
Immunobiology and Immunotherapy of Hematologic Malignancies

Keywords:
AIDS
CDR
chemokine
cytotoxic T lymphocyte
VH–VL

Research: Our laboratory is focused on testing the hypothesis that idiotypic determinants of B cell tumor-derived Ig (Id) can serve as a tumor-specific antigen for therapeutic vaccine development. Our principal objective is to obtain conclusive proof for the cancer vaccine concept; i.e., simply that it is possible to induce an immune response against a self protein, which is inherently poorly immunogenic, in human patients. In a pilot study of Id vaccination in nine patients with lymphoma, Dr. Kwak originally observed in vitro responses which were specific for autologous idiotype, but they were primarily antibody mediated and relatively weak in magnitude. Accordingly, our goals for vaccine development are: (1) to increase the potency of Id vaccine formulations, and (2) to develop formulations which are more effective in activating the cellular arm of the immune response. Our working hypothesis is that achieving the eventual goal of demonstrating clinical efficacy will depend on the ability of Id vaccines to elicit sustained, potent cell-mediated responses.

A central element of our laboratory research program is its translational orientation and the bidirectional flow of material from the laboratory to the clinic. For example, recombinant Id vaccines, comprised solely of the Ig variable region genes that have been arranged in tandem at the DNA level and expressed in live vectors and as naked DNA fused with chemokine genes, have been produced. We anticipate that comparative preclinical studies of these refined, second generation Id vaccines in small animal models of lymphoma, primarily addressing mechanism, will lead to improved vaccine formulations for clinical testing.

The current phase III clinical trial of Id vaccination in previously untreated patients with follicular lymphomas features a new formulation of the prototype Id-KLH conjugate vaccine. Our preliminary observations suggest that GM–CSF is a potent immunological adjuvant. CD8+ T cell responses, capable of lysing autologous tumor targets, and molecular remissions have

been observed in the vast majority of vaccinated patients. A second active clinical trial is that of HLA-matched sibling marrow transplant donor Id immunization in multiple myeloma. It is designed to test the hypothesis that adoptive transfer of tumor Id-specific immunity from donor to recipient with either unmanipulated bone marrow or donor lymphocyte infusions can be achieved as a strategy for specifically augmenting a graft-versus-tumor effect.

Among our long-term goals is the elucidation of the molecular basis of idiotype recognition by human T cells. Our recent characterization of a human idiotype-specific CD4+ T cell line, demonstrating blocking of proliferation in vitro by anti-MHC class II antibodies, has provided preliminary evidence for T cell recognition of idiotypic determinants which have been processed and presented as peptides in combination with MHC molecules. Our aim is to establish idiotype-specific T cell clones from immunized patients on the two clinical protocols above as unique reagents for mapping studies with defined tumor Ig variable region peptides.

A separate area of research interest is to develop novel vaccine carriers for cancer and AIDS. One example is the core antigen of hepatitis B (HBcAg), whose ability to self assemble into spherical particles and expose foreign epitopes on their surfaces makes it attractive as a potential carrier for tumor antigens. Chimeric HBcAg particles expressing mucin or mutated Ras oncogene fragments, as model tumor antigens, have been expressed in *Escherichia coli* and purified as 27nm-like particles and are being characterized and tested for immunogenicity.

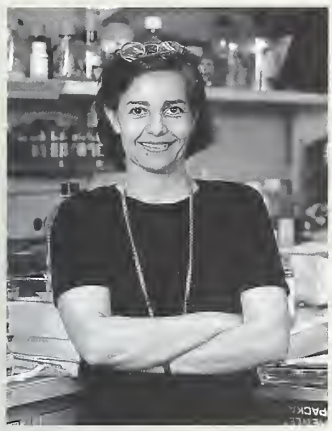
Recent Publications:

Kwak LW, et al. *Lancet* 1995;345:1016–20.

Biragyn A, et al. *Nat Biotechnol* 1999;17:253–8.

Bendandi MB, et al. *Nat Med* 1999;5:1171–7.

Biragyn A, et al. *Nat Med* 2000;6(9):966–8.



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Biography: *Dr. Tosato attended the La Sapienza Medical School in Rome, Italy, where she received her M.D. in 1973 and completed her residency in medicine at the Catholic University in Rome. In 1976, she came to the NCI where she became a Clinical Associate in the Pediatric and Medicine Branches and subsequently a visiting fellow in the Metabolism Branch. In 1983, Dr. Tosato began working at the Food and Drug Administration and in 1999 returned to the NCI as a senior investigator.*

Experimental Transplantation and Immunology Branch
Angiogenesis and Tumorigenesis

Keywords:
angiogenesis
animal models
chemokines
Epstein-Barr virus
Kaposi's sarcoma-associated herpesvirus

Research: Our work is directed toward furthering our understanding of the mechanisms underlying tumorigenesis with the aim of developing novel therapeutic approaches to cancer. A particular interest of our group has been B cell tumorigenesis in the context of T cell immunodeficiency utilizing Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV, also named HHV–8) as tools for investigation. EBV can growth-immortalize B cells in vitro and in vivo, but usually infects asymptomatically normal adult individuals. In the context of severe T cell immunodeficiency states, the virus can cause lymphoproliferative disease and contributes to the pathogenesis of other malignancies. KSHV is consistently detected in primary effusion lymphoma (PEL), a peculiar effusion lymphoma occurring in AIDS. The complex biology of EBV and KSHV in the human host provides an opportunity for investigating the following:

- the mechanisms of B cell infection/immortalization by EBV, particularly the contribution of virus-induced cytokines;
- intrinsic factors regulating the tumorigenicity of EBV or KSHV-infected/immortalized cells, particularly oncogenes and tumor suppressor genes;
- host responses to EBV or KSHV-infected cells with a particular emphasis on cytokines, chemokines, and regulators of tumor angiogenesis;
- characterization of natural inhibitors and stimulators of angiogenesis produced by EBV or KSHV-infected cells;
- preclinical cancer models as tools for the development of novel cancer therapies.

Research in our laboratory is focused on novel experimental approaches to cancer treatment using preclinical mouse models of cancer. In one such model, growth and regression of human Burkitt's lymphoma, breast adenocarcinoma, colon carcinoma, lung adenocarcinoma, T cell ALL, Wilms' tumors, and neuroblastoma transplanted into nude mice is reproducibly regulated by EBV-immortalized cells or B cells transduced with the EBV latency gene LMP–1. Investigation of the mechanisms responsible for tumor regression in this murine model supported the essential role of vascular

damage and thrombosis causing tumor necrosis. We found the chemokines IP–10/Crg-2 and Mig to be expressed at high levels during tumor regression, and to act as potent inhibitors of angiogenesis in vivo. Expression of human IP–10 in Burkitt's lymphoma cells markedly reduced their growth potential in nude mice. In addition, inoculation of IP–10, Mig, or IL–12, which stimulates the secretion of IP–10 and Mig, into established Burkitt's tumors, caused extensive tumor necrosis associated with massive vascular damage and intravascular thrombosis. We are currently investigating the mechanisms by which IP–10 and Mig act as angiogenesis inhibitors.

Certain EBV-immortalized cells secrete in the culture supernatant a potent antiangiogenic factor that directly inhibits endothelial cell growth in vitro. An endothelial cell inhibitor was purified from supernatant of an EBV-immortalized cell line and identified as N-terminal fragments of calreticulin. The purified recombinant N-terminal domain of calreticulin (amino acids 1–180) and a 61-amino-acids fragment of calreticulin (encompassing amino acids 103–164) inhibited the proliferation of endothelial cells, but not cells of other lineages, and suppressed angiogenesis in vivo. When inoculated into athymic mice, both fragments inhibited experimental tumor growth, including Burkitt's lymphoma, breast adenocarcinoma, colon carcinoma, Wilms' tumor, rhabdomyosarcoma, melanoma, and lung carcinoma. Recently, we have demonstrated that the N-domain of calreticulin binds to laminin and through this binding can regulate endothelial cell attachment to the laminin component of extracellular matrix. Additionally, structure/functional studies have defined distinct peptides within the N-domain of calreticulin that mediate the laminin-binding and the endothelial cell-binding activities of this molecule. Current efforts are focused on delineating the mechanisms by which these molecules target the endothelium, the molecular changes that occur in endothelial cells exposed to these inhibitors, and their utility as potential cancer therapeutics utilizing different preclinical models.

In summary, our group has a strong interest in translational cancer research particularly on the interplay of immunity, cytokines/chemokines, and angiogenesis in tumor growth and metastasis formation. The ultimate goal of these studies is to provide valuable information on which to base the development of new cancer therapeutics.

We have collaborated with Joshua Farber, Elaine Jaffe, Hynda Kleinman, and Robert Yarchoan, NIH; and Michael Detmar and Frederick Wang, Harvard Medical School.

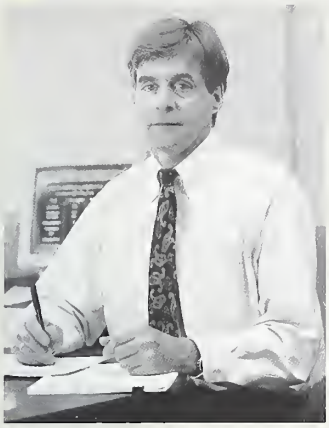
Recent Publications:

Pike S, et al. *J Exp Med* 1998;188:2349–56.

Yao L, et al. *Blood* 1999;93:1612–21.

Aoki Y, et al. *Blood* 1999;93:4034–43.

Pike S, et al. *Blood* 1999;94:2461–8.



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Biography: *Dr. Wilson received his B.A. and M.S. in biology from Stanford University in 1975. In 1981, he received his Ph.D. in neurobiology and M.D. from Stanford, and he completed residency training in internal medicine at Stanford in 1984. From 1984 to 1987, he was a clinical associate in the Medicine Branch, NCI, where he completed a fellowship in medical oncology. Dr. Wilson was special assistant to the director, Division of Cancer Treatment, NCI, from 1988 to 1995. In 1995, he joined the former Medicine Branch as a senior oncologist.*

Experimental Transplantation and Immunology Branch
Clinical Treatment Strategies and Biology of Lymphomas

Keywords:
acute (adult) T cell lymphoma/leukemia
CLL
drug resistance
HIV
lymphoma
T cell biology

Research: Although significant strides have been made in the treatment of Hodgkin's disease (HD) and non-Hodgkin's lymphomas (NHL), major clinical advances have plateaued over despite the development of new chemotherapy regimens and strategies. In an attempt to improve the therapy for lymphomas, we have focused our studies on several promising areas of research: (1) strategies to reverse drug resistance; (2) schedule dependence of cytotoxic agents and dose intensity; (3) development of novel agents, with a focus on T cell toxins; and (4) identification of clinical mechanisms of drug resistance.

Novel therapeutic strategies for lymphoma. In vitro, overexpression of the mdr-1 gene product, P-glycoprotein (Pgp), in tumor cells can confer high-level resistance to natural product derived cytotoxics-anthracyclines, vinca alkaloids, epipodophyllotoxins, and taxanes. It has been reported that Pgp was detectable by immunohistochemistry in 1/49 (2 percent) of untreated but was detectable in 6/8 (75 percent) treated lymphomas, suggesting that Pgp conferred drug resistance. To test this hypothesis, we developed and tested a mdr-1 reversal strategy in relapsed lymphomas using EPOCH (doxorubicin/vincristine/etoposide over 96 hrs days 1 to 4, prednisone daily days 1 to 5, cyclophosphamide bolus day 5) and dexverapamil. Based on our results showing EPOCH to be effective and well tolerated, we began a phase II study of EPOCH in previously untreated patients with aggressive lymphomas. In this study, EPOCH doses are escalated within patients to the maximum tolerated dose (MTD). Endpoints are dose-intensity, efficacy, toxicity, and molecular markers of drug resistance. Early results show a high complete response rate of 89 percent with an EFS of 77 percent at 2 years median followup. Accrual continues to this trial.

We have recently developed and tested a "second generation" EPOCH regimen (EPOCH II) to replace stem cell transplant for lymphomas requiring high-dose intensity including poor prognosis untreated aggressive lymphomas, potentially curable relapsed lymphomas, and low-grade lymphomas. This regimen is based on experimental/clinical observations that

suggest infusion schedules may improve the therapeutic index of natural product-derived cytotoxics, and that high-dose alkylator therapy can overcome drug resistance in lymphoma. An important component is the study of immune modulation with IL–2 and peripheral blood stem cells (PBSC) on the generation of natural killer (NK) and lymphokine activated killer cells (LAK), immune recovery, and eradication of microscopic disease posttherapy. This approach is based on several lines of evidence: (1) T cells are largely eradicated by intensive chemotherapy; (2) clinically relevant immune compromise is associated with T cell depletion; (3) T cell repopulation is accomplished through the thymus if the mature T cell population has been exposed to chemotherapy (but only after prolonged periods of time in older patients); (4) T cell repopulation pathways can be studied utilizing selected cell surface determinants; and (5) T cell repopulation can be influenced by cytokines, including IL–2 and IL–6. Additionally, in animal models, mature T cell precursors present in the PBSC can promote return of immunocompetence and possibly antitumor effects. Preliminary results show EPOCH II to have a response rate of 90 percent with 66 percent complete. Patients were able to tolerate 100 percent of the planned dose intensity. IL–2 treatment acutely increased the CD4 cells, all within the CD4RO subset, and increased NK and LAK cells. However, this effect was not sustained after discontinuation of IL–2, and when long-term immunoreconstitution is compared between the IL–2 and non-IL–2 groups, there was no difference.

In HIV-associated lymphomas, we are testing the efficacy and toxicity of EPOCH chemotherapy followed by interleukin 12. IL–12 is a TH1 cytokine that can stimulate conversions of a TH2 to TH1 phenotype in vitro. Furthermore, it has antitumor activity in murine models. We are also investigating the effect of chemotherapy on HIV plasma viral load and CD4 counts. We have had a longstanding interest in lymphomatoid granulomatosis, an EBV lymphoproliferative disorder that has similarity to posttransplant EBV LPSs. We are currently investigating the efficacy of α-interferon and EPOCH chemotherapy and assessing the immunological deficiency associated with this disorder. Examination of tumor tissues to assess potential mechanisms of drug resistance is an ongoing effort in our group. We have demonstrated the association of clinical drug resistance with p53 mutation and low proliferation rate in relapsed lymphomas. Currently, we are studying other novel cell cycle proteins such as p27 in lymphomas. Recently, we have been interested in testing novel protein kinase inhibitors in lymphomas. One such inhibitor, UCN–01, has shown marked synergy with fludarabine in a variety of human cell lines. To further assess this drug, we will soon begin a phase I study of UCN–01 and fludarabine in patients with relapsed and refractory indolent lymphomas.

Recent Publications:

Bishop P, et al. *J Clin Oncol* 2001; in press.

Little R, et al. *J Clin Oncol* 1998;16:584–8.

Walsh TJ, et al. *Clin Infect Dis* 1998;42:2391–8.

Wilson WH, et al. *J Clin Oncol* 1998;16:2345–51.

Clinical Trials:

Daniel Fowler	**95–C–0086:** The collection of peripheral blood lymphocytes and marrow progenitor cells from normal volunteers and volunteers with chronic myelogenous leukemia
	95–C–0146: A pilot study of autologous T cell transplantation and IL–2 administration for the enhancement of immune reconstitution after dose-intensive chemotherapy for breast cancer
	99–C–0143: A pilot study of donor Th2 cells for the prevention of graft-versus-host disease in the setting of nonmyeloablative, HLA–matched allogeneic peripheral blood stem cell transplantation
Ronald Gress	**96–C–0104:** A pilot study of paclitaxel/cyclophosphamide and high-dose melphalan/etoposide with autologous progenitor cell transplantation for the treatment of metastatic and high-risk breast cancer
Samir Khleif	**94–C–0096:** Vaccine therapy and detection of immunologic responses with tumor-specific mutated Ras peptides in cancer patients
	95–C–0105: Vaccine therapy with tumor-specific mutated p53 or Ras peptides alone or in combination with immunotherapy with peptide-activated lymphocytes (PAL cells) along with subcutaneous IL–2
	95–C–0154: Vaccine therapy and detection of immunologic responses with HPV–16 E6 and E7 peptides in patients with metastatic or locally advanced cervical cancer
	97–C–0141: Vaccine therapy with tumor-specific mutated Ras peptides and IL–2 or GM–CSF for adult patients with solid tumors
	97–C–0144: Cellular immunotherapy with autologous T lymphocytes stimulated with the patient's tumor-specific mutated Ras peptides
	98–C–0139: Vaccine therapy with tumor-specific mutated VHL peptides in adult cancer patients with renal cell carcinoma
	99–C–0137: Vaccine therapy with tumor-specific p53 peptides in adult patients with low burden adenocarcinoma of the ovary
	99–C–0138: Vaccine therapy with tumor-specific p53 peptides in adult patients with adenocarcinoma of the breast
Larry W. Kwak	**96–C–0133:** Active specific immunotherapy for follicular lymphomas with tumor-derived immunoglobulin idiotype antigen vaccines
	97–C–0030: Active immunization of sibling bone marrow transplant donors against purified myeloma protein of the recipient undergoing allogeneic bone marrow transplantation
	97–C–0033: Evaluation of cellular and humoral immunity against the idiotype in multiple myeloma patients undergoing autologous transplantation after idiotype vaccination in an attempt to decrease the risk of relapse
	97–C–0060: A phase II efficacy study of Roleron-A in hairy cell leukemia
	97–C–0077: Vaccination of follicular lymphomas with tumor-derived immunoglobulin idiotype combined with QS–21 adjuvant

Clinical Trials (continued):

Larry W. Kwak	**00–C–0050:** A randomized trial of patient-specific vaccination with conjugated follicular lymphoma-derived idiotype with local GM–CSF in first complete remission
Giovanna Tosato	**96–C–0113F:** A pilot/dose-finding study of the toxicity, anti-Kaposi's sarcoma (KS) activity, and immunologic activity of interleukin 12 administered to patients with AIDS-associated KS
Wyndham Wilson	**91–C–0156:** A phase I study of interleukin 1 with ifosfamide, CBDCA, and etoposide with autologous bone marrow transplant in metastatic carcinoma and lymphoma
	93–C–0133: EPOCH chemotherapy in previously untreated patients with aggressive non-Hodgkin's lymphoma
	94–C–0074: Treatment of lymphomatoid granulomatosis (LYG) with α-interferon for grade I/II and EPOCH/α-interferon for grade III
	95–C–0073: A randomized study of EPOCH II versus EPOCH II and immunotherapy in lymphomas
	97–C–0040: Interleukin 12 and EPOCH chemotherapy in AIDS-related lymphoma
	97–C–0178: Fludarabine treatment of chronic lymphocytic leukemia: cDNA microarray gene expression analysis and preclinical bone marrow transplant/immunotherapy studies
	98–C–0136: Analysis of molecular markers of drug resistance in tumor biopsies from previously untreated aggressive non-Hodgkin's lymphoma
	98–C–0164: A multicenter study (PGA2003) to assess the efficacy of 506U78 in patients with chronic lymphocytic leukemia who are refractory to fludarabine and alkylator therapy
	99–C–0127: A phase I and pharmacokinetic study of UCN–01 and fludarabine in relapsed or refractory low-grade lymphoid malignancies

Genetics Branch

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Among the defining concepts of the Genetics Branch is one that underlies its basic research focus and others that form the foundation of its clinical and translational research activities. The concept that underlies basic research is that cancer is a genetic disease caused by genetic instability. That instability is a function of all the inherited and acquired effects that mediate plasticity and alterability at the level of DNA. The success of molecular genetics over the past two decades has been the identification of genes involved in pathways of growth and development, and the identification of the mechanisms by which the normal regulation and/or products of these genes are altered in cancer. The elucidation of the necessary and sufficient factors that govern genetic instability, the description of the common and disparate themes among different types of instability, and the cataloging of distinct patterns of gene expression in tumors compared to the normal tissues from which they arise are within the purview and distinct perspective of this branch.

There is, in addition, a clinical/translational mantle that this branch is called upon to shoulder. At the clinical level this includes patient cancer risk screening, education, counseling, genetic testing (for those who choose to be tested, with testing provided in a setting that is attentive to all the ethical and legal aspects of the testing decision), and the development of appropriate surveillance and prevention options that take account of category of risk that an individual patient, family, or population represents. The translational research that supports this clinical enterprise consists of four components: (1) molecular diagnostics; (2) genotype/phenotype correlations; (3) the development of biomarkers that can be used for risk assessment and as intermediate endpoints for chemoprevention trials; and (4) targeted therapy and assays for active agents based on the underlying genetics and mechanism(s) of genetic instability that distinguish a tumor from the normal cells from which it arose. The Genetics Branch also is the site for the intramural NCI initiative to develop a repository and database for high-resolution FISH mapped STS-tagged BAC clones spaced at 1–2 Mb intervals across the human and murine genomes. We hope that this repository and database will serve as a general resource to the entire biomedical community for exploration of cancer-specific chromosomal aberrations.

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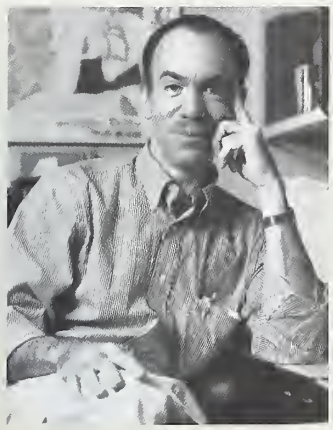

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Biography: *Dr. Kirsch received his S.B. from the Massachusetts Institute of Technology and his M.D. from Harvard University. He completed clinical training in pediatrics at Children's Hospital, Boston, and in pediatric hematology/oncology at the NCI. He was a postdoctoral fellow with Dr. Philip Leder in the Laboratory of Molecular Genetics, National Institute of Child Health and Human Development before joining the NCI in 1983.*

Genetics Branch
The Causes and Consequences of Chromosomal Aberrations

Keywords:

biomarkers of cancer risk
chromosomal aberrations
embryonic development
genetic counseling/testing
genetic instability

Research:

Functional Characterization of Genes Involved in Growth and Development

We have used the cloning of cancer-associated chromosomal aberrations as a means of discovering genes that play roles in growth and development. In the past this strategy led to our discovery of six genes. We characterized the genomic and cDNA structures of each of these six genes and then turned to the functional characterization of a subset of the six. We have successfully generated homozygous "null" animals for the neuronal lineage-specific bHLH transcription factors NHLH1 and NHLH2 and for the immediate early response gene SIL, activated at the G_0–G_1 transition, and implicated in the etiology of T cell ALL when juxtaposed to another bHLH transcription factor discovered in this laboratory (the hematopoietic lineage determiner SCL). The phenotype of the NHLH2 "knock-out" animal is hypogonadal, obese, and sex-drive deficient. Both males and females show a disruption of the normal hypothalamic-pituitary axis that controls sexual development at puberty. The female defect appears capable of being bypassed by the presence of normal male pheromones. The SIL knock-out animals die between embryonic day 8.5 and 10.5. The affected fetuses show loss of normal regulation of left-right symmetry of body axis, turning, and cardiac development. The NHLH1 knock-out mouse is capable of full embryonic maturation and birth. Analysis of phenotypic characteristics for the NHLH1 "null" animal demonstrates an adult-onset locomotor defect. A subpopulation suffers premature death, apparently due to a disruption of autonomic nervous system regulatory control. We have thus generated three model systems for studying the effect of two bHLH and one immediate early gene on embryonic development, cellular proliferation, and lineage determination. These systems are providing the basis for our next phase of investigations into these issues.

A Cancer Genetics Program

Having established a cancer genetics program within this branch, we serve and utilize as a resource the patient population of the NCI as well as the Bethesda Naval Hospital and the NSABBP cooperative group. The program

has two aspects: (1) There is a patient-oriented pathway which begins with patient assessment and eligibility determination and leads through an education and counseling component to the opportunity to be tested for germline gene mutation that would predispose to colorectal or breast cancer. Following the determination of germline status, the patient is apprised of the result and then introduced to and evaluated for cancer surveillance and prevention options. (2) Within the laboratory four research projects have been developed. The first focuses on molecular diagnostics and looks to define an algorithm and a specific defensible marker set for identifying a malignancy as microsatellite unstable and defective in nucleotide mismatch recognition and repair. The second project correlates specific gene mutation in MSH2, MLH1, PMS1, PMS2, MSH6, MSH3, BRCA1, and BRCA2 with the presentation, histopathology, response to therapy, family penetrance, related molecular markers, and ultimate outcome of patients with colorectal, endometrial, ovarian, or breast cancer. The third project is attempting to develop a biomarker that correlates with risk for the development of colorectal cancer. The fourth project takes as its starting point the difference between RER+ malignancies and the RER– nonmalignant tissue from which they arose, and is focused on the development of anticancer therapy that highlights this difference.

Refined Molecular Cytogenetics and Genetic Instability

We have adopted the techniques of spectral karyotyping (SKY), comparative genomic hybridization (CGH), and fluorescent in situ hybridization (FISH) for the determination of structural and numerical chromosomal aberrations in selected cancers and cancer cell lines. We are working collaboratively to correlate these data with gene expression data from the same samples. We are also determining the rates and persistence of genetic instability in normal and malignant cells. This endeavor has also led us toward the elucidation of common themes and mechanisms underlying genetic instability in general.

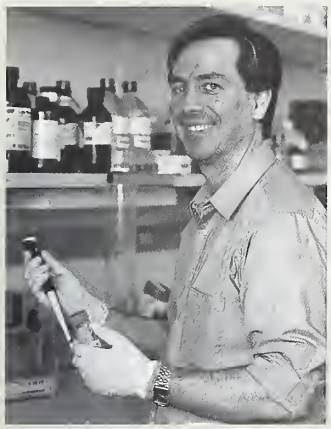
Recent Publications:

Kirsch IR, et al. *Nat Genet* 2000;24:339–40.

Tonon G, et al. *Genes Chromosomes Cancer* 2000;27:418–23.

Kirsch IR, et al. *Semin Hematol* 2000;37:420–8.

Cheung VG, et al. *Nature* 2001;409:953–8.



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Biography: *Dr. Aplan received a B.S. in biophysics (1979) and an M.D. (1983) from Pennsylvania State University in 1983. From 1983 to 1987, he trained in pediatrics and was chief resident at the Children's Hospital of Buffalo. He received fellowship training in pediatric hematology/oncology at the NCI from 1987 to 1992 and served as assistant, then associate, professor of pediatrics and microbiology and immunology at Roswell Park Cancer Institute from 1992 to 1999. He moved to the NCI in 1999 where he is an investigator in the Genetics Branch.*

Genetics Branch
Molecular Mechanisms of Leukemic Transformation

Keywords:
chromosomal translocations
leukemia
oncogenes

Research: In broadest terms, our laboratory studies genomic instability associated with the development of malignant disease. Our general model system focuses on the nonrandom chromosomal translocations associated with leukemogenesis; we have divided our investigations into two broad categories. The first category involves the evaluation of genes disrupted by chromosomal translocations as candidate oncogenes and how aberrant regulation of these genes may lead to malignant transformation. The second category focuses on defining the molecular mechanisms which cause these nonrandom translocations.

- In regard to the evaluation of genes at translocation breakpoints, the gene that has been investigated most thoroughly is *SCL*. We have developed a transgenic mouse model of T cell leukemia by crossing transgenic mice that overexpress *SCL* (also known as *TAL1* or *TCL5*) with transgenic mice which overexpress *LMO1* (also known as *TTG1* or *RBTN1*). All of the double transgenic mice develop aggressive T cell malignancies that are clinically, morphologically, and immunophenotypically similar to human T cell acute lymphoblastic leukemia (ALL). Further investigation of these leukemias suggests that *SCL* and *LMO1* exert their oncogenic effect through a functional inactivation of bHLH E-proteins (*E2A* and *HEB*). Additionally, examination of thymocytes obtained from mice prior to the onset of a frank malignancy shows that there are clear differences between the double transgenic mice and the control littermates in terms of thymocyte number, immunophenotype, proliferative index, and clonality prior to the onset of frank leukemia. Serial analysis of thymocytes from *SCL/LMO1* mice demonstrates a developmental block in T cell development prior to the leukemia. Future plans regarding this model include refining it by using cre-lox technology to control *SCL* expression in postnatal thymocytes, and crossing the *SCL* and *LMO1* transgenes onto a *scid* background to determine if TCR gene rearrangements are required for *SCL/LMO1* leukemic transformation. Experiments designed to investigate the biochemical basis for *SCL* action include a yeast two-hybrid screen that has demonstrated an interaction between SCL and the p44 subunit of the basal transcription factor TFIIH. The interaction has been confirmed using GST fusion proteins as well as coimmunoprecipitation of the

native proteins. This interaction, which is unique to SCL among the bHLH proteins tested, is not surprising given that SCL possesses a transcription activation domain; we are currently trying to determine the functional relevance of this finding.

- A novel t(14;21)(q11;q22) chromosome translocation has recently been cloned from a patient with T cell ALL. The translocation involves a V(D)J recombinase-mediated event between TCRα and a region on 21q22. We have recently found two transcripts near this breakpoint; one of these transcripts encodes a novel bHLH protein that is not usually expressed in hematopoietic cells but is highly expressed in leukemic cells that have undergone a t(14;21)(q11;q22) translocation.

- Two novel translocations have recently been cloned from patients with myelodysplasia following multiagent chemotherapy for a primary malignancy. These therapy-related myelodysplastic syndromes (t-MDS) both generate in frame fusions of the *NUP98* gene with two distinct partner genes (*HOXD13* and *TOP1*). The oncogenic potential of the *NUP98* fusions is being evaluated using stable transfection experiments and transgenic mice.

- The second general area of investigation is focused on the molecular mechanisms that cause chromosomal translocations. Of particular interest, because of the clinical relevance, are those translocations that can be induced by genotoxic chemotherapy.

Both the *AML1* and *MLL* genes are frequently translocated in patients who develop acute myeloid leukemia following treatment with chemotherapeutic regimens that incorporate topoisomerase II poisons. Specific sites within the breakpoint cluster regions of both the *AML1* and *MLL* genes that are uniquely sensitive to double-strand DNA cleavage induced by topoisomerase II poisons have been identified. The possibility that this cleavage event is the initiating event for translocations induced by topoisomerase II poisons is currently being investigated.

- Numerous studies employing either RT–PCR or FISH have clearly demonstrated that translocations leading to a fusion of the *AML1* and *TEL* genes occur very frequently, present in 30 percent of patients with B cell precursor ALL. However, no genomic breakpoints involving this translocation have been reported. In an effort to gain clues as to the mechanism of these translocations, genomic breakpoints were cloned and sequenced. Of note, the *TEL* breakpoints are clustered within a 4.0 kb segment; near a 240 bp purine/pyrimidine repeat region. In collaboration with Dr. Bill Burhans, neutral-neutral 2D gel electrophoresis is being used to determine if this purine/pyrimidine tract is a site for replication fork "pausing," which might make the region more susceptible to DNA double-strand breaks.

Collaborating with us are William Burhans, Kenneth Gross, and Thomas Shows, Roswell Park Cancer Institute; Pieter deJong, Parke-Davis; Carolyn Felix and Warren Pear, University of Pennsylvania; Ilan R. Kirsch and Thomas Ried, NIH; and Martin Stanulla, Hannover Medical School, Germany.

Recent Publications:

Chervinsky DS, et al. *Mol Cell Biol* 1999;19:5025–35.

Ahuja H, et al. *Blood* 1999;94:3258–61.

Zhao XF, et al. *J Biol Chem* 1999;274:1388–93.

Wang JJ, et al. *Proc Natl Acad Sci* 2000;97:3497–502.

Ahuja H, et al. *Genes Chromosomes Cancer* 2000;29:96–105.

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Genetics Branch
Molecular Determinants of Faithful Chromosome Transmission, Chromatin Structure, and Cell Cycle Regulation

Keywords:
cell cycle regulation
chromosome transmission
functional genomics
Saccharomyces cerevisiae
serial analysis of gene expression (SAGE)

Research: A fundamental requirement of the cell division cycle is the maintenance, replication, and segregation of chromosomal DNA. Failure of complex mechanisms involved in maintaining genome integrity has been implicated in cancer, aging, and congenital birth defects. Research in our laboratory focuses on the molecular mechanisms of high fidelity chromosome transmission, the organization of chromatin structure, and the regulatory mechanisms that ensure the proper execution of the cell cycle in *Saccharomyces cerevisiae* and the study of its human homologs. We are also interested in the application of genome technologies such as serial analysis of gene expression (SAGE) and DNA microarrays for analysis of transcription profiles and the identification of small nonannotated open reading frames (NORFs).

Molecular Determinants of Faithful Chromosome Transmission
CEN DNA sequences and the trans-acting kinetochore components (centromere-specific DNA binding proteins) are required for high fidelity chromosome transmission. Additionally, a higher order chromatin structure provides a framework for interactions of histones, CEN DNA, and the kinetochore. We have characterized genes that are important for the structure/function of the kinetochore and studied the corresponding human homolog. We analyzed the phenotype of the *ctf* (chromosome transmission fidelity) mutants in genetic screens for kinetochore integrity. From a total of

29 *ctf* mutants, 5 tested positive in these screens. We have studied two of these mutants, s138 and s141, and will pursue studies on the other three putative kinetochore mutants in the future. The gene complementing the s138 mutation was shown to be the *Saccharomyces cerevisiae SPT4* gene. We showed that the *spt4* mutants exhibit genetic interactions with mutations in cis-acting CEN DNA sequences and trans-acting kinetochore proteins. In collaborative efforts with Dr. Fred Winston's laboratory at Harvard, we have shown that a human homolog of *SPT4*, HsSPT4, is able to functionally complement the spt- as well as the chromosome missegregation phenotypes of *Saccharomyces cerevisiae spt4* mutants. We are studying the role of Spt4p in chromosome transmission and would like to determine if Spt4p is a component of the heterochromatin that may be present at the *Saccharomyces cerevisiae* centromere. We will also use yeast cDNA microarrays to identify downstream targets of Spt4p. Further studies of the second putative kinetochore mutant s141 showed that the mutation is allelic to a nucleoporin mutation *nup170*. The *Saccharomyces cerevisiae NUP170* and *NUP157* genes are highly homologous to each other and have a mammalian counterpart, *NUP155*. We are currently pursuing studies to determine the molecular role of Nup170p in chromosome transmission.

Functional Characterization of the CHD Family of Genes

We have studied a budding yeast homolog ScChd1p of the mammalian CHD (chromo-ATPase/helicase-DNA-binding domain) gene(s) in collaboration with Dr. Francis Collin's laboratory. The CHD gene family is highly conserved with homologs in mouse, *Drosophila*, and *Tetrahymena*. The CHD domains are important for several functions such as DNA repair, gene transcription, and heterochromatin formation. Intriguingly, ScChd1p is the only *Saccharomyces cerevisiae* protein that has all three domains characteristic of CHD proteins. Our results suggest that ScChd1p may be important for transcriptional regulation and we propose that alteration of gene expression by CHD genes might occur by modification of chromatin structure, with altered access of the transcriptional apparatus to its chromosomal DNA template. Yeast cDNA microarrays will be used to determine the role of ScChd1p in transcriptional regulation. The full length human CHD genes or human-yeast chimeras will be used to test the complementation of *chd* phenotypes in *Saccharomyces cerevisiae*.

SAGE Analysis to Identify Cell Cycle-Regulated Open Reading Frames (ORFs) and Nonannotated Open Reading Frames (NORFs)

We have used SAGE to identify, quantitate, and compare global gene expression patterns from hydroxyurea-arrested (S-phase), nocodozole-arrested (G2/M phase), and logarithmically growing cells of *Saccharomyces cerevisiae*. SAGE analysis was done in collaborative efforts with Dr. Hieter, Dr. Vogelstein, and Dr. Kinzler. SAGE has permitted the identification of at least 302 previously unidentified transcripts from NORFs corresponding to proteins with <100 amino acids, some of which are expressed in a cell cycle-regulated manner. The genome sequencing efforts have not annotated any ORF with <100 amino acids in length. Several of the NORF genes are evolutionarily conserved and have homologs in either human, mouse, or *C. elegans*. Further studies have shown that transcription of one of these, *NORF5/HUG1* (hydroxyurea, ultraviolet, gamma induced), is induced by DNA damage and this induction requires *MEC1*, a homolog of the ataxia

telangiectasia-mutated (ATM) gene and genes in the *MEC1* pathway. Overexpression of *HUG1* is lethal in combination with a *mec1* mutation in the presence of DNA damage or replication arrest, whereas a deletion of *HUG1* rescues the lethality due to a *mec1* null allele. Future studies will shed light on the role of *HUG1* in the DNA damage and replication arrest-induced pathways regulated by cell cycle checkpoint genes. Identification of a human homolog of *HUG1* may further our understanding of similar pathways in humans.

We have collaborated with Philip Hieter, Centre for Molecular Medicine and Therapeutics; Michael Lichten, NIH; Mark Winey, University of Colorado; and Richard Wozniak, University of Alberta.

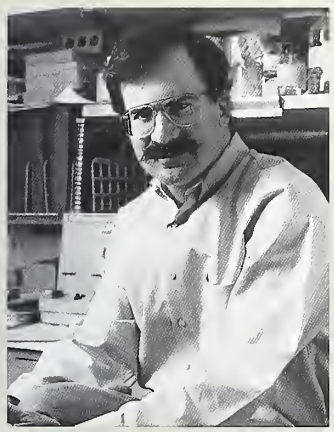
Recent Publications:

Basrai MA, et al. *Mol Cell Biol* 1996;16:2838–47.

Velculescu VE, et al. *Cell* 1997;88:243–51.

Basrai MA, et al. *Mol Cell Biol* 1999;19:7041–9.

Kerscher O, et al. *Genetics* 2001;157:1543–53.



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Genetics Branch
Mechanism of Oncogene Action in Tumorigenesis

Keywords:
animal models
cell cycle
clinical trial
lung cancer
retinoblastoma gene

Research: Our research effort is focused on investigating the genetic basis for human lung tumors and to develop new strategies for clinical trials. We have studied the role of the RB tumor suppressor pathway in human cancer and have demonstrated that the RB/p16 tumor suppressor pathway is inactivated in 100 percent of small cell lung cancer (SCLC) and non-SCLC. In preclinical studies, we have shown that p16 protein expression can be induced in approximately 40 percent of non-SCLC samples with the pharmacological agent decitabine (5aza2'deoxycytine). We are also analyzing lung tumors in a colony of RB(+/–) mice to determine the effect of RB versus p16 inactivation as the initial genetic hit. These mice develop a high frequency of endocrine thyroid, pituitary, and pancreatic tumors, and we will study the genetic basis

of how RB inactivation facilitates the initiation or progression of these tumors. We are also examining the patterns of gene inactivation that discriminate between the neuroendocrine tumor, SCLC, and the more common non-SCLC carcinoma. We have also studied the functional properties of 5 different RB mutant alleles that were identified in the germline of at least 14 distinct families with the phenotype of incomplete penetrance of familial retinoblastoma. We have shown that three missense mutants that localize to the "RB pocket" are temperature-sensitive for RB pocket protein binding, suggesting a model for the phenotype of incomplete penetrance in these families. We have initiated a program to investigate the genetic basis of rare lung tumors focusing on lung mucoepidermoid carcinoma.

Among our collaborators are Gregory Otterson, Ohio State University Cancer Center; and Jerold Ward, NIH.

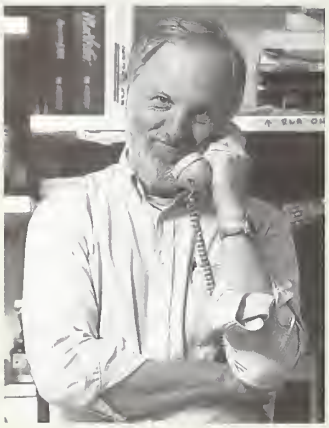
Recent Publications:

Otterson G, et al. *Am J Hum Genet* 1999;65:1940–6.

Kaye F, et al. *FEBS Lett* 2000;467:348–52.

Niklinski J, et al. *Mol Cell Biol* 2000;20:5276–84.

Modi S, et al. *Oncogene* 2000;19:4632–9.



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Genetics Branch
Molecular Pathogenesis of Multiple Myeloma and Other Plasma Cell Tumors

Keywords:

B lymphocyte tumors
cancer genetics
molecular cytogenetics
translocation

Research: Our work is focused on understanding the molecular pathogenesis of multiple myeloma (MM) and other human plasma cell (PC) tumors, such as Waldenstrom's macroglobulinemia (WM). Our long-term goal is to use this information to generate effective therapies for these presently incurable malignancies. MM tumors arise from postgerminal center B cells that have undergone somatic hypermutation, receptor editing, antigen selection, and IgH switch recombination to generate a long-lived PC. There is an ordered pathway of clinical progression from premalignant monoclonal gammopathy

of uncertain significance (MGUS) to intramedullary MM to extramedullary MM (PC leukemia) to MM cell line. We are using a panel of 30 MM cell lines as well as primary tumor samples for the following approaches:

- **IgH translocations:** Dysregulation of oncogenes by translocation to one of the Ig loci is a seminal event in many B cell malignancies. These translocations, which appear to be mediated primarily by errors in normal VDJ recombination, somatic hypermutation, or IgH switch recombination, are thought to occur as an early event in pathogenesis. As an example, murine plasmacytoma tumors are characterized by the invariable translocation of the *c-myc* oncogene into a switch region of the IgH locus, or into one of the IgL loci. For human MM, conventional karyotypic analysis detects IgH translocations (and only rarely the partner chromosome) in 20 to 30 percent of tumors with abnormal karyotypes, but Ig translocations involving the c-myc locus (8q24.1) are uncommon. By analyzing the MM cell lines with a combination of conventional karyotypic analyses, FISH analyses with specific Ig and painting probes, and molecular identification of translocation breakpoints, we have determined the following: (1) IgH translocations are present in at least 36/39 (92 percent) cell lines; (2) it is not unusual to have 2 or even 3 IgH translocations in a cell line; (3) most IgH translocation breakpoints involve IgH switch regions, some involve JH regions, and some involve other IgH sequences; and (4) there is a diverse but nonrandom array of at least 20 translocation partners (and oncogenes), including 4p16.3 (*FGFR3* and *MM.SET*), 11q13 (*cyclin D1*), 6p21 (*cyclin D3*), and 16q23 (*c-maf*) that we have identified in a majority of tumors, as well as less frequent but recurrent partners such as 1q21(*MUM2* and *3*), 6p25(*IRF–4*), 8q24(*c-myc*), 9p13 (*PAX–5*), 18q21(?*bcl-2*), and 20q11(*mafB*) that we, and others, have identified. Our preliminary metaphase FISH analyses show the presence of IgH translocations in a maximum of nearly 70 percent of advanced primary MM tumors, and others have found IgH translocations in about 50 percent of premalignant MGUS tumors. We are testing the hypothesis that many IgH translocations occur early in pathogenesis but that others occur as late progression events. We are also trying to determine if different translocation partners predict the course, prognosis, and response to therapy of MM.

- **IgL translocations:** We are beginning to study these translocations which appear to occur in about 20 percent of MM cell lines and primary tumors.

- ***C-myc* dysregulation:** We have determined that there is selective expression of one *c-myc* allele in all 13 informative MM lines. In addition, 28/32 (88 percent) MM cell lines have karyotypic abnormalities of the *c-myc* locus, which is often juxtaposed to a strong Ig enhancer, providing a structural explanation for tumor specific dysregulation. The incidence of karyotypic abnormalities of c-myc is about 47 percent in advanced primary MM tumors, and sometimes heterogeneous within a tumor, indicating that *c-myc* is dysregulated as a late event as the tumor enters a more aggressive phase. We are using both interphase and metaphase FISH plus fiber FISH to determine the molecular anatomy of the *c-myc* karyotypic abnormalities, with the goal of cloning translocation/insertion breakpoints.

- **Other projects:** (1) Development of a transgenic model for MM using oncogenes dysregulated by translocations in MM; (2) determination of gene expression in MM cell lines and tumors using the "lymphochip," in collaboration with Dr. L. Staudt; (3) determination of mutations in *N*- and

K-ras, p53, FGFR3, and other genes involved in tumor progression; and (4) molecular genetic and cytogenetic analysis of Waldenstrom's macroglobulinemia.

Collaborating with us are Bart Barlogie, Jeffrey Sawyer, and John Shaughnessy, University of Arkansas; P. Leif Bergsagel, Weill Medical College of Cornell University; Rafael Fonseca, Mayo Clinic; Ilan Kirsch, Thomas Ried, and Louis Staudt, NIH.

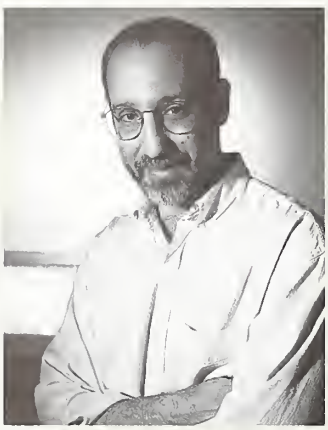
Recent Publications:

Gabrea A, et al. *Mol Cell* 1999;3:119–23.

Shou Y, et al. *Proc Natl Acad Sci* 2000;97:228–33.

Chesi M, et al. *Blood* 2001;97:729–36.

Shaughnessy J, et al. *Blood* 2001;98:217–23.



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Biography: *Stan Lipkowitz received an A.B. in 1977 and an M.D. and Ph.D. in 1984 from Cornell University. He trained in internal medicine at the New York Hospital, came to the NCI as a medical oncology fellow in 1987, and joined the laboratory of Dr. Ilan Kirsch. In 1992, he established his own laboratory where he studies molecular and cell biology of breast cancer cells. Dr. Lipkowitz is also an associate professor of medicine and molecular and cell biology at the Uniformed Services University of the Health Sciences.*

Genetics Branch
Molecular Control of Growth, Differentiation, and Death in Breast Cancer Cells

Keywords:
apoptosis
breast cancer
cbl proteins
cell signaling
death receptors

Research: Our laboratory investigations are focused on identifying genes that regulate growth, differentiation, and programmed cell death of normal and malignant mammary epithelial cells to gain understanding of the molecular pathways that control the fate of mammary cells. Ultimately this knowledge will be used to develop rational strategies for the treatment of breast cancer. In one project we are studying the role of cbl proteins in regulating epidermal growth factor receptor (EGFR) function. Growth of breast cancer cells is stimulated in response to a variety of growth factors via receptor tyrosine kinases such as the EGF family of receptors. We have cloned and begun to characterize cbl-b, a new gene which encodes a protein with striking homology to the c-cbl proto-oncogene. Cbl proteins are novel signal transduction molecules downstream of receptor tyrosine kinases. Genetic studies in the nematode *C. elegans* suggest that the cbl homolog is a suppressor of EGF receptor signaling. We have demonstrated that cbl-b associates with the EGFR and inhibits signaling by the receptor in

mammalian cells. We are now investigating the biochemical mechanisms of this inhibition. More recently, we identified and cloned a third mammalian cbl gene (cbl-3) that encodes a protein that is more closely related to the primitive cbl proteins found in *C. elegans* and *Drosophila*. We are now studying its effects on EGFR signaling.

In a second project, we are investigating the expression and function of death receptors of the TNFR family (e.g., TNFR, Fas, Dr3, Dr4, and Dr5) in normal and malignant breast epithelial cells. Ongoing studies are focused on exploring the normal physiologic role of the death receptors in mammary epithelial cells, the relationship of malignant transformation and the death receptor pathways, and the modulation of these pathways in breast cancer cells in vitro and in vivo.

We have collaborated with Phil Dennis, Frederic Kaye, and Allan M. Weissman, NIH; Josef Penninger, University of Toronto; Yosef Yarden, Weizmann Institute.

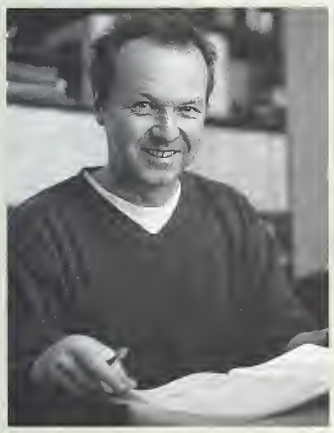
Recent Publications:

Keane MM, et al. *Cancer Res* 1999;59:734–41.

Cuello M, et al. *Gynecol Oncol* 2001;81:380–90.

Keane MM, et al. *Breast Cancer Res Treat* 2000;64:211–9.

Ettenberg SA, et al. *Mol Cell Biol Res Commun* 1999;2:111–8.



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Biography: *Dr. Ried received his M.D. from the Max Planck Institute for Biomedical Research in Heidelberg. After postdoctoral training at the University of Leiden and at Yale University, Dr. Ried joined the National Human Genome Research Institute at the NIH.*

Genetics Branch
Molecular Cancer Cytogenetics

Keywords:

carcinogenesis
chromosome aberrations
early cancer detection

Research:

Identification of Recurring Chromosomal Aberrations in Human Cancers and Development of Diagnostic Tools for Chromosomal Markers of Tumor Progression

Our laboratory has developed and applied molecular cytogenetic techniques—namely, comparative genomic hybridization (CGH) and spectral karyotyping (SKY)—to identify recurring chromosomal aberrations in solid tumors and hematological malignancies. CGH is a screening test for chromosomal imbalances in tumor genomes and is used to analyze sequential

changes in solid tumor progression after tissue microdissection of histologically defined lesions during the genesis of solid tumors. SKY allows color karyotyping of human chromosomes, therefore greatly facilitating the analysis of complex chromosomal aberrations in cancer cells. SKY was applied to delineate the structural organization of chromosomal aberrations and to identify novel recurring translocations in carcinomas, sarcomas, and hematological malignancies. We are particularly interested whether: (1) we can identify a recurring and tumor-specific pattern of chromosomal gains and losses; (2) SKY analysis of hematological malignancies can contribute to a genetic classification of leukemias; (3) we can correlate patterns of chromosomal aberrations with tumor stage, tumor phenotype, additional pertinent genetic parameters, and the clinical course; (4) how genomic imbalances influence gene expression patterns; and (5) this pattern of recurring and stage-specific chromosomal aberrations bears significance in terms of diagnosis, differential diagnosis, and prognostication of solid tumors when translated to cytological specimens using interphase cytogenetics.

Development of Molecular Cytogenetic Techniques for the Analysis of Chromosomal Aberrations in Murine Models of Human Cancers

The abundance of cytogenetic information in hematological malignancies and solid tumors has produced both diagnostically and prognostically relevant information and has contributed to the positional cloning of cancer-causing genes. However, efforts to understand the sequence of genetic aberrations during carcinogenesis and the biology of tumor progression and attempts to establish test systems for novel therapeutics depend increasingly on animal models of human cancers. Murine models of human carcinogenesis are widely used to delineate genetic mechanisms that determine tumor initiation and progression, and improved methods for genetic manipulation open new avenues to study biological pathways of tumorigenesis.

Karyotyping of mouse chromosomes is extremely demanding because all mouse chromosomes are acrocentric. Consequently, data on recurring chromosome aberrations in mouse models are rare. We have therefore developed molecular cytogenetic techniques that facilitate the characterization of chromosomal aberrations. The potential of comparative genomic hybridization and spectral karyotyping as genome scanning methods for detection of chromosomal aberrations in mouse tumors was explored by analyzing different mouse models of human carcinogenesis: (1) chemically induced plasmacytomas in BALB/c mice; (2) mammary gland tumors that developed in transgenic animals that overexpress the c-myc gene under the control of the MMTV-promoter or that are deficient for the breast cancer suppressor gene BRCA1; and (3) thymomas that occurred in mice deficient for the ataxia telangiectasia (Atm) gene. Future research will focus on the questions of whether (1) the distribution of chromosomal gains and losses in mouse carcinomas is similar to that observed in human cancers; (2) the pattern and mechanisms of chromosomal aberrations are similar to those in human cancers—i.e., is the translocation-induced activation of oncogenes important in mouse models for hematological malignancies, and do chromosomal imbalances define mouse models for human carcinomas?; (3) different induction of mouse tumors, such as the overexpression of different oncogenes, will result in specific karyotypic changes and can therefore assist in the elucidation of genetic pathways; (4) the establishment

of syntenic maps of chromosomal aberrations can be used to identify chromosomes and chromosomal regions that are important for tumorigenesis across species boundaries with increased resolution; and (5) molecular cytogenetics can contribute to the validation of mouse models of human carcinogenesis.

Identification of Mechanisms of Chromosomal Aberrations

The analysis of a large number of human and mouse solid tumors has established the relevance of genomic imbalances as the premier genetic aberration in cancers of epithelial origin. Ninety-five percent of all aberrations result in copy number changes reflecting gains and losses of specific chromosomes, chromosome arms, and chromosomal regions. Therefore, solid tumors differ in their cytogenetic aberration pattern from hematological malignancies in that they are characterized by chromosomal translocations, 50 percent of which are balanced, reciprocal ones. The dominance of chromosomal gains and losses, particularly at early stages of carcinogenesis, suggests that an impairment of chromosome segregation fidelity plays a central role in the genesis of epithelial cancers. Based on this observation, we have identified the following questions: (1) are there abnormalities of cellular structures that regulate chromosome segregation, thereby causing the emergence of chromosomal aneuploidy, and what role does the centrosome, as the major organizer of the spindle apparatus, have in the proper segregation of chromosomes?; and (2) is there a karyotypic difference between diploid and aneuploid tumors, and can aberrations in centrosome number and function lead to chromosomal aneuploidy in human and mouse tumors? We plan to address these problems by developing live cell imaging in combination with the use of green fluorescent protein technology to explore the sequence of chromosomal copy number changes as related to centrosome aberrations, and by developing model systems to study the effects of extra copies of specific chromosomes in normal cells.

Recent Publications:

Veldman T, et al. *Nat Genet* 1997;15:406–10.

Xu X, et al. *Mol Cell* 1999;3:389–95.

Ghadimi BM, et al. *Genes Chromosomes Cancer* 2000;27:183–90.

Difilippantonio M, et al. *Nature* 2000;404:510–4.

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Biography: *Roscoe R. Stanyon obtained a Ph.D. in 1982 from the Pennsylvania State University, University Park, PA, with a thesis on chromosome evolution in primates. In 1987 he was a recipient of a Humboldt fellowship at the Institute of Anthropology and Human Genetics, University of Munich, Germany. In 1988 he became associate professor of physical anthropology at the University of Genoa, Italy, and received tenure in 1992. In November of 1998 he came to the NCI.*

Genetics Branch
Mechanisms of Chromosome Rearrangements in the Evolution of the Genome

Keywords:
chromosomal rearrangements
comparative genomics
cytogenetics
flow cytometry
fluorescence in situ hybridization
laser microdissection
phylogenetics

Research: Studies of the genome are now entering a phase focusing on comparative and functional genomics. Of significant importance is resolving the evolutionary history of the human genome as it relates to both genome organization and chromosomal architecture. Comparative genome analysis offers the potential to interpret the evolutionary dynamics of gene organization within chromosomes to reveal the forces governing conservation of synteny and to specify the adaptive rationale behind genome organization. Chromosomal instability is a hallmark of cancer cells. Similarly, during the course of mammalian chromosome evolution, a tremendous degree of reshuffling has occurred. The next challenge for comparative genome analysis is to better understand the patterns and rates of chromosomal evolution and apply these to reveal the processes involved in breaking and reshaping genomes. Identifying breakpoints precisely in cancer and evolution is a first and essential step in understanding the mechanisms and causes of genome evolution in both human phylogeny and disease. Comparative molecular cytogenetics can now literally dissect the evolution and origin of human chromosomes, and translocation or interchromosomal rearrangements can now be identified down to the sequence level using the CCAP BAC arrays. Recent large-scale comparative sequencing efforts have begun to provide some insight into chromosome breakpoints. Genomic features such as Alu sequences flanking the sites of recombination may result in susceptibility to chromosome rearrangements. Other sequences such as transposons, minisatellites, and duplicons may be responsible for promoting chromosome rearrangements. Fragile sites, which often appear to lie associated with breakpoints in chromosomal rearrangements in both cancer and evolution, may contain such repeat sequences. It will be necessary to take into account the evolutionary age of breakpoints by more precise studies of multiple mammalian genomes and their interpretation within a phylogenetic perspective.

The core services offered by the facility combined with resources of the Cancer Chromosome Aberration Project (**http://cgap.nci.nih.gov/Chromosomes/CCAP**) comprises the entire spectrum of advanced molecular cytogenetic techniques:

- generation of chromosome painting probes from flow-sorted human and mouse chromosomes;
- FISH analysis with Ccap BAC clones;
- multicolor spectral karyotyping (SKY) of human and murine cancer samples;
- chromosome microdissection; and
- generation of BAC arrays for aCGH analysis (human).

Among our collaborators are Stephen O'Brien, Thomas Ried, and Robert Wiltrout, NIH.

Recent Publications:

Stanyon R, et al. *Cytogenet Cell Genet* 1999;84:150–5.

O'Brien SJ, et al. *Science* 1999;286:458–81.

Stanyon R, et al. *Am J Primatol* 2000;50:95–107.

Muller S, et al. *Proc Natl Acad Sci* 2000;97:206–11.

Clinical Trials:

Frederic Kaye	**96–C–0128:** A pilot study of correlation between atypical malignant cells in sputum and fluorescent bronchoscopy
Ilan Kirsch	**95–HG–0165:** Outcomes of education and counseling for HNPCC testing
	96–C–0061: Genetic factors and interrelationships for cancer risk-related behaviors and complex traits
	98–C–0037: A phase I/II multiple dose safety and efficacy study of a selective inhibitor of cyclo-oxygenase (NSC 58635) in HNPCC patient and carriers
	99–C–0081C: Methods in education for breast cancer genetics

HIV and AIDS Malignancy Branch

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The HIV and AIDS Malignancy Branch (HAMB) conducts laboratory and clinical research in AIDS, AIDS-related malignancies, and viral-induced tumors. The principal aims of this translational research program are to develop novel therapies for these diseases based on an understanding of their pathogenesis. Clinical research is currently directed towards HIV infection in both children and adults as well as AIDS-related malignancies. Some areas of focus include antiangiogenesis approaches for Kaposi's sarcoma; immunologic and vaccine approaches for HIV infection; and novel anti-HIV therapies. Included in the clinical research effort are studies of the neuropsychological and psychosocial aspects of HIV infection. Some areas of laboratory focus include the regulation of HIV infection, the effects of redox stress on HIV protease, Kaposi's sarcoma-associated herpesvirus (KSHV), also called human herpesvirus-8 (HHV–8), and the regulation of splicing in human papilloma virus.

The branch is currently growing. Drs. Steven Zeichner and Zhi-Ming Zheng recently joined as investigators and Dr. Richard Little as a senior oncologist. Dr. Hiroaki Mitsuya, a long-time collaborator with members of the branch, joined it in 2001. Recruiting efforts are ongoing for an additional principal investigator in the area of clinical pediatric HIV research.

Investigators in the branch have previously made substantial contributions to the development of such AIDS therapies as zidovudine (AZT), didanosine (ddI), and zalcitabine (ddC) in adults and children, and paclitaxel for the therapy of Kaposi's sarcoma. Accomplishments during the past 2 years have included: the development of ritonavir and indinavir in HIV-infected children; the identification of thalidomide as an active agent for Kaposi's sarcoma; the identification of a novel protease inhibitor and nucleoside analog as anti-HIV agents; the identification of a new regulatory site on the long terminal repeat of HIV; and the identification of the transcriptional program of KSHV. The branch provides a unique environment in which to conduct translational research on AIDS and AIDS-related malignancies. Training opportunities are available for laboratory research and for clinical research in children and adults. Members of the branch have longstanding collaborations with Dimiter Dimitrov of the Laboratory of Experimental and Computational Biology, Jeff Trent of the National Human Genome Research Institute, Gene Shearer of the Experimental Immunology Branch, and Crystal Mackall of the Pediatric Oncology Branch.

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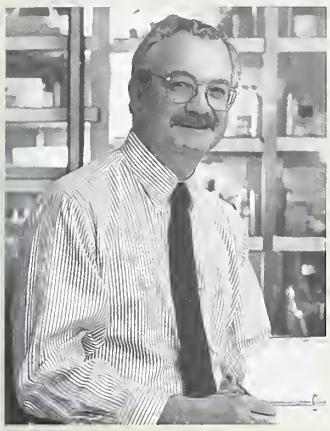

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Biography: *Dr. Yarchoan received his B.A. from Amherst College and his M.D. from the University of Pennsylvania. He trained in internal medicine at the University of Minnesota and Immunology in the Metabolism Branch, NCI. He then joined the laboratory of Dr. Samuel Broder, where he played a major role in the discovery and development of effective therapies for HIV infection. Dr. Yarchoan was section chief in the Medicine Branch from 1991 to 1996 and was named chief of the newly formed HIV and AIDS Malignancy Branch in 1996.*

HIV and AIDS Malignancy Branch
Development of Novel Therapies for HIV- and AIDS-Related Malignancies in Adults

Keywords:
AIDS
angiogenesis
antiretroviral therapy
HIV
Kaposi's sarcoma
Kaposi's sarcoma-associated herpesvirus
lymphoma
vaccines

Research: The main focus of our research is to develop novel therapies for HIV infection and HIV-related malignancies and to increase our understanding of the pathogenesis of these diseases. One area of principal interest is Kaposi's sarcoma (KS). Recent studies indicate that this AIDS-related malignancy is caused by a newly discovered herpesvirus called Kaposi's-associated herpesvirus (KSHV) or human herpesvirus-8 (HHV–8). Initial studies by our group have sought to identify the incidence of KSHV/HHV–8 infection in patients in various risk groups. We have recently shown that hypoxia can induce KSHV replication. Our group previously showed that paclitaxel was highly effective as a therapy for patients with advanced KS. Our group is now exploring approaches that are based on an understanding of the pathogenesis of this disease but that do not involve the use of cytotoxic chemotherapy. There is evidence that production of virally encoded and cellular angiogenesis-inducing factors by KSHV-infected cells is important in the pathogenesis of KS, and this makes antiangiogenesis approaches attractive to consider. We are studying in the laboratory the regulation and activity of these angiogenic factors. Trials are presently under way to evaluate the antiangiogenic agents thalidomide and IL–12 in this disease. Preliminary results suggest that these agents have activity at doses that are well tolerated. We are also exploring the combination of IL–12 and a liposomal anthracycline. It is anticipated that further studies will explore the mechanisms by which these may work and expand upon these results. Our group is also exploring whether the antiherpes drug cidofovir, which has substantial in vitro activity against KSHV/HHV–8, will induce remissions of Kaposi's sarcoma in patients with some residual immune function. In collaboration with the Experimental Transplantation and Immunology Branch, our group is also exploring the use of an infusional regimen and IL–12 in patients with AIDS-related lymphoma.

Another area of focus is the development and evaluation of effective antiretroviral therapy. An important target for such therapy is the HIV protease. This enzyme is a dimer, each half of which contains two cysteines.

These cysteines are highly preserved among strains of HIV, and modification of these cysteines by glutathionylation can profoundly increase or suppress the HIV protease activity. In particular, upon exposure to oxidative conditions, glutathionylation of a conserved cysteine at position 95 (Cys 95) at the dimer interface completely shuts off HIV–1 protease activity. Interestingly, HIV–2 protease has no cysteines, but has a conserved methionine at position 95 that acts in a similar manner. Occasional HIV–1-infected patients on long-term protease inhibitor therapy have mutations in Cys 95, and we are attempting to understand the benefit to the virus of this mutation and use this as a tool to understand the role of the Cys 95 regulatory mechanism. We are also attempting to devise therapeutic protease inhibitors based on this understanding.

In the clinic, our group is conducting an initial study of a peptide-based HIV vaccine in patients with early HIV infection who are on combination therapy with anti-HIV drugs. A trial of a NYVAC-based HIV vaccine to boost the immune response to HIV in infected patients is planned.

Finally, working within the Pediatric HIV Working Group, we are studying the long-term administration of protease-based therapy and the immuno-reconstitution attained with highly active antiretroviral therapy in children with HIV infection. Children have reasonably preserved thymus glands, and thus serve as a model to study the potential of HIV-infected patients to have a restoration of immune function upon inhibition of HIV replication. It is possible that the results of these studies will be used as a platform to study therapeutic HIV vaccine strategies in HIV-infected children.

We have collaborated with Jay Berzofsky, William Figg, Genoveffa Franchini, James Kelley, Rod Levine, Crystal Mackall, Hiroaki Mitsuya, George Pavlakis, James Pluda, Gene Shearer, Giovanna Tosato, and Paul Wingfield, NIH; and Kathleen Staskus, University of Minnesota.

Recent Publications:

Davis DA, et al. *J Virol* 1999;73:1156–64.

Little RF, et al. *J Clin Oncol* 2000;18:2593–602.

Jankelevich S, et al. *J Infect Dis* 2001;183:1116–20.

Davis DA, et al. *Blood* 2001;97:3244–50.

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Biography: *Dr. Little received his B.S. from Tulane University and his M.P.H. and M.D. from the University of South Carolina. He completed his clinical training in internal medicine at the Boston City Hospital and in hematology and oncology in the Hematology Branch, National Heart, Lung, and Blood Institute, and the Medicine Branch of the NCI. He then joined the Laboratory of Tumor Cell Biology where he worked on retroviral strategies for gene transfer. Currently he is a senior oncologist in the Retroviral Diseases Section of the HIV and AIDS Malignancy Branch, which he joined in 1996.*

HIV and AIDS Malignancy Branch
Clinical Research in HIV Infection and AIDS-Associated Malignancies

Keywords:
AIDS
HIV
Kaposi's sarcoma
lymphoma
vaccines

Research: Dr. Little's research has focused on clinical investigations in AIDS-related malignancies, developing treatment strategies for resistant HIV and treatment vaccines for HIV infection.

Treatment strategies for AIDS-related non-Hodgkin's lymphoma have included reduced doses of chemotherapy given concurrently with antiretroviral therapy, but the prognosis is poor—with a median survival of about 11 months in favorable prognosis patients, using standard therapies. A concern is that antiretroviral drugs given concurrently with chemotherapy may result in reduced dose intensity of chemotherapy due to pharmacokinetic interactions and overlapping toxicity, thus adversely affecting the curative potential of the antilymphoma therapy. To optimize delivery of chemotherapy, antiretroviral therapy can be suspended, but it is unclear if suspension of antiretroviral therapy during chemotherapy will adversely affect the underlying AIDS. A focus of Dr. Little's research is to study viral and immunologic parameters associated with optimal chemotherapy administration, and to study the potential for subsequent immune reconstitution in HIV-infected patients.

Kaposi's sarcoma, a highly vascular tumor, may serve as a model for new treatment approaches that are directed against tumor blood supply. In collaboration with other members of his branch, Dr. Little's clinical investigations have also focused on novel treatments using these approaches for this tumor.

Another aspect of his research is directed toward immunologic function in HIV infection, and to study how immune responses can be potentially modified to help control HIV longer term in conjunction with antiretroviral therapy. These clinical studies involve the use of cytokines, HIV peptide treatment vaccines, and highly active antiretroviral therapy.

Collaborating with us are Jay Bersofksy, Genoveffa Franchini, James Pluda, John Tisdale, Giovanna Tosato, Wyndham Wilson, and Robert Yarchoan, NIH.

Recent Publications:

Little R, et al. *J Clin Oncol* 2000;18(13):2593–602.

Little R, et al. *Curr Opin Oncol* 2000;12:438–44.

Yarchoan R, et al. AIDS-associated cancers. In: *Cancer Principles and Practice of Oncology* 6th ed. Devita VT, et al., editors. Philadelphia: Lippincott Williams & Wilkins, 2001.

Little R, et al. *JAMA* 2001;285(14):1880–5.

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Biography: *Dr. Mitsuya obtained his M.D. and Ph.D. in Kumamoto University School of Medicine in Japan. After receiving immunology/hematology/oncology training at Kumamoto University Hospital, Dr. Mitsuya joined the National Cancer Institute in 1982 and began studying the outcomes of infection by human T cell leukemia virus type 1, the first known human pathogenic retrovirus. In 1984, Dr. Mitsuya steered his attention to human immunodeficiency virus or HIV. In 1985, he identified three nucleoside reverse transcriptase inhibitors (NRTIs: 3′-azido-2′,3′-dideoxythymidine or AZT, 2′,3′-dideoxyinosine or ddI, and 2′,3′-dideoxycytidine or ddC) now widely used in the treatment of acquired immunodeficiency syndrome (AIDS), and guided much of their preclinical development. Dr. Mitsuya has been chief of the Experimental Retrovirology Section since 1991.*

HIV and AIDS Malignancy Branch
Development of Therapies for HIV Infection

Keywords:
AIDS
antiviral therapy
chemokine receptor inhibitors
dideoxynucleosides
drug resistance
HIV
protease inhibitors
reverse transcriptase inhibitors

Research: In the therapy of HIV infection and AIDS, we have lately seen what is called cautious optimism. It is now evident that an antiviral intervention brings about clinical benefits in both symptomatic and asymptomatic patients and that protease inhibitors (PIs) administered with NRTIs block HIV replication more profoundly and persistently than ever. This significant progress was possible mainly in combination with NRTIs and PIs or highly active antiretroviral therapy (HAART). In 1985, utilizing an in vitro drug testing system involving human target cells Dr. Mitsuya created himself, he showed for the first time that AZT had potent anti-HIV activity in vitro. Based on his data, AZT was ultimately selected for the clinical trial at the NCI and he collaborated in a series of clinical studies establishing AZT as the first drug for AIDS therapy. Concomitantly, Dr. Mitsuya discovered that other 2′,3′-dideoxynucleosides (ddNs) were also active against HIV in vitro. Of these, ddI and ddC ultimately came into clinical use as important anti-HIV drugs. Dr. Mitsuya also showed that ddNs served as substrates for HIV reverse transcriptase, which upon interaction with the latter undergoes viral DNA chain termination, and the ddNs are active against a number of human retroviruses under certain conditions, thus establishing the general conditions for the antiretroviral effect of ddNs.

Since these epochal studies, Dr. Mitsuya has continued to produce important insights into the potential therapy of AIDS. In 1993 to 1994, Dr. Mitsuya showed for the first time that NRTIs can be classified into two groups: (1) cell-activation-dependent ddNs such as AZT and 2′,3′dideoxy-2′,3′-didehydrothymidine (d4T) that are preferentially phosphorylated, yield higher ratios of ddN 5′-triphosphate (ddNTP)/2′-deoxynucleoside 5′-triphosphate (dNTP), and exert more potent anti-HIV activity in activated cells than in resting cells; and (2) cell-activation-independent NRTIs including ddI, and ddC that produce higher ratios of ddNTP/dNTP and exert more potent activity against the virus in resting cells. These findings have provided the basis for the design of currently available combination chemotherapy with NRTIs.

Another major focus of Dr. Mitsuya's research is the drug-resistant HIV problem. In 1992, Dr. Mitsuya showed that HIV develops resistance to AZT more readily than ddI and ddC and that HIV develops a set of novel mutations which confer multidrug resistance (MDR) on HIV. Dr. Mitsuya and his group has continued and extended the study of MDR HIV variants. The MDR-conferring mutations have been identified in 6 of 36 patients (~20 percent) receiving combination chemotherapy in Dr. Mitsuya's study. A number of groups in the United States and Europe have increasingly confirmed that the emergence of MDR–HIV is an alarming problem in the therapy of HIV infection and prompt measures need to be taken to address this issue.

In response to the drug-resistant HIV problems and in collaboration with intramural and extramural scientists, Dr. Mitsuya's group has identified several promising protease inhibitors (PIs) which are extremely potent against wild-type HIV and HIV variants resistant to currently available PIs. One such PI, JE–2147 (AG–1776), has a flexible component that may be related to its anti-HIV activity against a wide spectrum of multi-PI-resistant variants. Another extremely potent novel PI is UIC–94003 (TMC–126) that, unlike other PIs, binds to the main chains of the protease active site amino acids, a unique property presumably enabling UIC–94003 (TMC–126) to be active against multi-PI-resistant HIV variants. Dr. Mitsuya has most recently identified novel CCR5 antagonistic spirodiketopiperazine derivatives (e.g., E913) that are extremely potent against macrophage-tropic HIV. Dr. Mitsuya and his group have a wide range of scientific and practical interests in a number of modalities of antiretroviral intervention and both national and international active collaborations are always in progress.

We have collaborated with Edward Arnold, Rutgers University; John Erickson, Tibotec, Rockville, MD; Arun Ghosh, University of Illinois at Chicago; Victor Marquez and Robert Yarchoan, NIH; Masao Matsuoka, Kyoto University, Kyoto, Japan; Thomas Merigan, Stanford University, Stanford, CA; and Jiri Zemlicka, Karmanos Cancer Institute, Detroit, MI.

Recent Publications:

Mitsuya H, et al. In *Textbook of AIDS Medicine*. Baltimore: Williams and Wilkins, 1999.

Yoshimura K, et al. *Proc Natl Acad Sci USA* 1999;96:8675–80.

Kosalaraksa P, et al. *J Virol* 1999;73:5356–63.

Kodama E, et al. *Antimicrob Agents Chemother* 2001;45:1539–46.

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Biography: *Dr. Wood received her M.D. from Duke University School of Medicine and completed clinical training in internal medicine and pediatrics at Baylor College of Medicine Affiliated Hospitals in Houston, Texas. She received further subspecialty training in Allergy and Immunology at the National Institute of Allergy and Infectious Diseases before joining the NCI in 1992. As a Senior Immunologist in the Pediatric HIV Working Group, her clinical research involves investigation of antiretroviral and immunomodulatory therapies in children and adolescents with HIV infection.*

HIV and AIDS Malignancy Branch
Immune-Based Therapies for HIV Infection

Keywords:
AIDS
HIV
immunodeficiency
immunotherapy

Research: Despite the documented therapeutic advances associated with the development of highly active antiretroviral therapy (HAART), viral resistance, metabolic complications, and drug toxicities pose major limitations for long-term control of viral replication. Impaired functional immune responses persist to recall and HIV-specific antigens even in the setting of significant immune reconstitution. Consequently, Dr. Wood's research has focused increasingly on investigating immune-based therapies to promote immune reconstitution and augment control of viral replication.

Interleukin 2 (IL–2) is a critical T cell-derived mediator of both cellular and humoral immune function involved in promoting the proliferation, activation, and differentiation of T cells, B cells, and natural killer cells. Administration of IL–2 in adults has been shown to be associated with improvement in CD4 counts as well as reduction in reservoirs of resting CD4 cells that contain replication-competent HIV. We have been conducting an ongoing pilot study to determine the safety, tolerance, and pharmacokinetics of low- and high-dose recombinant IL–2 given in combination with licensed antiretroviral agents. Preliminary results reveal that subcutaneous IL–2 can be administered safely to children and teens on an outpatient basis and is not associated with acute or chronic elevations in plasma HIV RNA levels. The most common side effects are fever, local injection site reactions, and nasal congestion. High dose IL–2 has been associated with a higher incidence of persistent fever despite antipyretics, often requiring dose reduction. Improvements in both CD4 count and cellular immune function have been observed in patients with varying degrees of immune suppression, although

these improvements are more pronounced in patients with absolute CD4 counts greater than 500 cells/mm3 at baseline. Neither baseline viral load nor antiretroviral treatment regimen has been predictive of responsiveness to IL–2.

HIV–1 Immunogen is a therapeutic vaccine being studied to determine its safety and immunogenicity in children and adolescents with HIV infection. It consists of inactivated whole killed HIV–1 virus that contains all viral proteins except for gp120, which is lost in the inactivation process. Administered as an intramuscular injection every 3 months for a total of five injections, this study has examined cellular and humoral responses associated with vaccination and its effect on HIV viral replication. Initially a double-blind, dose-ranging phase I study, interim analysis revealed that in children receiving antiretroviral therapy, those receiving a 10-unit dose of vaccine had a more sustained reduction in plasma HIV RNA levels than those who received the 2.5-unit dose. This downward trend in RNA levels was statistically significant. In addition, the higher dose was associated with greater development of humoral and lymphoproliferative responses. Importantly, vaccine administration was not associated with any observed immediate increases in HIV RNA levels. The study was amended to allow all patients to receive the 10-unit (adult equivalent) Immunogen dose. Analysis of lymphoproliferative (LP) responses to HIV–1 and recall antigens demonstrated a strong and highly significant correlation between development of a positive LP response to HIV–1 Immunogen and p24 antigen. This suggests that therapeutic vaccination can promote in vivo HIV-specific immune responses that may contribute to long-term control of HIV infection. In addition, HAART therapy was found to be associated with higher LP responses and a shorter time to their development. Future studies are planned examining structured treatment interruption (STI) in patients on HAART receiving therapeutic vaccination with HIV–1 Immunogen to determine how these responses may influence and alter the kinetics of viral replication.

An ongoing challenge to the management of HIV infection in children is inadequate viral suppression despite highly potent antiretroviral therapy. Hydroxyurea (HU) is a cytostatic chemotherapeutic agent that exhibits antiretroviral activity in vitro as well as in vivo when administered in combination with didanosine (ddI) and/or stavudine (d4T), even in the presence of viral resistance. We are conducting a pilot study investigating virologic and immunological responses in heavily treatment-experienced patients given ddI/d4T/efavirenz in combination with hydroxyurea for 48 weeks. Preliminary analysis has demonstrated viral decay kinetics during the first week of therapy similar to those observed in patients receiving their first HAART regimen. Median time to viral nadir was 6 weeks although all patients exhibited some degree of viral rebound by 32 weeks. Despite unsustained virologic responses, patients exhibited immunological reconstitution with an increase in total CD4 cells consisting primarily of naive CD45RA subset. A substantial decrease in T cell activation has been observed despite persistent viral replication, suggesting an independent treatment effect on immune dysregulation. The study treatment regimen is being

modified to replace efavirenz with Kaletra, a second-generation protease inhibitor with a favorable antiviral activity profile against resistant viral isolates, in an attempt to further maximize sustained virologic responses.

Preclinical studies of IL–7, in collaboration with Drs. Crystal Mackall, Terry Fry, and Robert Yarchoan, have demonstrated strong inverse correlations between IL–7 levels and CD4 counts, particularly the CD4 CD45RA+ naive cell subset, in both children and adults with HIV infection. This effect, also observed in CD4-depleted patients receiving cancer chemotherapy, suggests that IL–7 may play a critical role in restoring T cell homeostasis following T cell depletion and have a potential utility as a therapeutic intervention to promote T cell regeneration in patients with cancer and HIV infection.

Finally, the dramatic therapeutic advances and improved clinical outcomes associated with HAART have also been associated with the emergence of metabolic and lipodystrophy syndromes that appear to have mitochondrial toxicity as a common denominator in their pathogenesis. In collaboration with Drs. Steve Zullo and Henry Wiener, we are examining the effects of various protease inhibitors on in vitro systems and examining mitochondrial protease processing and mitochondrial toxicity in an attempt to further elucidate the pathophysiology observed with these clinical syndromes.

Among our collaborators are Josephine Cox, Silvia Ratto-Kim, and Merlin Robb, Henry M. Jackson Foundation; Dimiter Dimitrov, Terry Fry, Marianna Gerschenson, Crystal Mackall, Gene Shearer, Shizuko Sei, Thomas Walsh, Jon Wigginton, Chad Womack, Robert Yarchoan, NIH; Henry Wiener, Purdue University; and Steve Zullo, NIST.

Recent Publications:

Fry TJ, et al. *Blood* 2001:97(10):2983–90.

Jankelevich S, et al. *J Infect Dis* 2001;183(7):1116–20.

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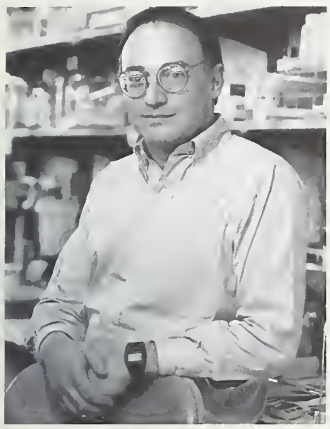

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Biography: *Dr. Zeichner received an M.D. and Ph.D. from the University of Chicago as part of the Medical Scientist Training Program. He served as a resident in pediatrics and a fellow in pediatric infectious diseases at the Children's Hospital of Philadelphia. Dr. Zeichner is also an associate professor in pediatrics at the Uniformed Services University of the Health Sciences.*

HIV and AIDS Malignancy Branch
Molecular Pathogenesis of HIV, Herpesviruses, and Related Malignancies

Keywords:
AIDS
cDNA microarray
gene expression
herpesviruses
HHV–8/KSHV
HIV
Kaposi's sarcoma

Research: Our laboratory investigates the pathogenesis of HIV disease and related opportunistic infections and malignancies at a molecular level. We study the interactions of HIV and other viruses with their host cells, particularly the contribution of cellular genes and gene products to viral replication and pathogenesis. We also have a special interest in pediatric HIV disease pathogenesis and hope to understand the basis for some of the unique features of this disease. Our work involves three principal areas: investigating cellular factors involved in HIV replication, cloning and characterizing cellular genes that control HIV gene expression, and studying the clinical features of pediatric HIV disease.

Our studies of the cellular factors involved in HIV replication center on identifying and characterizing cellular genes differentially expressed during HIV infection. We hypothesize that one set of cellular growth conditions is optimal for normal cell growth, that another set of conditions is optimal for viral replication, that HIV has evolved the means to alter the host cell to favor viral replication, and that at least some of these alterations occur through changes in transcription. We employ newly available cDNA microarray technology, which can provide quantitative expression information for several thousand genes simultaneously, to catalog the cellular genes that are differentially expressed during HIV infection. Using cell lines that express the viral gene products, purified viral gene products, and drugs acting at different stages during the viral replication cycle, we are attributing the observed changes in cellular gene expression to particular times during the viral replication cycle and to individual viral gene products. We have identified several cellular genes that appear to be differentially regulated and are continuing to characterize them and investigate the consequences of their differential expression. In the future, we plan to assess the contribution of selected cellular genes to viral replication and pathogenesis.

In complementary work, we are using cDNA array technology to study other viruses, including HHV–8 (Kaposi's sarcoma-associated herpesvirus) and HHV–6, the etiologic agent of roseola. We have produced arrays that contain

virtually all the open reading frames of these viruses and are using the arrays to describe their transcription programs. The details of the transcription programs should provide additional insights into viral pathogenesis strategies. Further studies using our viral arrays will enable us to understand how viral transcription changes under different conditions. We are investigating the cellular response to viral replication and compare the patterns of cellular gene expression during the replication of the viruses to ascertain whether any common features characterize the cellular response to different viruses, and investigate the clinical implications of the viral gene expression programs.

In more clinically related work, we aim to understand the special features of pediatric HIV disease. For example, infants infected with HIV often experience a more fulminant disease course than adults and have higher viral loads. We hypothesized that these differences may, in part, reflect differences in the interaction of HIV with the host cells of children and adults. HIV replicates preferentially in actively dividing cells, so one possible explanation for the differences between infants and adults could be that infants have more actively dividing cells. One way of indirectly measuring cell turnover involves measuring the telomere shortening rate, since the telomeres of most nongermline cells are believed to shorten by some relatively constant amount with each cell division. We recently found that the telomeres of the peripheral blood mononuclear cells (PBMCS, which include lymphocytes infected by HIV) of uninfected infants shorten more rapidly than those of adults, ~270 bp/y compared to ~50 bp/y. This finding suggests that the PBMCs of infants include more dividing cells. Infants may therefore have a larger pool of cells capable of effectively supporting HIV replication, potentially leading to the higher viral loads and a more rapid disease course. Additional studies are under way to confirm these findings using other approaches and to more thoroughly characterize the turnover rates in various populations of infant cells.

The therapy of HIV infection is becoming ever more challenging, given the rapid ability of the virus to evolve to become resistant to available therapies. It is also difficult to develop new antiretroviral therapies because new drugs must be tested in combination with other drugs in order to give the patient the maximum possible benefit from therapy. In our clinical work, we are investigating the optimization of antiretroviral therapy in children and new potential therapies for pediatric HIV disease. We are also investigating how HIV evolves within the pediatric host and how measurements made at the beginning of antiretroviral therapy may be able to predict the long-term response to therapy.

Collaborating with us are Mike Baseler, Robert Biggar, Mike Bittner, Dimiter Dimitrov, K.T. Jeang, Frank Maldarelli, Paul Meltzer, H. Mitsuya, Charles Rabkin, Jeff Trent, S. Venkatesan, and Robert Yarchoan, NIH; Andy Bonwit, Christie Holland, and Stephen Teach, Children's Hospital National Medical Center; Christian Brandler, Massachusetts General Hospital; Mary Caserta, University of Rochester; Jae Jung, New England Regional Primate Center; Paul Palumbo, University of Medicine and Dentistry of New Jersey; and John Sleasman, University of Florida.

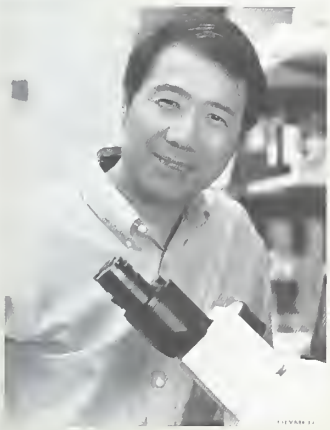
Recent Publications:

Mueller BU, et al. *AIDS* 1998;12:F191–6.

Zeichner SL, et al. *Blood* 1999;93:2824–30.

Paulose-Murphy M, et al. *J Virol* 2001;75:4843–53.

Ng P, et al. *Oncogene* 2001; in press.



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Biography: *Dr. Zheng received his medical and virological training in China and was a Yale-China exchange scholar at Yale University from 1981 to 1984. After returning to China, Dr. Zheng served as an associate professor, chief of the Clinical Virology Laboratory, and deputy as well as acting director of the Virus Research Institute at Hubei Medical University, China. He received his Ph.D. from the University of South Florida School of Medicine in 1994. He then joined the NCI as an IRTA fellow with Dr. Carl Baker in the Laboratory of Tumor Virus Biology and became a member of the senior staff in 1997. He moved to the HIV and AIDS Malignancy Branch as an investigator in 2000. His research interests center on the RNA processing and tumorigenesis of tumor viruses including papillomaviruses and Kaposi's sarcoma-associated herpesvirus.*

HIV and AIDS Malignancy Branch
Viral RNA Splicing and Tumorigenesis

Keywords:
cervical cancer
DNA tumor viruses
Kaposi's sarcoma-associated herpesvirus
papillomaviruses
RNA
RNA processing
RNA-protein interaction
RNA splicing
splicing
tumorigenesis

Research: Pre-mRNA splicing is one of the most important steps in control of gene expression. This essential step involves intron removal from a primary transcript and exon ligation to form a real message. In many cases, the mechanisms that regulate RNA splicing remain poorly understood. DNA tumor viruses such as cervical cancer-associated human papillomaviruses (HPV) and Kaposi's sarcoma-associated herpesvirus (KSHV) have several critical genes undergoing regulation by RNA splicing at the posttranscriptional level. Our research focus is to understand the mechanisms that control viral RNA splicing and to look for new tools and molecular targets for antiviral and anticancer therapy at the RNA level. Present studies in our laboratory focus on (1) identification of viral cis elements that are involved in regulation of the RNA splicing of viral structural and nonstructural genes including viral oncogenes in high- and low-risk HPVs and KSHV; (2) characterization of cellular splicing factors and viral proteins involved in processing of RNA splicing; and (3) development of RNA interference as a new tool for antiviral and anticancer therapy.

We have been utilizing HPV–16 and –18 E6 and E7 RNA transcripts as a first step to approach our goal. The E6 and E7 genes of HPV–16 and –18 are two major viral oncogenes and are expressed in almost every cancer cell of cervical carcinoma. E6 and E7 proteins inactivate cellular tumor suppressor proteins p53 and pRb, respectively, and play key roles in the induction of human cervical cancer. However, expression of E6 and E7 is complicated not

only with their transcription as a bicistronic mRNA, but also with alternative splicing of their primary transcripts from which a large portion of E6 has been removed through using two alternative 3′ splice sites within E6 coding regions. These alternatively spliced RNA species are termed E6*I and E6*II and form the majority of early viral transcripts both in cervical tumors and in tumor-derived cell lines. Ironically, transcripts for unspliced, full-length E6 are in extremely low abundance and sometimes it is hard to detect them in many tumors or tumor-derived cell lines. We are analyzing HPV–16 and –18 E6–E7 RNA splicing patterns in cervical cancer-derived cell lines with various strategies and have successfully established an in vitro splicing system to identify the viral cis element that controls the alternative splicing of the E6–E7 pre-mRNAs. Works are in progress on the characterization of these elements and their interaction with splicing factors and viral proteins.

Another approach in our laboratory is to determine how cellular splicing factors are involved in viral RNA splicing. We are using a bovine papillomavirus type 1 (BPV–1, a prototype virus in the papillomavirus family) late pre-mRNA as our model to address this question since this pre-mRNA has two alternative 3′ splice sites (3′ss). Switching from one 3′ ss to another in the splicing of this transcript relates to keratinocyte differentiation and involves viral cis elements interacting with cellular splicing factors. We have established a series of cell lines with stable transfection of BPV–1 late genes. These cell lines have a cellular splicing factor ASF/SF2 under the control of a tetracycline (tet)-repressible promoter. Using these cell lines, we have demonstrated that the splicing factor ASF/SF2 is required for such a switch of 3′ ss usage, but is not essential. Activation of the cells doesn't recover tet-repressed ASF/SF2 expression, but restores the default pattern of 3′ ss utilization though activation of other splicing factors. Currently, we are focusing on the characterization of transcription and polyadenylation coupling with this feature of the splicing.

Kaposi's sarcoma-associated herpesvirus (KSHV) or human herpesvirus (HHV–8) is a newly identified human gamma herpesvirus strongly associated with development of KS, body cavity-based B-cell lymphoma, and Castleman's disease. Currently, our lab is focusing on the KSHV K8 and K8.1, which are two juxtaposed but unrelated genes posited from nt 74850 to nt 76730 of the virus genome. However, both genes share a single poly (A) site at nt 76714. The K8 gene consists of 4 exons and 3 introns, which are alternatively spliced during the viral gene expression. The K8.1, although sharing the exon 4 with K8, utilizes the intron 3 of K8 as its own coding region, which is also alternatively spliced to the exon 4. We have extensively profiled the RNA splicing patterns of the K8 and K8.1 in KSHV+ JSC–1 cells and identified the presence of a bicistronic pre-mRNA which has three alternative 5′ splice sites in its intron 3. Further experiments show that this bicistronic RNA could be used as a precursor of both K8 and K8.1 mRNAs. Our current focus is (1) to understand how three 5′ splice sites in this bicistronic RNA are alternatively selected during the viral life cycle and B cell differentiation, and (2) to identify viral cis elements and trans-acting factors involved in this regulation.

Recent Publications:

Zheng ZM, et al. *Proc Natl Acad Sci USA* 1998;95:14088–93.

Zheng ZM, et al. *J Virol* 1999;73:29–36.

Zheng ZM, et al. *J Virol* 2000;7413:5902–10.

Zheng ZM, et al. *J Virol* 2000;74:10612–22.

Clinical Trials:

Richard Little	**01–C–0067:** A phase II study of liposomal doxorubicin and interleukin 12 in AIDS-associated Kaposi's sarcoma followed by chronic administration of interleukin 12
L. Wiener	**01–C–0203:** Long-term survival with HIV: A study of the psychological and behavioral factors associated with the transition from adolescence to young adulthood
Lauren Wood	**99–C–0118:** A pilot study of hydroxyurea in combination with stavudine, didanosine, and efavirenz in pediatric patients with HIV–1 infection
Robert Yarchoan	**94–C–0159:** A phase I protocol for the evaluation of the safety and immunogenicity of vaccination with synthetic HIV envelope peptides in patients with early human immunodeficiency virus infection **96–C–0113:** A pilot/dose-finding study of the toxicity, anti-Kaposi's sarcoma (KS) activity, and immunologic activity of interleukin 12 administered to patients with AIDS-associated KS **98–C–0041:** A pilot study of the immunologic reconstitution in HIV–1-infected children receiving highly active antiretroviral therapy with combination ritonavir, nevirapine, and stavudine (HAART) **00–C–0193:** A study of the effects of potent anti-HIV therapy on parameters hypothesized to be related to the pathogenesis of Kaposi's sarcoma (KS) in HIV-infected individuals **01–C–0038:** Collection of blood, bone marrow, tumor, or tissue samples from patients with HIV infection, KSHV infection, viral-related premalignant lesions, and/or cancer **01–C–0158:** A protocol to assess vascularity in Kaposi's sarcoma lesions utilizing noninvasive imaging techniques
Steven Zeichner	**99–C–0134:** A long-term observational study of immunologic reconstitution in HIV–1-infected children who are receiving combination protease inhibitor and reverse transcriptase inhibitors **01–C–0025:** A phase I study of capravirine (AG 1549), a novel nonnucleoside reverse transcriptase inhibitor in children with HIV infection

Medical Oncology Clinical Research Unit

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The Medical Oncology Clinical Research Unit (MOCRU) serves as an implementation arm for scientific concepts derived from laboratories across the Center for Cancer Research. The unit provides a creative, integrated, and interactive scientific environment for the development of new strategies for the detection, prevention, and treatment of human malignancies, principally focusing on highly prevalent forms of cancer, including cancers of the gastrointestinal tract, breast, ovary, lung, and prostate, and lymphomas. The members of the unit plan and implement clinical investigations utilizing strategies based on outstanding preclinical scientific investigations. In addition to the conduct of clinical science, the unit provides consultation in medical oncology for the Clinical Center, NIH, and fosters the training and development of medical oncologists through maintenance of an outstanding Accreditation Council on Graduate Medical Education (ACCME)-approved medical oncology fellowship program and ongoing recruitment and development of clinical and scientific investigators. The branch also participates with the National Naval Medical Center Hematology/Oncology Department and Breast Care Center in the delivery of medical care, through both clinical investigations and the delivery of standard care, to patients with malignancy or those at high risk for the development of cancer.

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John Janik	Head, Immunotherapy Clinical Research Section *(Description of research can be found in the Metabolism Branch section, page 181.)*
Richard Little	Head, AIDS Malignancy Clinical Research Section *(Description of research can be found in the HIV and AIDS Malignancy Branch section, page 149.)*
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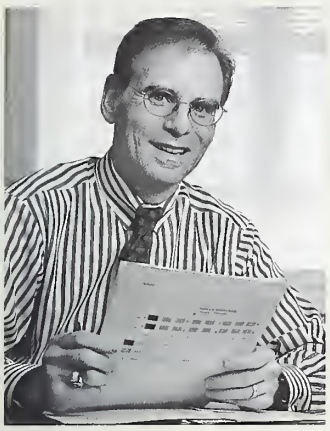

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Biography: *Dr. Dahut received his M.D. from Georgetown University in Washington, DC. He completed clinical training in internal medicine at the National Naval Medical Center in Bethesda, MD, followed by training in hematology and medical oncology at the Bethesda Naval Hospital and the Medicine Branch of the NCI. Dr. Dahut worked as an attending physician in the NCI–Navy Medical Oncology Branch until 1995. He then joined the faculty of the Lombardi Cancer Center at Georgetown University before returning to the former NCI Medicine Branch in 1998 as head of the prostate cancer clinic. Dr. Dahut's primary research interest has been in the development of novel therapeutic strategies for the treatment of adenocarcinoma of the prostate.*

Medical Oncology Clinical Research Unit
New Therapeutics for Patients With Genitourinary Malignancies

Keywords:
angiogenesis
clinical trial
pharmacology
prostate cancer
vaccines

Research: Prostate cancer is the most common cancer in men, with an estimated 180,000 new cases and 37,000 deaths in 1999. It is an extremely heterogeneous disease with an extremely varied clinical course. Although many patients do not manifest any evidence of disease after initial local therapy, approximately 40 percent of those treated surgically will eventually develop recurrent persistent prostate cancer. Many patients will be asymptomatic for many years, while others will develop rapidly progressive metastatic cancer. The research program of the Prostate Cancer Clinic has been focused on attempting to develop novel therapies for each disease state that a prostate cancer patient may experience. The treatment needs to balance the biology of the tumor at that point with the clinical goals of maintaining quality of life and prolonging survival. In addition, in the unique environment of the NCI we have been able to combine our clinical trials with important biologic endpoints.

The increasing use of the serum prostate specific antigen (PSA) has resulted in the earlier detection of prostate cancer and also its recurrence in asymptomatic men. A rising PSA after local therapy may antedate clinical manifestations of disease by many years, yet is a reliable marker of prostate cancer. Although the use of androgen ablation is effective therapy, it is not without the side effects of loss of libido, weight gain, and osteopenia. In addition, patients will eventually develop androgen independence manifested by a second rising PSA while on hormonal therapy. Thus therapies are needed to control or eradicate the growth of the tumor while maintaining the quality of life in patients whose only sign of cancer may be an abnormal blood test. Angiogenesis (blood vessel growth) appears to have an important role in the progression of prostate cancer. Thalidomide, initially approved in Europe as a sedative in the 1960s but not initially approved in this country because of teratogenic effects, has been shown to inhibit angiogenesis. We have studied thalidomide in patients with advanced

metastatic prostate cancer who have failed hormonal therapy. We have seen patients benefit with a fall in their PSA and an improvement in symptoms (particularly impressive in light of our preclinical work that indicated that thalidomide may increase PSA secretion). It is our belief that thalidomide may be even more effective in patients with minimal disease. Thus we have developed a clinical trial in patients with a rising PSA after local therapy to determine if, after a short course of hormonal therapy, thalidomide can allow one to stop the hormonal therapy and maintain an undetectable PSA.

Most patients with recurrent prostate cancer will eventually commence androgen ablation. In time, the PSA will again rise, although often without clinical evidence of disease. There is no standard therapy for these asymptomatic patients and surprisingly there is not good data about the time interval until they develop demonstrable metastatic disease. We have designed a clinical trial to test the ability of recombinant pox viruses expressing PSA and the costimulatory molecule B7.1 plus GM–CSF and IL–2 to prevent hormone-resistant prostate cancer from developing lesions that can be seen on bone scans or CT scans. This vaccination strategy will be evaluated by determining changes in the T cell precursor assay as measured by the Elispot assay. Unfortunately, many prostate cancer patients will eventually develop metastatic disease, which is progressing despite androgen ablation. There is a renewed interest in cytotoxic therapies, especially chemotherapy. We are studying docetaxel, a particularly promising compound in combination with thalidomide. Biopsies will be done to assess the effect on angiogenesis as well as apoptosis. The development of effective treatment for all patients with prostate cancer is of paramount importance. Along with the above trials, we are designing novel combinations of surgery and radiotherapy, and continuing the development of new agents in phase I trials.

Our collaborators are Philip Arlen, William Figg, Jean Grem, J. Michael Hamilton, Steve Libutti, Marston Linehan, Eddie Reed, Jeff Schlom, and Chris Takimoto, NIH; Dan Petrylak, Columbia University; Oliver Sartor, Louisiana State University; and Eric Small, University of California–San Francisco.

Recent Publications:

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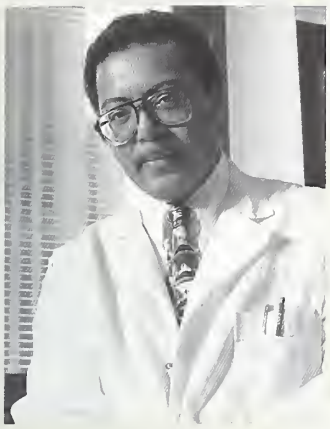
Bharga AP, et al. *Clin Cancer Res* 1999;8:1989–95.



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Biography: *Dr. Gause received his M.D. from Michigan State University, College of Human Medicine. He completed clinical training in internal medicine at Emory University in Atlanta, GA, followed by training in medical oncology and tumor immunology at the NCI. Dr. Gause worked in the extramural community at the Howard University Cancer Center and presently serves on its advisory committee. His major interests are in tumor vaccine development.*

Medical Oncology Clinical Research Unit
Cancer Immunotherapy

Keyword:
vaccines

Research: The objectives of our research are to develop immunotherapeutic models for the treatment of various cancers. At the present time, we are evaluating the clinical and immunological activity of two vaccines: idiotype vaccines in patients with advanced stage indolent lymphomas and B7 transfected melanoma cell line vaccines in patients with melanoma. We are also evaluating the ability of various biological agents to act as adjuvants.

The indolent follicular lymphomas (FL) are follicular small-cleaved cell (FSC) and follicular mixed lymphomas (FM). Stage I and II patients comprise only 10 to 15 percent of all cases of follicular lymphomas and are best managed with radiation therapy. Eighty-five percent of patients with follicular lymphomas present with stage III or IV disease. The optimal management of these patients remains controversial and there is no standard therapy that is considered curative.

Immunoglobulin (Ig) molecules are composed of heavy and light chains, which possess highly specific variable regions at their amino termini. The variable regions of heavy and light chains combine to form the unique antigen-recognition site of the Ig protein. These variable regions contain determinants that can themselves be recognized as antigens, or idiotypes. B cell malignancies are composed of clonal proliferation of cells synthesizing a single antibody molecule with unique variable regions in the heavy and light chains. B cell lymphomas are neoplasms of mature resting and reactive lymphocytes, which generally express synthesized Ig on the cell surface. The idiotypic determinants of the surface Ig of a B cell lymphoma can thus serve as a tumor-specific marker for the malignant clone.

We performed a phase I clinical study to evaluate the ability of an idiotype vaccine to elicit tumor-specific T cell immunity as measured by the ability of the patient's T cells to specifically lyse their own tumor cells in vitro and to exert antitumor effects as measured by the elimination of t(14;18)-bearing cells from the peripheral blood of uniformly treated FL patients in first CR. Eleven patients were PCR positive in PBMC at study entry

(prechemotherapy), as well as at prevaccination. However, eight of these patients converted to PCR negativity immediately after completion of vaccination.

Based on this phase II data, we plan to evaluate, in a phase III setting, the effect of idiotype vaccine on disease-free survival in patients with grade I/II follicular center cell lymphoma, stage III/IV.

The second vaccine trial is directed towards patients with melanoma. Recent investigations have identified several potential immunologic targets against melanoma. At least six different peptide fractions have been eluted from HLA–A2 melanoma cell lines that are recognized by HLA–A2 CTL. Two proteins, tyrosinase and a second undefined 10 kd protein, contain the peptides that are recognized by the HLA–A2 melanoma-specific CTL. Two immunogenic peptide sequences of tyrosinase have been identified. The expression of tyrosinase and the 10 kd protein appears to be restricted to melanocytes and melanomas. One hundred percent of cell lines in some series express the tyrosinase gene. HLA–A2 CTL can mediate tumor regression in vivo in a murine model. The CTL developed specific cytolytic activity against autologous and HLA–A2-matched allogeneic melanomas but not against the allogeneic determinants of the stimulator melanoma cell line.

Shared melanoma antigens presented by HLA–A1 (present on 25 percent of the Caucasian population) have also been identified and the gene sequence determined. The antigen, MAGE–1, is a normal protein expressed on 40 to 50 percent of melanomas and some other tumors but not on most normal tissues. Similar to the HLA–A2 system described above, HLA–A1 CTL specific for MAGE–1 will kill allogeneic melanomas also expressing HLA–A1 and MAGE–1. A second melanoma-specific antigen belonging to the MAGE family (MAGE–3) can be presented by HLA–A1. MAGE–3 appears to be present on 60 to 70 percent of HLA–A1 melanoma patients.

In order for vaccines to be successful, these antigens have to be presented to effector cells of the immune system. Effective antigen presentation to T cells in vivo requires both the crosslinking of the T cell receptor by the peptide/MHC complex and a second costimulatory signal. The costimulatory signal can be generated by an interaction between the molecule B7 on the antigen-presenting cell (APC) and its ligand CD28 on the surface of the T cell. B7 is a member of the immunoglobulin gene superfamily. It is a transmembrane glycoprotein with two immunoglobulin-like extracellular domains and a core molecular mass of 30 kd. B7 binds to two antigens on T lymphocytes, CD28 and CTLA4; with the former, which is consitutively expressed on 95 percent of CD4+ T cells and 50 percent of CD8+ T cells, and with the latter, whose expression is induced upon T cell activation. The interaction between B7 on the APC and its ligand on the T lymphocyte markedly enhances T cell activation costimulatory signal produced by the B7/CD28 or B7/CTLA4 interaction induces T cell tolerance.

We have recently completed a phase I study of a melanoma vaccine. Patients who were HLA–A1 and/or HLA–A2 were vaccinated with irradiated melanoma cell lines that were also HLA–A1 or –A2 positive and transfected with B7. There was no systemic toxicity, and tumor regression or stabilization

was seen in several patients. Preliminary results show that some patients mounted a cytotoxic T lymphocyte (CTL) response against the B7-transfected melanoma cell lines used in the vaccine. The addition of an adjuvant can strengthen the immunologic effect of a vaccine. This next trial will use IL–2, an agent with efficacy against melanoma and the ability to expand CTL responses.

Our collaborators include Nelson Chao and David Rizzieri, Duke University; Brendan Curti, Pennsylvania State University; John Janik, Larry Kwak, Seth Steinberg, Douglas Swartzentruber, and Dennis Taub, NIH; William Kopp, NCI–Frederick; and David Liebowitz, University of Pennsylvania.

Recent Publications:

Janik JE, et al. *Clin Immunol* 1999;93(3):209–21.

Bendandi M, et al. *Nat Med* 1999;5(10):1124.

Curti BD, et al. *J Clin Oncol* 1998;16(8):2752–60.

Gause BL, et al. *Cancer Invest* 1998;16(6):374–80.

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Medical Oncology Clinical Research Unit
Medical Oncology Fellowship Program

The Fellowship Training Program in Adult Medical Oncology at the National Cancer Institute is a unified teaching experience in clinical oncology and research that is conducted at the National Cancer Institute (NCI) and the National Naval Medical Center (NNMC), Bethesda, MD. This fellowship program provides a unique opportunity for physicians interested in academic careers to develop and integrate both their clinical and basic research interests. The clinical portion of the fellowship consists of primary responsibility for the clinical care of inpatients and outpatients with a broad spectrum of adult malignancies. It introduces the participants to the process of designing and conducting clinical trials. During the first clinical year, there is an emphasis on exposing fellows to the basic science and clinical medicine of oncology. During the research years, the fellow will join one of the several hundred available investigators within the National Cancer Institute to acquire the skills necessary to become an independent biomedical

investigator with emphasis on laboratory or clinical science. The NCI guarantees at least 2 years of protected research time to all of its medical oncology fellows. At the end of 3 years (or 4 years for oncology-hematology trainees), fellows may compete for an additional 3 years of salary support to extend their period of mentored research training. The goal of the program is to foster the development of physician-scientists who have excellent clinical and research skills.

During the first year of medical oncology training, fellows devote 100 percent of their time to clinical training in inpatient and outpatient care. All first-year fellows spend 6 months at the NIH Clinical Center (NIH–CC) and 6 months at the NNMC. In the second and third years, 20 percent of the fellows' time is spent in the care of outpatients in either the NIH–CC or NNMC. This continuity clinic ensures that fellows in the program extend their experience with a wide spectrum of human cancer. The remaining time in the second and third years is protected time for research in any of the NCI clinical teams or any NIH laboratory.

The NIH–CC emphasizes the development of new approaches to the treatment of cancer and its complications. Current areas of clinical emphasis include breast cancer, prostate cancer, ovarian cancer, lymphomas, Hodgkin's disease, chronic leukemia, and new drug testing; colorectal, pancreas, lung, gastric, and cervical cancer; and bone marrow transplantation and AIDS and its associated malignancies. Patients with AIDS constitute approximately 10 percent of the total branch patient population; nearly all these patients are involved in studies of experimental antiretroviral compounds. Training in clinical diagnostic procedures such as bone marrow examination, thoracentesis, and paracentesis is a routine part of the first-year experience. Collaboration with related clinical units in the Clinical Center such as Radiation Oncology, Surgery, and Critical Care Medicine is very close.

The NNMC is located in Bethesda adjacent to the NIH campus. It is a tertiary referral hospital that serves active duty military personnel and their families, retired military and their spouses, and some embassy, Congressional, and other nonmilitary personnel. Patients are referred from the military system around the world with all types of malignant disease including solid tumors and hematological malignancies. There is a large population of young adults with malignant diseases characteristic of this age group (e.g., germ cell tumors). The program has a 16-bed inpatient service and also provides consultation in oncology and hematology to the NNMC. In addition, civilian patients who are eligible for NCI studies can be admitted to the program for care and study. The studies at NNMC have focused on lung cancer, breast cancer, and gastrointestinal cancers and cancer vaccines. Roughly 15 percent of patients enrolled are in NCI clinical trials and 85 percent of patients are involved in standard oncologic management.

The Medical Oncology Clinical Research Unit (MOCRU) also has a joint Hematology/Oncology Training Program in collaboration with the Hematology Branch of the National Heart, Lung, and Blood Institute (NHLBI) and the Hematology Department of the NIH's Clinical Center. This program combines the separate training programs of hematology and medical oncology into a series of clinical rotations encompassing the first

18 or 22 months of a fellow's training. Fellows whose principal interest is hematology have a primary appointment in the NHLBI and their chief clinical exposure is in clinical hematology that includes 6 months of clinical oncology in the Medicine Branch, NCI. Fellows who have a primary interest in medical oncology have their appointment with the NCI. Their principal clinical exposure is in clinical oncology with an additional 6 months of hematology rotations. The first 12 months of training are exactly like those for medical oncology alone. In the second year, they undertake an additional 6 months of clinical hematology that usually includes the following: 2 months' ward hematology (NHLBI), 1 month's leukemia service (Johns Hopkins Transplant Unit), 2 weeks' transfusion medicine, 6 weeks' consult hematology and coagulation, and 1 month's rotation at the Washington VA Hospital. Following completion of the 18-month clinical period, the fellow does laboratory or clinical research for up to 4 years of fellowship along with continued outpatient clinical trials.

Offered throughout the program are lectures, research seminars, conferences, journal club, teaching rounds, and conferences relevant to clinical oncology and cancer research. Most of these are organized specifically for medical staff fellows. These include a core curriculum lecture series in clinical oncology, which meets once a week throughout the year and offers lectures by members of the senior staff across the spectrum of issues in neoplastic disease. At both the NIH–CC and the NNMC, the formal didactic lectures and teaching rounds total approximately 10 hours per week. Fellows are responsible for preparing class presentations and lecture material for about 33 percent of the lecture time. Pathology teaching sessions are held in both the NIH–CC and NNMC in addition to multidisciplinary clinical conferences, walk rounds, and a variety of disease-specific multidisciplinary meetings. In addition, there are 10 to 15 research seminars and lectures given per day throughout the NIH; these are advertised in a weekly announcement and are regularly open to the entire NIH community.

Keywords:
cancer immunotherapy
clinical trials
vaccine

Research: Dr. Hamilton's specific areas of clinical research are clinical trials in tumor vaccines targeting PSA and CEA and in gastrointestinal cancer phase I studies. The tumor vaccines focus on vaccinia and fowlpox vectored antigens with expansion of the immunologic response via cytokines and costimulatory molecules.

Our collaborators are Phillip Arlen, Jay Berzofsky, William Dahut, Jean Grem, Samir Khleif, and Jeffrey Schlom, NIH.

Recent Publications:

Grem JL, et al. *J Clin Oncol* 2000;18:3952–63.

Pai-Scherf LH, et al. *Clin Cancer Res* 2000;6:1720–30.

Simon RM, et al. *J Clin Oncol* 2001;19:1848–54.

Grem JL, et al. *Cancer Chemother Pharmacol* 2001;47:117–25.

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Biography: *A graduate of Marquette University in Milwaukee, WI, Dr. Zujewski received her M.D. at the University of Minnesota in 1988 and completed her internal medicine residency at the University of Washington Affiliated Hospitals in 1991. A medical oncology fellowship brought her to the University of Washington and the Fred Hutchinson Cancer Research Center from 1991 to 1992; after that, she joined the NCI as a senior staff fellow where, in 1997, she became a senior medical oncologist.*

Medical Oncology Clinical Research Unit
Clinical and Translational Trials in Breast Cancer Prevention and Treatment

Keywords:
breast cancer
chemoprevention
clinical trial

Research: Dr. Zujewski is spearheading the breast cancer prevention initiative within the Medical Oncology Clinical Research Unit (MOCRU) of the NCI. She has conducted pilot chemoprevention trials in women at high risk of developing breast cancer. These trials are designed to evaluate the toxicities and pharmacokinetics of potential breast cancer prevention agents with the ultimate goal of selecting agents suitable for large-scale testing. An important component of this work involves obtaining breast tissue before and after therapy to identify candidate molecular markers that may be useful as surrogate endpoints in clinical trials. Her initial work has been with tamoxifen, which may be considered the prototype selective estrogen receptor-modulating agent (SERM), and fenretinide, a retinoid that has been effective in preventing mammary cancer in animal models. She is currently conducting a clinical trial of raloxifene, a second generation SERM, in premenopausal women to study its safety and its effect on steroid hormone levels, bone metabolism, and lipid profiles, Raloxifene may be an ideal candidate for study as a breast cancer prevention agent, having potential antiestrogenic effects in the breast and uterus while maintaining beneficial effects in the bone and lipids. Although this agent has been tested in postmenopausal women, its safety in premenopausal women has not been determined. Raloxifene may be an appropriate breast cancer prevention agent in the latter group, provided adequate safety data can be provided.

Dr. Zujewski is also interested in developing molecularly targeted therapeutics for use in the treatment of advanced breast cancer. She conducted the first clinical trial of R115777, the first farnesyl protein transferase inhibitor to enter clinical studies. This agent was designed to target the p21 ras signal transduction, a step critical for normal cell signaling. Preclinical data suggest this agent will be effective in breast cancer, a cancer that is often dependent upon growth factors that signal through the ras pathway. In conjuction with the laboratory of Dr. Edison Liu, Dr. Zujewski plans to design future trials of R115777 and other agents to incorporate molecular profiling with cDNA microarray analysis.

Dr. Zujewski is the chairperson of the Breast Cancer Think Tank steering committee. The Breast Cancer Think Tank is a paradigm for the operation of programs that cross branches and divisions within the Intramural Research Program of the NCI. She also participates in the Research Planning Group of the National Naval Medical Center's Breast Care Center.

Recent Publications:

Zujewski J, et al. *J Natl Cancer Inst* 1998;90(21):1587–9.

Zujewski J, et al. *Cancer Therapeutics* 1998;1:302–7.

Caruso RC, et al. *Arch Ophthalmol* 1998;116:759–63.

Conley B, et al. *J Clin Oncol* 2000; 91(18):275–83.

Clinical Trials:

William L. Dahut

99–C–0052: A randomized phase II trial of high-dose ketoconazole plus alendronate versus high-dose ketoconazole in patients with androgen-independent metastatic prostate cancer

00–C–0033: A randomized phase II trial of weekly docetaxel plus thalidomide versus weekly docetaxel in metastatic androgen-independent prostate cancer

00–C–0080: A blinded randomized crossover phase II study of oral thalidomide versus placebo in patients with stage DO androgen-dependent prostate cancer

Jo Anne Zujewski

84–C–0216: A multimodality treatment approach to patients with inflammatory cancer of the breast and locally advanced noninflammatory stage III breast cancer and stage IV breast cancer

93–C–0153: A prospective randomized, phase III trial of FLAC (5-fluorouracil, leucovorin, Adriamycin, Cytoxan) chemotherapy with GM–CSF (granulocyte-macrophage colony-stimulating factor) versus PIXY321 in advanced breast cancer

94–C–0056: A pilot trial of tamoxifen and 4–HPR (4–N-hydroxyphenyl retinamide) in persons at high risk for developing breast cancer

96–C–0007: Antimetabolite induction, high-dose alkylating agent consolidation, and retroviral transduction of the MDR1 gene into peripheral blood progenitor cells followed by intensification therapy with sequential paclitaxel and doxorubicin for stage IV breast cancer

96–C–0032: A pilot trial of sequential chemotherapy with antimetabolite induction, high-dose alkylating agent consolidation with peripheral blood progenitor cell support, and intensification with paclitaxel and doxorubicin for patients with high-risk breast cancer

96–C–0080: A phase I trial of tamoxifen and 9-cis-retinoic acid in breast cancer patients

98–C–0123: A phase II trial of two doses of raloxifene in premenopausal women at high risk for developing invasive breast cancer

99–C–0180: An assessment of effects of raloxifene on salivary estradiol and progesterone levels

00–C–0079: Susceptibility to breast cancer

Metabolism Branch

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Since its inception in 1956, the Metabolism Branch has been an exemplar of translational research. Its investigators combine basic research, preclinical investigation, and drug development with innovative, therapeutic clinical trials that have as their scientific basis fundamental research performed in the branch. One primary interest of the branch concerns the regulation of the immune response and the definition of disorders of immunoregulation that underlie immunodeficiency and neoplastic diseases. A second interest has been in the hormonal control of normal and malignant growth. The fundamental laboratory-based studies concern:

- molecular biology of human lymphoid malignancies (Lou Staudt); characterization of gene expression patterns in human lymphomas and leukemias using the cDNA microarray lymphochip to provide a new molecular classification of leukemia and lymphoma;
- T lymphocyte recognition of antigens presented by the major histocompatibility complex (MHC)-encoded molecules and its applications to vaccines for AIDS and cancer (Jay Berzofsky);
- identification and purification of novel interleukin molecules including IL–15, codiscovered by Dr. Waldmann's group, as well as the molecular analysis of the multichain IL–2/IL–15 receptors expressed on normal and malignant lymphocytes (Thomas Waldmann);
- definition of the molecular defects in patients with primary immunodeficiency diseases (David Nelson);
- identification of novel modulators of apoptotic cell death pathways and evaluation of their therapeutic potential (Colin Duckett);
- analysis of the mechanisms of action of insulin-like growth factors that, in addition to being primary stimulators of normal growth, also play a role in preventing apoptosis and promoting the growth of certain cancer cells (Peter Nissley); and
- development of vectors for gene therapy of cancer (John Morris).

A second focus of the branch is to translate fundamental insights into new approaches for prevention, diagnosis, and treatment of human cancer and immunodeficiency diseases. Studies on T lymphocyte recognition of antigens presented by MHC-encoded molecules provide the molecular basis for trials evaluating cancer vaccines aimed at inducing cytotoxic lymphocytes reactive with mutant oncogene products common in human cancers, and developing peptide vaccines for HIV (Jay Berzofsky). An additional clinical emphasis has as its scientific basis the demonstration that IL–2 receptors are constitutively

expressed by leukemic cells but not by normal cells. This difference in receptor expression is being exploited in clinical trials employing humanized anti-IL–2R antibodies armed with α- and β-emitting radionuclides to treat patients with leukemia (John Morris, John Janik, David Nelson, and Thomas Waldmann).

Metabolism Branch Staff

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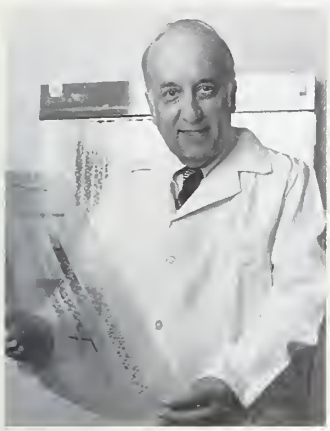

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Biography: *Dr. Waldmann received his M.D. from Harvard Medical School. He joined the NCI in 1956 and has been chief of the Metabolism Branch since 1973. He studies the IL–2/IL–2 receptor system in growth of normal and neoplastic cells. He codiscovered IL–15 and introduced various forms of IL–2/IL–15R-directed therapy for leukemia. His honors include the Ehrlich medal, Stratton, Lila Gruber, Simon Shubitz, Milken, Artois-Baillet Latour, Bristol-Myers Squibb prizes, and election to the National Academy of Sciences, American Academy of Arts and Sciences, and Institute of Medicine, NAS.*

Metabolism Branch
The IL–2/IL–15 Receptor System: A Target for Cancer Therapy

Keywords:
acute (adult) T cell lymphoma/leukemia
animal models
breast cancer
cancer immunotherapy
cell signaling
cellular immunity
clinical trial
cytokines
gene therapy
immunotherapy
interleukin 2
leukemia
monoclonal antibodies
null mice
radioimmunotherapy
retroviruses
T lymphocytes
target antigens

Research: Our work on basic and clinical immunology focuses on the regulation of the human immune response and how its dysregulation can lead to autoimmune, immunodeficiency, and malignant disorders. We apply insights gained in fundamental research to the development of new approaches to the treatment of patients. Our recent studies focus on the critical role played by the receptor for interleukin 2 (IL–2) on the growth and differentiation of normal and neoplastic T cells. Resting cells do not express high affinity IL–2 receptors (IL–2R), but receptors are rapidly expressed on T cells after activation. We defined two of the IL–2R subunits, IL–2Rα and IL–2Rβ, that together with IL–2Rγ are participants in the high affinity form of the receptor. As part of our study of HTLV–1-associated adult T cell leukemia (ATL), we codiscovered the cytokine IL–15 that stimulates T cell proliferation and is essential to NK cell development. We demonstrated that although IL–2 and IL–15 share two receptor subunits and many functions, they provide distinct contributions to adaptive immune responses. IL–2 is pivotally involved in activation-induced cell death (AICD) to achieve tolerance to self. In contrast, IL–15 inhibits this process. IL–15 stimulates the development and maintenance of $CD8^+$ memory phenotype T cells. IL–2 inhibits their survival. In light of their opposing effects on AICD and memory cells, IL–15 may be superior to IL–2 in the treatment of cancer and as a component of vaccines. IL–15 protein is posttranscriptionally regulated by multiple controlling elements that impede translation including 13 upstream AUGs of the 5′ UTR, 2 unusual signal peptides, and the C terminus of the mature protein. The two signal peptide isoforms and alternative processing of one isoform direct the intracellular trafficking of IL–15 into the nucleus, cytoplasm, and secretory ER pathways. The complex intracellular trafficking patterns of IL–15 with its impediments to translocation added to the impediments to translation may be required due to the potency of IL–15 as an inflammatory cytokine. In terms of a more positive role, intracellular infection may relieve the burdens on translation and translocation to yield effective IL–15 expression and secretion.

In T and NK cells, the IL–15 receptor includes IL–2/15Rβ and γ_c that are shared with IL–2, and an IL–15-specific receptor subunit, IL–15Rα. Mast cells respond to IL–15 with a receptor system that does not share elements with the IL–2 receptor but uses a novel IL–15RX subunit. In mast cells, IL–15 signaling involves JAK2/STAT5 activation rather than the JAK1/JAK3 and STAT5/STAT3 system used in activated T cells.

We have demonstrated a role for IL–15, IL–2, and their receptors in HTLV–1-associated ATL and tropical spastic paraparesis (TSP). Transactivation of IL–2, IL–2R, IL–15, and IL–15Rα by HTLV–1 tax protein supports a role for these cytokines in the pathogenesis of HTLV–1-associated diseases. Spontaneous proliferation of T cells ex vivo in TSP and the persistence of antigen-specific CD8 T cells were abrogated by the simultaneous addition of antibodies to IL–2 and IL–15 receptors, supporting the view that there are autocrine loops involving these cytokines and their receptors in this disease.

One of our most crucial contributions was the recognition that the IL–2R represents an extraordinarily useful therapeutic target. The scientific basis for this approach is that resting cells do not express IL–2Rα whereas this receptor subunit is abundantly expressed by a variety of malignant cells including the leukemic cells in adult T cell leukemia. The FDA approved our humanized anti-Tac (Zenapax) for use in humans to prevent acute kidney transplant rejection. Hu-anti-Tac therapy led to reduction in HTLV–1 proviral load and spontaneous lymphoproliferation in TSP patients and provided effective treatment for intermediate and posterior uveitis. In a clinical trial involving ^{90}Y-anti-Tac (anti-IL–2Rα) therapy for patients with HTLV–1-associated ATL, we observed a partial or complete remission in over 50 percent of patients. New agents under active development include humanized antibodies directed to cytokine receptors shared by IL–2 and IL–15 (e.g., IL–2/15Rβ) antibodies as well as these antibodies armed with α-emitting radionuclides (^{212}Bi, ^{213}Bi, ^{211}At), geldanamycin linked to an anti-HER–2 mAb, as well as small molecular agents that inhibit the tyrosine kinase JAK–3, which is required for IL–2, IL–4, IL–7, IL–9, IL–21, and IL–15 action. Thus, new insights concerning receptors and signaling pathways used by malignant cells taken in conjunction with the ability to produce humanized antibodies armed with radionuclides are providing a novel perspective for the treatment of select neoplastic diseases.

Among our collaborators are Martin Brechbiel, Jorge Carrasquillo, William Eckelman, Steven Jacobson, Robert Kreitman, Henry McFarland, Roland Martin, Robert Nussenblatt, and Ira Pastan, NIH; Sandor Damjanovich, University of Debrecen, Hungary; and Gillian Wharfe, University of the West Indies.

Recent Publications:

Waldmann TA, et al. *Annu Rev Immunol* 1999;17:19–49.

Waldmann TA, et al. *Immunity* 2001;14:105–10.

Mandler R, et al. *J Natl Cancer Inst* 2000;19:1573–81.

Phillips KE, et al. *Cancer Res* 2000;60:6977–84.

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Biography: *Dr. Morris received his M.D. from Upstate Medical Center in Syracuse, NY, then completed a residency in internal medicine at Mount Sinai Hospital in New York City, where he served as an assistant professor of medicine and neoplastic diseases. Board-certified in internal medicine and medical oncology, Dr. Morris worked for the Clinical Gene Therapy Branch of the National Human Genome Research Institute from 1995 to 1999, when he joined the NCI's Metabolism Branch's Clinical Program.*

Metabolism Branch
Clinical Trials Evaluating Cytokine-Directed Therapy, Antitumor Vaccines, and Gene Therapy of Cancer

Keywords:
acute (adult) T cell lymphoma/leukemia
adenovirus
anaplastic large cell lymphoma
cancer immunotherapy
CD30
clinical trial
cytokines
dendritic cells
gene therapy
immunotherapy
interleukin 2
leukemia
lymphokines

Continued on page 182

Research: The goals of the Metabolism Branch Clinical Trials Team (CTT) are to effectively translate the preclinical laboratory findings of the branch into human clinical trials to determine the safety and therapeutic efficacy of these approaches. The CTT is involved in developing cytokine/growth factor receptor-directed therapies for treatment of cancer, particularly interleukin 2 receptor-directed therapy. The high-affinity interleukin 2 receptor (IL–2Rα, Tac, CD25) is highly expressed on the malignant T cells of certain lymphoproliferative disorders, but not on the normal resting cells of these patients. In our initial study, 6 of 19 patients receiving murine anti-Tac for Tac-expressing adult T cell leukemia (ATL) had a partial or complete remission. In an attempt to improve on the efficacy of this approach, murine anti-Tac was conjugated to the β-emitting isotope yttrium-90 (^{90}Y). Nine of 16 evaluable patients treated with the radiolabeled antibody achieved a response; however, this approach was limited by the development of human antimouse antibodies (HAMA) to murine anti-Tac. In an attempt to prevent an antibody response, a humanized form of anti-Tac (Hu-anti-Tac) was generated. An ongoing Phase I/II study determined the maximum tolerated dose of

Keywords (continued):
lymphoma
monoclonal antibodies
radioimmunotherapy
retroviruses
T lymphocytes
target antigens

^{90}Y-labeled Hu-anti-Tac to be 25 mCi and tumor responses were seen in almost half of the patients treated. Studies in the laboratories of the branch showing the in vivo efficacy of unmodified Hu-anti-Tac in the MET–1 mouse model of ATL have been translated into a phase I/II clinical trial of unmodified Hu-anti-Tac for patients with ATL (Protocol 00–C–0030). This trial rapidly accrued patients and completed phase I. Preliminary results show a dose-dependent ability of Hu-anti-Tac to saturate surface IL2Rα on ATL cells in the blood and lymph nodes with little toxicity.

Another antibody, Mikβ–1, directed against the common β-chain (CD122) of the interleukins 2/15 receptor, has been developed. T cell large granular cell leukemia (LGL) expresses the β-chain of the receptor in higher numbers than normal cells. The binding of IL–2 or IL–15 to this receptor is necessary for the function and survival of these cells in vitro. The CTT is investigating the usefulness of murine Mikβ–1 for treatment of patients with clonal T cell LGL with hemocytopenias.

New cytokine/receptor-directed agents under development by the CTT include humanized antibodies directed at unique and shared cytokine receptors (e.g., humanized Mikβ–1); receptor-directed antibodies armed with α-emitting radionuclides (^{212}Bi, ^{213}Bi, ^{211}At); anti-CD2 (MEDI 507), anti-CD30 (HeFi-1), and anti-CD52 (CAMPATH–1H) monoclonal antibodies for the treatment of T cell lymphomas, anaplastic large cell lymphoma, or relapsed ATL respectively; geldanamycin conjugated anti-HER–2/*neu* monoclonal antibody for treatment of breast cancer, as well as molecular targeted small molecules that inhibit the tyrosine kinase JAK–3 required for the action of interleukins 2, 4, 7, 9, and 15; and epidermal growth factor receptor-signaling inhibitors as a treatment for advanced head and neck cancer. Also under development is a systemic three-step pretargeted monoclonal antibody system using a streptavidin-linked monoclonal antibody directed against the B cell marker CD20 expressed on B cell lymphomas; this is followed by treatment 24 hrs later with a biotin conjugated "clearing agent" that removes unbound streptavidin antibody from the circulation. Next, a biotinylated radionuclide is administered that binds to the streptavidin-linked monoclonal antibody, specifically targeting the radioactivity to the lymphoma.

Another major clinical focus is the development and testing of mutant peptide-pulsed dendritic cells as a vaccine for cancer. Our current trial is open to patients with advanced colorectal cancer who are HLA–2.1 positive and whose tumor expresses a mutated *ras* gene. Autologous monocytes obtained by leukapheresis are matured ex vivo using IL–4, GM–CSF, and CD 40 ligand into antigen-presenting dendritic cells. Patients are reinfused with autologous dendritic cells that have been pulsed with a synthetic peptide matching the specific mutation found in their tumor. Patients are then monitored for toxicity, evidence of immune responses, tumor response, and survival. A second protocol using a peptide-pulsed dendritic cell vaccination against tumors with mutations of the p53 gene is available for patients with stage III nonsmall cell lung cancer.

Employing adenoviral-mediated gene transfer of the *herpes simplex* virus-thymidine kinase (HSV-*tk*) gene, we used animal models to develop a clinical trial of escalating dose ganciclovir and HSV-*tk* gene transfer for the treatment

of cutaneous melanoma lesions. Recently we developed and tested in preclinical models an HSV-*tk*-expressing replicating adenovirus (Ad.OW34) in preparation for a clinical trial of this oncolytic vector for patients with advanced carcinoma of the head and neck. In addition to the advancement of gene therapy, specific monoclonal antibody projects and cancer vaccines, the CTT maintains a research interest in the natural history, epidemiology, complications, and treatment of adult T cell leukemia/lymphoma and other HTLV–1-associated diseases. Research in these areas has led to publications on the infectious complications and HLA frequencies in ATL.

Correlation of tumor antibody saturation and clinical efficacy represents an important area of investigation in understanding the antitumor activity of monoclonal antibodies. Our ongoing study of unlabeled anti-Tac has shown differential tumor saturation in skin, lymph nodes, and peripheral blood that is dependent on the dose of infused antibody. Two new clinical trials will examine the clinical activity and tumor saturation of Campath-1H, which binds to CD52, and HeFi-1, which binds to CD30. Our trial of HeFi-1 will also evaluate in vitro apoptosis in tumor specimens from patients to correlate with tumor saturation and clinical efficacy.

Collaborating with us are Riad Agbaria, Ben-Gurion University, Negev, Israel; Martin Brechbiel, Fabio Candotti, Jorge Carrasquillo, Charles Corter, Tom Fleischer, Laurie Herscher, Steven Jacobson, Elaine Jaffe, Eric Kass, Robert Kreitman, Henry McFarland, Richard Morgan, Robert Nussenblatt, Ira Pastan, W. Jay Ramsey, Elizabeth Read, Carter van Waes, Jon Wigginton, and Wyndham Wilson, NIH; Barrie Hanchard and Gillian Wharfe, University of the West Indies; and Oliver Wildner, Humbolt University, Berlin, Germany.

Recent Publications:

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Janik J, et al. *Clin Immunol* 1999;93:209–21.

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Metabolism Branch
T Lymphocyte Recognition of Antigens and Applications to Vaccines for AIDS and Cancer

Keywords:
cancer immunotherapy
cellular immunity
HIV
IL–13
mucosal immunity
NKT cells
oncogenes
sarcoma
T lymphocytes
tumor immunosurveillance
vaccine design

Research: We are studying mechanisms by which T cells recognize antigens presented by major histocompatibility complex (MHC)-encoded molecules (such as HLA in humans) and factors determining which structures are likely to be recognized, and applying these principles to the design of synthetic vaccines for AIDS, cancer, and viruses that cause cancer.

At a basic level, we studied the molecular basis for antigenic peptide interaction with MHC molecules and T cell receptors (TCR) by examining the role of different amino acids of the antigen. We identified the first sequence pattern associated with T cell epitopes and defined amino acid residues involved in binding to MHC molecules. Replacement of certain residues enhanced antigenic potency of peptides, producing more potent vaccines described below. To understand the role of TCR affinity in function, we selectively grew cytotoxic T lymphocyte (CTL) lines of high and low avidity for the same HIV peptide-MHC complex. High avidity CTLs were much more effective at clearing virus (HIV-recombinant vaccinia) from immunodeficient mice than low avidity CTL, implying that quality of CTL is as important as quantity for clearing virus and probably cancer cells. We showed the mechanism involved killing cells earlier in infection when less virus was produced, and lysing cells more rapidly. However, the high avidity CTLs were also more susceptible to tolerance induction. High antigen densities caused apoptotic death of high but not low avidity CTL, mediated by TNF and the TNF–receptor II and decreasing Bcl-2, explaining how virus can delete CTL effective for viral clearance. We also developed ways to steer immune responses toward desired phenotypes with combinations of cytokines in adjuvant with antigen for immunization. GM–CSF was overall the most effective cytokine for enhancing most responses. IL–12 was synergistic both with GM–CSF and with TNF in enhancing CTL activity, and the combination of all three was synergistic for protection against infection with recombinant vaccinia expressing gp160, through an interferon-γ dependent mechanism and also increased antigen presentation.

For HIV vaccine design, we identified broadly recognized helper and CTL epitopes of HIV envelope and reverse transcriptase. Multideterminant helper regions of the HIV envelope were determined to induce help in mice and humans of multiple MHC types, to overcome the problem of MHC polymorphism. These "cluster" helper determinants were combined with a CTL and neutralizing antibody epitope to produce a vaccine which elicited neutralizing antibodies, T-helpers, and CTL. This prototype vaccine is in clinical trials, and appears able to elicit all three responses in humans. Second generation vaccines are being developed by "epitope enhancement," altering sequences to enhance binding to MHC molecules without altering TCR recognition. Proof of principle has been obtained in mice, enhancing both a helper epitope of HIV and a CTL epitope of HCV presented by a human class I HLA molecule. Helper epitope enhancement also skewed the response to Th1 by a mechanism involving upregulation of CD40L on T helpers and resulting upregulation of IL–12 on dendritic cells.

Because much transmission of HIV is mucosal, we immunized mice intrarectally with a peptide vaccine and induced mucosal CTL and protection against mucosal transmission of a recombinant vaccinia virus expressing HIV envelope. We showed for the first time that CTL must be present in the mucosa itself to prevent mucosal viral transmission. Intrarectal IL–12 enhances protection and synergizes with GM–CSF. Finding that systemic immunization left the mucosal system naive, we immunized mucosally to circumvent the widespread problem that prior poxvirus immunity diminishes efficacy of vaccinia vectors, opening new possibilities for vaccines. We translated the mucosal vaccine studies to primates, showing mucosal was more effective than systemic immunization of Rhesus macaques to reduce plasma viral load, by reducing the major reservoir in the gut that seeds the bloodstream. This information is critical for designing an AIDS vaccine.

For viruses causing cancer, we identified HCV core and NS3 epitopes presented by HLA–A2.1 to human CTL, and modified the sequence of an HCV core epitope to enhance immunogenicity. We discovered a negative regulatory mechanism that dampens CTL-mediated tumor immunosurveillance, involving IL–13 made by NKT cells and acting through the IL–4R–STAT6 signaling pathway. Blockade of IL–13 prevented tumor recurrence. This could be applied to human tumor immunotherapy. We also targeted products of mutant and translocated genes in tumor cells as vaccines for cancer immunotherapy. Peptides corresponding to mutations in p53, coated on dendritic cells, elicited CTL that killed tumor cells expressing the mutation and suppressed established tumors in animals. Mutant ras peptides were found to bind to HLA–A2.1, and these and mutant p53 peptides are being studied in clinical trials to treat patients with cancers expressing these mutations. Fusion proteins produced by translocations in sarcomas were also found to create neoantigenic determinants. Peptides corresponding to these bound human HLA molecules and induced human CTL that killed human tumors expressing the fusion protein. A clinical trial of these peptides for immunotherapy of pediatric sarcomas is ongoing.

We have collaborated with Martha Alexander-Miller, Wake Forest University; David Carbone, Vanderbilt University; Mary Carrington, Charles Carter, Genoveffa Franchini, Jay Greenblatt, Ronald Gress, Michael Hamilton, Lee Helman, Pierre Henkart, Allan Hildesheim, Jerry Keith, Brian Kelsall, Morris Kelsey, Samir Khleif, Steven Leppla, Lance Liotta, Crystal Mackall, David Margulies, Franco Marincola, Louis Miller, Bernard Moss, Nancy Noben-Trauth, William Paul, Elizabeth Read, Barbara Rehermann, Mark Schiffman, Gene Shearer, Alan Sher, Warren Strober, and Robert Yarchoan, NIH; John Clements, Tulane University; Anne De Groot, Brown University; Charles DeLisi, Boston University; Debra Donaldson, Genetics Institute; Stephen Feinstone, FDA; Michael Good, Queensland Institute of Medical Research, Australia; Douglas Hanahan, University of California at San Francisco; Stephen Hoffman, NMRI; Anne Hosmalin, Hôpital Pitié-Salpetrière, Paris; Alan Landay, Rush Medical College; Hanah Margalit, Hebrew University, Jerusalem; John D. Minna, University of Texas Southwestern Medical Center; Isabella Quakyi, Georgetown University; Matthias Schnell, Thomas Jefferson University; Mutsunori Shirai, Yamaguchi Medical School; Hidemi Takahashi, Nippon Medical School, Tokyo; and Elaine Thomas, Immunex.

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Derby MA, et al. *J Immunol* 2001;166:1690–7.

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Biography: *Dr. Duckett received his Ph.D. from the University of London, England, in 1993. He carried out postdoctoral work at the University of Michigan, where he worked on the transcriptional regulation of HIV, and at the University of Chicago, where he codiscovered the IAP family of signal transduction intermediates and apoptosis regulators. He established his laboratory in the Metabolism Branch at the NCI in 1997, and currently studies the regulation of apoptosis by the tumor necrosis factor receptor superfamily.*

Metabolism Branch

Identification of Novel Modulators of the Apoptotic Cell Death Pathway and Evaluation of Their Therapeutic Potential for the Treatment of Cancer

Keywords:
apoptosis
CD30
IAP
lymphoma
NFκB
TGFß

Research: Apoptosis is a genetically determined, biochemically ordered process in which cells are induced to initiate a cellular suicide program in response to physiologic signals, cellular damage, or virus infection. In normal cells the apoptotic pathway is very tightly controlled, but deregulation of this cell death cascade has been found in many human diseases including cancer, AIDS, neurodegenerative diseases, and autoimmune disorders. The primary focus of my laboratory is the study of a family of genes known as the *iaps* (inhibitors of apoptosis) that suppress apoptotic cell death and that have been implicated in a number of human diseases. We and others have identified cellular *iaps* in species ranging from yeast to human. Much of our research is concentrated on one member of the human IAP family that we and others identified, termed X-linked IAP (XIAP; also called IAP-like protein-1/ILP–1).

We have recently identified two novel XIAP-related genes, which we have designated ILP–2 and ILP–3. ILP–2 is a potent inhibitor of the "intrinsic" or mitochondrial cell death pathway, characterized by cellular insults such as growth factor withdrawal and chemotherapy-induced death. However, in contrast to XIAP, we have found that ILP–2 does not inhibit the "extrinsic" death receptor pathway exemplified by Fas or tumor necrosis factor-mediated death. Our current and future direction is to understand the molecular basis of these effects. We have established an in vitro system which recapitulates the major biochemical events that occur in apoptosis, and in which the activities of IAPs can be evaluated. In addition, we have cloned a novel IAP-interacting protein, VIAF, that functions to regulate the activities of the IAPs.

We have also found that XIAP can function as a cofactor in the regulation of gene expression by transforming growth factor-β (TGFβ). XIAP associates with several members of the type 1 class of the TGFβ receptor superfamily, and potentiates TFGβ-induced signaling. While XIAP-mediated signaling was found to require the TGFβ signaling intermediate Smad4, the ability of XIAP

to suppress apoptosis was found to be Smad4-independent. These data implicate a role for XIAP in TGFβ-mediated signaling that is distinct from its anti-apoptotic functions.

A second project being undertaken in our laboratory involves the analysis of the signaling pathways utilized by CD30, a member of the tumor necrosis factor receptor superfamily whose expression is deregulated in numerous malignancies including Hodgkin's disease and anaplastic large cell lymphoma. Activation of CD30 by its cognate ligand or by crosslinking with agonistic antibodies can induce either proliferation or apoptotic cell death, depending upon the cellular context. We have examined the signal transduction pathways utilized by CD30. We have found that activation of CD30 leads to the proteolytic degradation of the intracellular signaling intermediate TRAF2, and in anaplastic large cell lymphoma cells this is thought to sensitize to proapoptotic stimuli. Our data suggest that CD30-directed targeting strategies may have great potential in the treatment of CD30-positive malignancies.

Among our collaborators are Larry Boise, University of Miami; Howard Fearnhead, David Nelson, Anita Roberts, and Richard Youle, NIH; Marie Hardwick, Johns Hopkins; Robert Korneluk, University of Ottawa; William Murphy, SAIC–FCRDC; Luigi Notarangelo, University of Brescia, Italy; Neil Perkins, University of Dundee; and Paolo Vezzoni, ITBN, Milan, Italy.

Recent Publications:

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Richter BWM, et al. *Mol Cell Biol* 2001; in press.

Reffey SB, et al. *J Biol Chem* 2001; in press.

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Biography: *Dr. Morris received his M.D. from Upstate Medical Center in Syracuse, NY, then completed a residency in internal medicine at Mount Sinai Hospital in New York City, where he served as an assistant professor of medicine and neoplastic diseases. Board-certified in internal medicine and medical oncology, Dr. Morris worked for the Clinical Gene Therapy Branch of the National Human Genome Research Institute from 1995 to 1999, when he joined the NCI's Metabolism Branch's Clinical Program.*

Metabolism Branch
Cancer Gene Therapy Section

Keywords:
adenovirus
dendritic cells
gene therapy
oncolysis
tumor vaccines

Research: The goals of the Cancer Gene Therapy Section are to develop new and innovative gene transfer approaches for the treatment of cancer. Proposed strategies for "gene therapy" of cancer include replacement of defective tumor suppressor genes, antisense oligonucleotides to block the action of dominant oncogenes, expression of immune stimulatory molecules and cytokines, and the transfer of genes encoding enzymes that activate nontoxic drugs into cytotoxic agents locally in tumors. Despite initial optimism, clinical trials have reported few responses. The reason for this is the low efficiency of in vivo gene transfer achieved by current vectors. Early safety considerations required the use of viral gene transfer vectors that were replication-defective and therefore unable to infect or transduce tumor cells beyond the initial site of vector inoculation. Also contributory is that most current approaches to gene therapy of cancer represent at best local treatments. Our research efforts have focused on two areas: (1) improvement of viral gene transfer vectors, particularly the development of replication (oncolytic) adenoviral vectors, and (2) the use of adenoviral vector-mediated gene transfer for antitumor vaccination.

We constructed and tested a series of replicating adenoviral vectors expressing the herpes simplex virus-thymidine kinase (HSV-*tk*) gene. Mammalian cells expressing HSV-*tk* are killed when they are exposed to the ordinarily nontoxic antiviral drug ganciclovir (GCV). These vectors are lytic and exhibit greater in vivo antitumor activity compared to nonreplicating HSV-*tk* expressing adenoviral vectors in several different athymic nude mouse human tumor models. We compared two of the vectors, one lacking the adenovirus E1b 55 kD gene and one in which this gene was reinserted. The E1b 55 kD-intact adenovirus showed greater cytolytic activity. The E1b 55 kD-deleted vector required delayed administration of GCV for optimal tumor response; however, the E1b 55 kD-intact adenovirus had more robust replication and its efficacy was not enhanced by use of the prodrug. The E1b 55 kD-intact adenoviral vector induced more tumor responses and a greater median survival than the E1b 55 kD-deleted vector. Subcutaneous administration of the E1b 55 kD-intact adenovirus to cotton rats, a semipermissive species,

caused no significant toxicity. Further safety studies are planned in anticipation of a clinical trial for patients with advanced head and neck cancer. Additionally, we are studying ways of restricting the replication of these vectors by placing the E1-region genes' expression under the control of tumor-specific promoters.

In a second approach, we are attempting to enhance antitumor immune responses using adenoviral-mediated transfer of tumor antigen genes such as mutant *ras* or HER-2/*neu* into murine bone marrow-derived dendritic cells (DCs) and transferring these cells to mice bearing tumors that express these antigens. DCs play a pivotal role in the immune response including generation of antitumor immunity. The inability of the immune system to eradicate tumors may be due to ineffective tumor antigen presentation by DCs as well as attenuation of DC costimulatory signals. Clinical trials using autologous DCs pulsed with peptide sequences of tumor-specific antigens are undergoing evaluation in the Metabolism Branch as a cancer vaccine strategy. A limitation of this approach is the requirement to have prior knowledge of the binding affinity of different antigen-derived peptides for various MHC molecules. Using recombinant adenoviral vectors to introduce tumor antigens into DCs offers a potential alternative. An advantage of this approach is that DCs can process the intracellular expressed antigens and present an optimal sequence regardless of specific MHC. We studied the efficiency of adenoviral-mediated gene transfer into murine bone marrow-derived DCs and the phenotypic changes that it induced. Other studies are in progress examining the ability of this approach to induce specific antitumor immunity.

Recent Publications:

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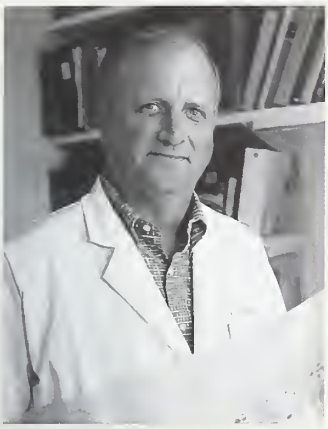

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Biography: *Dr. Nelson received his M.D. from the University of Kansas and trained in pediatrics at the University of Minnesota. He is board certified in pediatrics and allergy and immunology. His research interests are in human immunodeficiency disorders associated with cancer. He is the recipient of numerous awards including an honorary doctor of science from Washburn University, and the U.S. Public Health Service Outstanding Service and the Meritorious Service Medals. Dr. Nelson has been chief of the Immunophysiology Section since 1987.*

Metabolism Branch
Investigations of the Human Immune Response

Keywords:
cancer prone syndromes
immunodeficiency

Research:

Background, Goals and Structure
The Immunophysiology Section conducts clinical translational research on immunodeficiency states associated with cancer including the primary immunodeficiency diseases. The primary goal of this section is to generate new understandings of the human immune response and to use this knowledge to produce new approaches, which are then taken to the clinic for translation into novel diagnostics and/or therapeutics.

Project: Studies of Selected Primary Immunodeficiency Disorders
The goal of this project is to identify and characterize the genetic basis for certain of the primary immunodeficiency disorders, which are associated with an increased incidence of cancer. While these disorders are rare, their study has been extremely instructive in defining previously unsuspected elements of importance in immune function. Patients with CD40 ligand (CD40L) deficiency (X-linked hyper-IgM syndrome, XHIM) fail to produce certain cytokines in vitro and this can be reversed by recombinant CD40L. A new clinical trial has been initiated for the treatment of XHIM patients with recombinant CD40L in vivo. When stimulated with a new recombinant molecule, B lymphocyte stimulator (BLyS) in vitro, patients with common variable immune deficiency (CVID) make antibody and a clinical trial is being designed to use BLyS to treat CVID and IgA deficient patients in vivo. We are also examining a possible genetic basis for CVID by gene expression profiling using cDNA microarrays.

Other recent studies have focused on the Wiskott-Aldrich syndrome (WAS) and the responsible gene (WASP). A hindrance in the study of WASP was the lack of a cellular phenotype for WAS cells. We identified an abnormal phenotype in B cell lines from WAS patients showing abnormal F-actin expression, and corrected this phenotype by retroviral WASP gene transfer in vitro. In cultured normal human macrophages, WASP is associated with the small GTPase, Cdc42Hs, and F-actin in structures termed "podosomes."

To analyze WASP in podosomes, we studied macrophages from WAS patients and found they lacked podosomes. When WAS macrophages were subjected to a chemoattractant gradient, they failed to polarize and also failed to express normal cell surface projections termed "filopodia." Thus we defined a phenotype in two different WAS cell types that should be useful in the study of WASP function. Moreover, spontaneous genetic reversions in two WAS patients also corrected defective cellular phenotypes in both patients. As patient mutations in WASP failed to interfere with the interaction of WASP with any of its previously identified protein partners, we hypothesized that there might be other important protein partners. We searched for interacting proteins using the yeast two-hybrid system. WAS patient mutations in one of our gene isolates, HSPRPL2/WIP, impaired the WASP–WIP interaction, providing the first such evidence to date. We also identified an interacting protein CIP4, which bound both WASP and Cdc42Hs and also bound microtubules—perhaps linking the actin and the microtubule cytoskeletal systems. In addition we are currently pursuing studies on an international consortium of WAS patients to see if polymorphisms in host defense molecules will predict the clinical course of the WAS including which patients ultimately develop cancer. These studies may be useful in the clinical management of WAS patients.

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Tian L, et al. *J Biol Chem* 2000;275:7854–61.

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Metabolism Branch
Mechanism of Action of Insulin-Like Growth Factors

Keywords:
apoptosis
cell proliferation
IGF–I
IGF–I receptor
IGF–II
signaling

Research: Insulin-like growth factors (IGF–I and IGF–II) are important for normal fetal and postnatal growth of the whole animal. In addition, there is increasing evidence that IGFs play an important role in cancer. For example, in recent prospective studies, a high circulating level of IGF–I has been shown to be a risk factor for the development of breast cancer and prostate cancer. Moreover, overexpression of IGF–II is a feature of a number of human cancer cell lines and there is evidence that the IGF–II/mannose 6-phosphate receptor, which is important in a degradative pathway for IGF–II, is a tumor suppressor gene. Mutation of the IGF–II/mannose 6-phosphate receptor gene resulting in loss of function would result in increased concentration of extracellular IGF–II. IGF–I and IGF–II promote tumor cell growth by stimulating proliferation and/or inhibiting programmed cell death (apoptosis). Both of these actions are mediated by the IGF–I receptor. Therefore, we are focusing on understanding how the IGF–I receptor signals proliferation and inhibits apoptosis with the long-term goal of developing agents that block these pathways.

The IGF–I receptor is a member of the tyrosine kinase family of growth factor receptors. To understand the mechanism whereby the IGF–I receptor signals biologic responses to IGF–I and IGF–II, it is necessary to identify the intracellular proteins which interact directly with the receptor. We have used the yeast two-hybrid system to identify proteins which interact with the cytoplasmic domain of the IGF–I receptor. Proteins which bind to the autophosphorylated (active) receptor are candidates for being downstream effector molecules in IGF–I receptor signaling pathways. In this powerful yeast genetic system, cDNAs for potential binding partners are introduced into vectors which encode either a DNA binding module or the activation module of a transcription factor. Yeast are then cotransformed with the two vectors. If the test proteins interact, the function of the transcription factor is restored, resulting in the transcription of reporter genes. Using the yeast two-hybrid system, we showed that the adaptor protein Shc binds directly to the IGF–I receptor. By testing deletion mutants of Shc, we showed that the amino terminal portion of Shc rather than the SH2 domain of Shc accounted for the interaction with a phosphotyrosine-containing motif on the receptor.

We have also used the yeast two-hybrid system to identify new binding partners for the cytoplasmic domain of the IGF–I receptor. In this application, the cytoplasmic domain of the IGF–I receptor is inserted into the DNA binding vector and used to screen a library that has been inserted into the activation domain vector. A screen of a human fetal brain library has identified six independent clones. The library screen identified Grb10, an adaptor molecule that had previously been shown to interact weakly with the EGF receptor. The human homolog of p55 gamma, a member of the family of p85-related molecules which regulates PI–3 kinase, has also been identified. Two members of the 14–3–3 family of proteins have been identified in the library screen, and 14–3–3 family members have been shown to associate with a number of signaling molecules and oncogene products including Raf and middle T antigen. This is the first example of interaction of a 14–3–3 family member with a growth factor receptor. Interestingly, the two 14–3–3 isoforms do not bind the active insulin receptor and thus could potentially account in part for the differences in signaling between these two closely related receptors. In addition, the domain of the receptor that we have shown to be important for 14–3–3 binding has been implicated by others as being important for the transformation of cells accompanying IGF–I receptor overexpression. More recently, a member of the SOCS (suppressor of cytokine signaling) family, SOCS–2, has been identified in the library screen. Heretofore, SOCS proteins have been known as negative regulators of cytokine signaling. Our findings raise the possibility that SOCS proteins may also play a role in signaling by tyrosine kinase receptors.

Our collaborator is Richard Furlanetto, University of Rochester.

Recent Publications:

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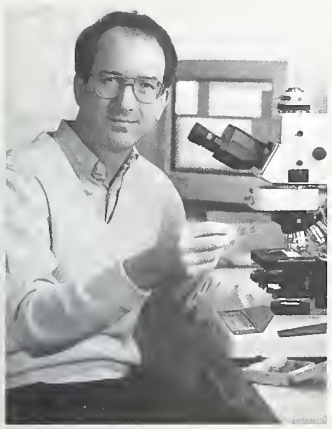

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Biography: *Dr. Staudt received his B.A. from Harvard College in 1976 and his M.D. and Ph.D. from the University of Pennsylvania in 1982. His Ph.D. thesis in the laboratory of Walter Gerhard defined somatic hypermutation as a specialized mechanism of antibody diversification. Following internal medicine training, he joined David Baltimore's laboratory where he cloned and characterized the lymphoid-restricted transcription factor Oct-2. He established his laboratory in the Metabolism Branch, NCI, in 1988, and currently studies the molecular basis of human lymphoid malignancies.*

Metabolism Branch
Molecular Biology of Human Lymphoid Malignancies

Keywords:
BCL–6
cDNA microarray
genomics
immune response
leukemia
lymphocyte
lymphoma

Research: Dr. Staudt's laboratory studies the molecular pathogenesis of human lymphoid malignancies. The laboratory has three overarching goals: (1) to establish a new molecular diagnosis of human lymphoid malignancies using gene expression profiling; (2) to elucidate the oncogenic pathways that result in malignant transformation of normal B lymphocytes; (3) to identify molecular targets for development of novel therapeutics for these cancers.

To provide a new molecular basis for the diagnosis of human lymphoid malignancies, Dr. Staudt's laboratory is exploiting DNA microarray technology to profile gene expression in these cancers on a genomic scale. In particular, the laboratory created a novel microarray, the lymphochip, that is enriched in genes that are expressed in and/or function in lymphocytes. The lymphochip is composed of over 18,500 cDNA clones, many of which were identified by high-throughput sequencing of cDNA libraries prepared from germinal center B cells and various types of lymphoma and leukemia. Lymphochips were initially used to profile gene expression in diffuse large B cell lymphoma (DLBCL), chronic lymphocytic leukemia (CLL), and follicular lymphoma as well as in a wide variety of normal lymphoid subsets. The assumption underlying this approach is that the genes that vary in expression during lymphocyte differentiation can be used to identify subsets within the lymphoid malignancies that are derived from different stages of differentiation. The central goal of these studies is to relate gene expression to clinical outcome, thereby establishing useful prognostic indicators and identifying potential targets for new therapies.

DLBCL has long been enigmatic in that 40 percent of patients can be cured by combination chemotherapy whereas the remainder succumb to this disease. By gene expression profiling, the laboratory discovered that DLBCL is actually two different diseases that are indistinguishable by current diagnostic methods. One subgroup of DLBCL, termed "germinal center B-like DLBCL," expressed genes that are hallmarks of the germinal center stage of B cell development whereas the other DLBCL subgroup, termed "activated B-like DLBCL," resembled mitogenically activated peripheral blood B cells in

gene expression. The stratification of DLBCLs by gene expression provided a molecular basis for the clinical heterogeneity in these patients: more than 75 percent of patients with germinal center B-like DLBCL were alive 5 years after treatment compared with fewer than 25 percent of patients with activated B-like DLBCL. This finding provided a clear demonstration that genomic-scale gene expression analysis can define clinically important subtypes of human cancer. A current effort in the laboratory is aimed at bringing this DLBCL subtype distinction into clinical practice.

Recently, the question has been raised whether the diagnosis of CLL comprises two distinct diseases, one in which the immunoglobulin genes of the leukemic cells are mutated (Ig-mutated CLL) and another one in which the immunoglobulin genes are unmutated (Ig-unmutated CLL). Patients with Ig-unmutated CLL have a much more aggressive disease requiring earlier treatment. Dr. Staudt's laboratory used gene expression profiling to test the hypothesis that CLL is two distinct diseases. This study revealed that all CLL patients shared a common gene expression signature demonstrating that CLL should be thought of as a single disease. Nonetheless, highly significant gene expression differences were found between Ig-mutated CLL and Ig-unmutated CLL. The CLL subtype distinction genes were enriched in genes that are modulated during signaling of B lymphocytes through the antigen receptor, raising the intriguing possibility that antigen stimulation may contribute to the pathogenesis of CLL. Furthermore, the CLL subtype distinction genes were formulated into a predictive test that distinguished Ig-mutated and Ig-unmutated CLL with 100 percent accuracy, a test which could be used clinically to guide treatment decisions for these patients.

The scope of the laboratory's gene expression profiling analysis of the lymphoid malignancies has been significantly expanded with the creation of the Lymphoma/Leukemia Molecular Profiling Project (LLMPP). This international collaborative project involves seven clinical groups that are sending patient samples and clinical data to the Staudt laboratory. The laboratory is accruing hundreds of samples, which should lead to a molecular definition of all types of lymphoid malignancies and should identify which genes affect clinical treatment response and outcome. The large sample size provided by the LLMPP will lead to the development of statistically robust diagnostic methods that will provide a new molecular definition of these malignancies that is useful clinically.

The Staudt laboratory's approach to elucidating the oncogenic mechanisms that generate lymphoid malignancies is based on a longstanding interest in the molecular control of normal lymphocyte differentiation by transcription factors. The laboratory is particularly interested in the normal development of germinal center B cells since many of the non-Hodgkin's lymphomas are derived from cells that have passed through this developmental stage. Previously, the laboratory showed that the most commonly translocated oncogene in non-Hodgkin's lymphomas, BCL–6, encodes a transcriptional repressor protein that is required for development of germinal center B cells.

To understand the molecular mechanisms by which BCL–6 controls lymphocyte differentiation and lymphomagenesis, the laboratory needed to identify which genomic target genes are repressed by BCL–6. Lymphochip

microarrays were used to profile the changes in gene expression that occurred in cells when BCL–6 function was manipulated, either by conditional overexpression or dominant-negative approaches. A particularly illuminating BCL–6 target was blimp-1, a gene that encodes a transcriptional repressor that is important for plasmacytic differentiation. Since BCL–6 expression is normally silenced in plasma cells, repression of blimp-1 by BCL–6 may control plasmacytic differentiation. Indeed, inhibition of BCL–6 function in a lymphoma cell line initiated changes indicative of plasmacytic differentiation, including decreased expression of c-myc and increased expression of the cell cycle inhibitor p27kip1. Translocations of BCL–6 in lymphomas prevent the physiological downregulation of BCL–6 expression that occurs during normal plasmacytic differentiation. These considerations led to a model in which BCL–6 translocations cause lymphomas by blocking terminal differentiation of germinal center B cells through inhibition of blimp-1 expression.

Finally, the Staudt laboratory is exploiting the insights gleaned from gene expression profiling of the lymphoid malignancies to identify new molecular targets in these diseases. The laboratory has discovered that a critical molecular difference between these two DLBCL subtypes is the activation of the NFκB pathway. Inhibition of this pathway in activated B-like DLBCL cells is lethal, thus validating NFκB as a molecular target in this subset of DLBCL patients. Future work will be aimed at understanding the molecular basis for constitutive NFκB activity in activated B-like DLBCL, with the goal of developing novel therapies that would be selective for this DLBCL subtype.

We have collaborated with James Armitage, John Chan, Tim Greiner, Jim Lynch, and Dennis Weisenburger, University of Nebraska Medical Center ; Leif Bergsagel, Cornell University Medical School; John Byrd and Michael Grever, Ohio State University School of Medicine; Kathryn Calame, Columbia College of Physicians and Surgeons; Elias Campo and Emilio Montserrat, University of Barcelona; Nicholas Chiorazzi, North Shore Hospital/Cornell; Joe Connors and Randy Gascoyne, British Columbia Cancer Center; Richard Fisher, Tom Grogan, Mike LeBlanc, and Tom Miller, SWOG; Elaine Jaffe, Michael Kuehl, Peter Lipsky, John Powell, Ed Sausville, Uli Siebenlist, Richard Simon, Bill Telford, and Wyndham Wilson, NIH; Thomas Kipps, UCSD Medical School; Stein Kvaloy and Erland Smeland, Norwegian Radium Hospital; Konrad Muller-Hermelink, University of Wuerzburg; and Anjana Rao, Harvard Medical School.

Recent Publications:

Alizadeh A, et al. *Cold Spring Harb Symp Quant Biol* 1999;64:71–8.

Alizadeh A, et al. *Nature* 2000;403:503–11.

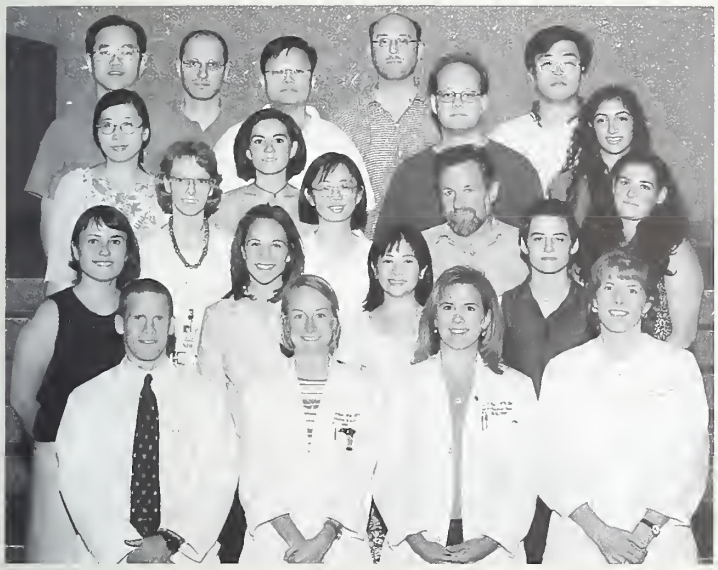
Shaffer AL, et al. *Immunity* 2000;13:199–212.

Staudt LM. *Trends Immunol* 2001;22:35–40.

Clinical Trials:

Jay A. Berzofsky	**97–C–0050:** A pilot study of tumor-specific peptide vaccination and IL–2 with or without autologous T cell transplantation in recurrent pediatric sarcomas
	97–C–0052: A pilot study of autologous T cell transplantation with vaccine-driven expansion of antitumor effectors after cytoreductive therapy in metastatic pediatric sarcomas
	99–C–0023: A phase I/II trial of vaccination with mutant ras peptide-pulsed dendritic cells in the treatment of HLA–A2.1-positive patients with colorectal cancer
	99–C–0142: A phase II trial of individualized mutant p53 peptide-pulsed cultured autologous dendritic cells in the adjuvant treatment of patients with locally advanced nonsmall cell lung cancer after standard therapy
John Morris	**98–C–0140:** A phase I study of intralesional administration of an adenovirus vector expressing the HSV–1 thymidine kinase gene (AdV.RSV–TK) in combination with escalating doses of ganciclovir in patients with cutaneous metastatic malignant melanoma. Accruing patients
	00–C–0030: A phase I/II study of the efficacy and toxicity of humanized anti-Tac (Zenapax) in the therapy of Tac-expressing adult T cell leukemia
David L. Nelson	**97–C–0143:** Investigation of the human immune response in normal subjects and patients. Accruing patients
Thomas A. Waldmann	**95–C–0054:** A phase I study of T cell large granular lymphocytic leukemia using the MiKβ–1 monoclonal antibody directed toward the IL–2R–β subunit
	96–C–0147: A phase I/II study of Tac-expressing adult T cell leukemia with yttrium-90 (^{90}Y)-radiolabeled humanized anti-Tac monoclonal antibody and calcium DTPA
	97–C–0110: A phase I/II study of Tac-expressing malignancies (other than ATL) with yttrium-90 (^{90}Y)-radiolabeled anti-Tac and calcium DTPA. Accruing patients
	99–C–0023: A phase I/II trial of vaccination with mutant Ras peptide-pulsed dendritic cells in treatment of HLA A 2.1-positive patients with colorectal cancer. Accruing patients
	00–C–0030: A phase I/II study of the efficacy and toxicity of humanized anti-Tac (Zenapax) in the therapy of Tac-expressing adult T cell leukemia

Neuro-Oncology Branch



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Primary tumors of the central nervous system (CNS) are the second leading cause of cancer mortality in people under the age of 34 and the fourth leading cause of cancer mortality in individuals under the age of 54. With the dramatic improvement in the treatment of childhood leukemia, tumors of the brain and spinal cord are quickly becoming the leading cause of cancer deaths in children in the United States. Despite dramatic advances in neurosurgery, radiation oncology, and imaging of the central nervous system, the prognosis of patients with the most common primary brain tumors (i.e. malignant gliomas), remains essentially unchanged over the last two decades with most patients surviving less than a year from the time of diagnosis. Clearly, current treatment is suboptimal and novel therapeutic approaches are needed.

The Neuro-Oncology Branch is one of the first trans-institutional initiatives at the National Institutes of Health. The branch will develop an integrated clinical, translational, and basic research program that will engage the strengths and resources of the NCI and the NINDS for the purpose of developing novel experimental therapeutics for children and adults with tumors of the brain and spinal cord. Toward this end, the translational laboratory efforts of the Neuro-Oncology Branch are focusing on new strategies for selective tumor targeting through gene transfer using neural and endothelial stem cells and novel genetic vectors, through the identification of tumor-selective processes such as angiogenesis, and through the identification of tumor-specific markers. Additionally, the branch is undertaking a large national study to create a molecular classification of gliomas so that physicians will more accurately be able to give a prognosis to patients as well as select more appropriate treatments that have a greater likelihood of being effective in any individual tumor.

There is a growing and significant interest in the neuro-oncology laboratory in neural stem cell biology. We have recently demonstrated that we can isolate neural stem cells not only from the brain of animals and humans (as many others have shown) but we can also isolate neural progenitor cells from the peripheral blood and bone marrow of adult animals and humans. These cells behave identically to those found within the brain; however, they represent a much more accessible reservoir for study and therapeutic manipulation. We have demonstrated the ability of these cells to migrate both to sites of tumor cell infiltration within the brain and to sites of neural tissue damage. We have begun to examine the genetics of these bone marrow-derived neural progenitor cells using microarray gene expression profiling through the

various stages of cell differentiation into the different lineages that make up the central nervous system (i.e., glial cells, oligodendrocytes, and neurons). There are a number of ongoing studies investigating issues related to the therapeutic use of these cells for antitumor purposes, for neural damage repair, and for better understanding their biology at a molecular/genetic level.

We were interested in developing a technology that would allow us to induce tumor-selective transgene expression from our genetic vectors based on aberrant signal transduction pathways that are intrinsic to the tumorigenic process itself. We became interested in the P16/cyclinD/CDK4/RB pathways secondary to its mutation/deregulation in almost 100 percent of malignant gliomas. Thus, a prediction that until now has not been demonstrated experimentally in vivo is that E2F responsive promoters should be more active in tumor cells relative to normal cells due to an excess of free E2F and loss of pRB/E2F repressor complexes. Thus, a prediction, that until now has not been demonstrated experimentally in vivo, is that E2F responsive promoters should be more active in tumor cells relative to normal cells due to an excess of "free" E2F and loss of pRB/E2F repressor complexes. We demonstrate that adenoviral vectors, containing transgenes driven by the E2F–1 promoter, can mediate tumor-selective gene expression in vivo, allowing for eradication of established gliomas with significantly less normal tissue toxicity than seen with standard adenoviral vectors. Our data indicate that derepression of the E2F–1 promoter occurs in cancer cells in vivo, a finding that can be exploited to design viral vectors that mediate tumor-selective gene expression. We now have demonstrated that freshly isolated human malignant gliomas have a heterogeneous mixture of tumor cells, some expressing high levels of E2F and others low. We plan on exploring the difference in the biology of these two different tumor cell populations through microdissection of the cells and gene expression profiling using cDNA microarrays (see below).

We identified a subpopulation of human and mouse hematopoietic stem cells that are actually endothelial progenitor cells (angioblasts). Human and mouse proliferation-competent, bone-marrow or peripheral circulation-derived endothelial progenitor-like cells (PBECs) were isolated, expanded, and genetically engineered ex vivo to express the beta galactosidase (β-gal) or thymidine kinase (TK) genes using retrovirus-mediated gene transfer. Genetically-labeled PBECs were transplanted into wild-type and sublethally irradiated mice and found to migrate and incorporate into the angiogenic vasculature of growing tumors while maintaining transgene expression. Ganciclovir (GCV) treatment resulted in significant tumor necrosis in animals previously administered TK-expressing PBECs. These results demonstrate the potential of using genetically modified PBECs as angiogenesis-selective gene-targeting vectors and demonstrate the potential of this approach to mediate nontoxic and systemic antitumor responses. These experiments have also taught us a more fundamental biologic principle which is that tumor-associated neovasculature is not just derived through angiogenesis but also through the embryonic process of vasculogenesis. We are exploring a number of in vitro and in vivo experiments to better understand this process and to elucidate the cellular and molecular biology of these PBECs.

It is known that human gliomas (brain tumors) are a heterogeneous group of tumors; however, there are no pathologic classification schemas that reproducibly allow us to separate out biologically similar tumors. We have initiated a very large cDNA microarray effort in collaboration with the Human Genome Project and the Cancer Genome Anatomy Project (CGAP) to develop a comprehensive and novel molecular classification schema for human gliomas based on a gene expression profile using cDNA microarray technology. We have constructed our own cDNA microarray "chips" which will be enhanced for new and selective genes thought to be important in glioma biology. This project will include hundreds of tumor specimens and offer an unprecedented opportunity for gene discovery, dissecting signal transduction pathways, and learning this exciting new technology.

We have a growing interest in better understanding the cellular, molecular, and genetic basis for drug- and radiation-induced neurotoxicity. We have established several proficient in vitro models of the blood brain barrier that appear to mimic the functional as well as the cellular and molecular phenotype of the in vivo barrier. This, along with our significant experience with neural stem cells and our animal models incorporating both chemotherapy and radiation therapy to the central nervous system, put us in a prime position to begin to unravel the pathophysiologic mechanisms behind drug- or radiation-induced demyelination, neuronal loss, and endothelial cell destruction.

Additionally, we also have projects in neurostem biology as it relates to glioma tumorigenesis and in random peptide and single chain antibody phage screening for identifying novel binding motifs to tumor cells and tumor-associated endothelium.

Finally, we are building an administrative and clinical infrastructure to begin to see and treat pediatric and adult patients with primary tumors of the central nervous systems at the Clinical Center at the NIH. This patient population has not historically been seen in an organized fashion at the NIH and thus we are spending a great deal of time and effort building such an infrastructure. We are developing close relationships with outside institutions and with the collaborative cancer groups, specifically the CNS Tumor Consortium, and have opened up a referral base for patients with CNS neoplasms and their physicians to obtain information and advice about potential therapeutic options. Through this flow of patients, we will have ready access to the relatively large numbers of patients that will be necessary to complete the early clinical trials of the novel therapeutic agents as they come out of the laboratory. It is our intention to conduct early pilot and feasibility trials within the clinical centers and then to export the most promising of these therapeutic approaches to the NCI-sponsored CNS Consortia for more extensive phase I/II trials. The most promising of these therapies, we hope, will then go on to large-scale national randomized trials. It is through this process that we hope to develop a unique NCI-sponsored, nationally coordinated therapeutics development program for tumors of the brain and spinal cord.

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Biography: *Dr. Fine is currently chief of the Neuro-Oncology Branch at NCI's Center for Cancer Research, and of the National Institute of Neurologic Disorders and Stroke. He received his B.A. from the University of Pennsylvania, Philadelphia, and his M.D. from Mount Sinai School of Medicine, New York. Dr. Fine completed both his internship and his residency in internal medicine at the Hospital of The University of Pennsylvania. Three years later he completed his fellowship in medical oncology at Harvard Medical School's Dana-Farber Cancer Institute, Boston, MA. Before joining the NIH in 2000, Dr. Fine was both director of the Neuro-Oncology Disease Center at the Dana-Farber Cancer Institute, and of the Neuro-Oncology Program at the Harvard Cancer Center. The NCI senior investigator serves on several committees, including the Brain Tumor Program Review Group and the American Joint Committee on Cancer. Dr. Fine also serves on the editorial boards of several journals, including the* Journal of Clinical Oncology, Neuro-Oncology, *and* The Oncologist. *He has received several distinguished awards, including the Dana-Farber Harvard Cancer Center's Clinical Investigator Award in 1999, the Emil Frei III Clinical Investigator Award in 1993, and the Brain Tumor Society Research Award in 1992. His research interests include tumor angiogenesis, therapeutic gene transfer, and neural stem cell biology.*

Neuro-Oncology Branch
Developmental Therapeutics for Improved Treatment of Brain Tumors

Keywords:
adenovirus vectors
angiogenesis
gene therapy
glioma
microarray
molecular markers

Research: The aim of the Neuro-Oncology Branch (NOB) is to develop an integrated clinical, translational, and basic research program that will engage the strengths and resources of both the NCI and the National Institute of Neurological Disorder and Stroke (NINDS) for the purpose of developing novel experimental therapeutics for children and adults with tumors of the central nervous system. Toward this end, the NOB has a growing laboratory effort devoted to developing new strategies for utilizing genetic vectors to explore basic biologic questions and develop novel therapeutic approaches that can be brought into the clinic. In addition, one of the major goals of the Neuro-Oncology Branch will be to closely align its efforts with the NIH-sponsored extramural effort in brain tumor basic and clinical research. In particular, it is the intention of the branch to set up close working collaborations with other NCI-sponsored collaborative clinical trials groups that have an interest in brain tumors for the purpose of synergizing the strengths of these groups (i.e., large patients accrual, outstanding data management) with some of the unique capabilities of the NIH intramural program (i.e., capability of doing highly novel, technically demanding, and expensive pilot trials). Through this alliance of basic and applied science, closely linked to individuals capable of conducting solid clinical investigation, a new generation of therapeutic breakthroughs will be realized.

We have collaborated with Edward Oldfield, NIH.

Recent Publications:

Manome Y, et al. *Nat Med* 1996;2:567–73.

Tanaka T, et al. *Nat Med* 1997;3:437–42.

Parr MF, et al. *Nat Med* 1997;3:1145–9.

Fine HA, et al. *J Clin Oncol* 2000;18:708–15.

Pediatric Oncology Branch

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The Pediatric Oncology Branch (POB) is dedicated to the study and treatment of childhood cancer. During the past decade, there has been considerable progress in understanding fundamental processes in the pathogenesis of pediatric cancers. New insights in the cell biology and molecular genetics of specific cancers have led to new targets for therapy. At the same time, advances in immunology are providing reagents capable of regulating immune function or altering host-defense systems in ways that may permit the use of current cytotoxic regimens in more rational and tolerable schedules and formulations, as well as the development of combined modality therapy utilizing both cytotoxic therapy and immunotherapy.

The Pediatric Oncology Branch has developed a close partnership and collaboration between basic and clinical scientists involved in studying pediatric oncology, immunology, genetics, and supportive care. Efforts are focused along several themes:

- **Utilization of the tools of molecular and tumor biology** to improve the diagnosis, classification, treatment, and outcome of childhood cancer. The integration of advanced technology approaches designed to detect specific "fingerprints" of pediatric tumors as well as the detection of specific mutations are major goals of all clinical studies and will allow the branch to become a model of translational clinical research.

- **Development of new treatment strategies** based upon laboratory findings of clinical interest as well as testing new agents (i.e., phase I and phase II clinical trials) that might eventually be integrated into front-line chemotherapy regimens. We evaluate principles of therapy (e.g., "therapeutic window," combination immunotherapy and chemotherapy) for tumors that have been shown to be refractory to current therapies. We also study the role of specific biologic therapeutics and combinations (for example, combination IL–2 plus IL–12) as they specifically relate to pediatric tumors.

- **Pursuit of strategies to decrease the complications of cancer therapy,** particularly the infections associated with neutropenia in cancer patients and the immunologically compromised host, in order to modulate or improve altered host-defense systems and permit the delivery of effective treatment.

Pediatric Hematology-Oncology Fellowship Program: Overview

The Pediatric Oncology Branch sponsors dynamic projects in clinical and laboratory research related to acute leukemia, non-Hodgkin's lymphomas (especially small, noncleaved cell lymphomas), Ewing's sarcoma, osteogenic

sarcoma, rhabdomyosarcoma, neuroblastoma, and CNS tumors. The Johns Hopkins/National Cancer Institute Pediatric Hematology-Oncology Fellowship Program, accredited by the Accreditation Council on Graduate Medical Education, provides eligibility for certification in pediatric hematology-oncology. The first year of the program is essentially all clinical and consists of 4 months of rotations on the POB inpatient and outpatient clinical services, 4 months on the Johns Hopkins University (JHU) pediatric inpatient and outpatient services, and 3 months on the JHU hematology service. The second and third years of the program focus on pursuing research based on the interests of the individual fellow and can be primarily at either the NCI or JHU.

The fellowship program is designed to enter a maximum of six new fellows per year in a 3-year training program. The goal is to train outstanding academic pediatric hematologists-oncologists in the clinical management of children with hematologic and oncologic disorders and to provide training in a rigorous research setting to ask important questions that will ultimately advance the state of knowledge in the biologic understanding of these disease processes. The program emphasizes the importance of integrating an understanding of pathophysiology into the development of improved treatment approaches to children with hematologic and oncologic disorders. The fellowship training program is designed to progressively increase responsibility of patient care, teaching, and independent research from the first through the third years. During the first year, fellows oversee all care of pediatric hematology-oncology patients. There is no formal house staff at the NIH Clinical Center, although fourth-year medical students and house staff from various medical schools around the country may rotate for one month on the Pediatric Oncology Service at the NIH. Fourth-year students/house staff on rotation are assigned "primary patients," while first-year fellows teach and supervise the student/resident and supervise care for patients not assigned to a student/resident. When no fourth-year student/house staff is rotating, the first-year fellow is directly responsible for patient care and supervises nurse practitioners and the trained pediatric oncology nursing staff. First-year fellows rotating at Johns Hopkins University are directly involved in supervising the house staff covering pediatric hematology and oncology patients. First-year fellows and the attending physician jointly see all patients. The attending physician supervises the house staff. Fellows interact extensively with other pediatric subspecialty fellows and work closely with surgical, radiation, and oncology fellows. At the NIH, there is a particularly close interaction with infectious disease fellows, who follow pediatric patients admitted with febrile neutropenic episodes. First-year fellows present formal didactic lectures on relevant topics, at both weekly chief-of-service rounds and at tumor boards. By the third year, fellows are required to present a formal conference on their research project. Third-year fellows spend at least one month as coattending physician on both the inpatient and outpatient services. During this month, fellows supervise and teach first-year fellows. They run daily work rounds and meet each day with the senior attending physician to review patient care issues and for formal "one-on-one" teaching from the senior staff member.

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Biography: *Dr. Helman received his M.D. magna cum laude in 1980 from the University of Maryland School of Medicine. He completed his clinical training in internal medicine at Barnes Hospital, Washington University, in 1983, and in medical oncology at the NCI Pediatric Oncology Branch and the former Medicine Branch in 1985. He has held positions of increasing responsibility at the NCI and became branch chief in 1997. Dr. Helman has a wide range of research interests and accomplishments spanning molecular genetics of human cancer and development of new therapeutic approaches to pediatric solid tumors.*

Pediatric Oncology Branch
Molecular Pathogenesis of Pediatric Sarcomas

Keywords:
animal models
Ewing's sarcoma family of tumors (ESFT)
IGF–I
IGF–II
immunotherapy
metastases
rhabdomyosarcoma

Research: Alterations of insulin-like growth factor signaling appear to be a common feature of many pediatric sarcomas including rhabdomyosarcoma (RMS), Ewing's sarcoma (ES), and osteogenic sarcoma (OS). In RMS, we have shown the IGF–II is markedly overexpressed and can function as an autocrine growth and survival factor. We have focused our attention on understanding how IGFs provide signals for cell survival. We found that mouse myoblast cells overexpressing IGF–II have an abnormal response to radiation-induced DNA damage. While parent control cells respond as expected to such DNA damage with G1 arrest, the IGF–II overexpressing cells fail to arrest in G1 after radiation despite normal induction of p53 and p21. This failure of the G1 checkpoint is associated with a failure to inhibit both CDC2 and CDK2 kinase activity. To confirm that this alteration was specifically related to alterations in the IGF-signaling pathway, we performed similar experiments on CHO cells that overexpress IGF–II from a tetracycline-regulatable promoter. These cells indeed behave in a similar manner to the IGF–II overexpressing myoblast cells with loss of the G1 checkpoint. We subsequently found that overexpression of IGF–II in mouse myoblasts also inhibits apoptosis. Inhibition of apoptosis is related to increased p70S6 kinase activity, and this appears to be downstream of activated PI3 kinase. These findings have direct implications for therapeutic intervention in tumors, such as rhabdomyosarcoma, that have activated IGF–II signaling.

A second major focus of the Molecular Oncology Section is to identify the molecular basis for metastases in pediatric sarcomas. We established a transplantable murine osteosarcoma cell line and identified subclones with high and low metastatic potential. Using cDNA microarray technology, we identified a group of genes whose expression is correlated with either high or low metastatic potential. We are evaluating the genes identified in this screen and have demonstrated similar patterns of expression in both human and canine osteosarcoma using tissue arrays. Functional studies are ongoing to determine the specific mechanism whereby expression of a specific gene or genes leads to increased metastatic potential.

Many pediatric sarcomas express tumor-specific translocations leading to expression of fusion proteins. The presence of these tumor-specific translocations also led us to explore the possibility that such alterations can generate novel and tumor-specific antigens that could be targeted by CTLs. We have been able to demonstrate in mouse models that antigen-presenting cells (APCs) pulsed with translocation-specific fusion peptides can be used to elicit MHC class I-restricted CTL that specifically recognizes cells pulsed with peptide used for APC vaccination. Furthermore, we have shown that these CTLs can specifically recognize mouse tumor cells that have been transfected with the full-length translocation-specific fusion cDNA, and these CTLs can be adoptively transferred to tumor-bearing mice and elicit an antitumor effect. These studies have led to the development of clinical studies using translocation-specific peptide vaccine strategies in ES and RMS. These studies are in the early stages.

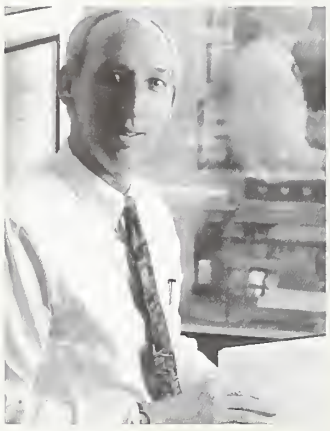
Recent Publications:

Zhang L, et al. *J Biol Chem* 1999;274:13118–26.

Khanna C, et al. *Clinical and Experimental Metastasis* 2000;18:261–71.

Khanna C, et al. *Cancer Research* 2001;61:3750–9.

Dupont J, et al. *J Biol Chem* 2001;276:26699–707.



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Biography: *Dr. Balis received his B.S. in zoology from the University of North Carolina in 1971 and his M.D. from Vanderbilt University in 1975. He completed a pediatric residency at Vanderbilt Children's Hospital in 1978 followed by 4 years of fellowship training in pediatric hematology/oncology at the Children's Hospital in Seattle and the Fred Hutchinson Cancer Research Center. Dr. Balis came to the Pediatric Oncology Branch in 1982 as a clinical associate and became a senior investigator in the branch in 1988. He is board certified in pediatrics and pediatric hematology/oncology.*

Pediatric Oncology Branch
Clinical Pharmacology and Drug Development of Anticancer Drugs for Childhood Cancers

Keywords:
brain tumor
childhood tumors
clinical trial
drug development
molecular targets
pharmacokinetics
pharmacology

Research: The research objective of the Pharmacology and Experimental Therapeutics Section is to develop innovative therapeutic approaches for childhood cancers through the clinical development of novel investigational agents and the study of the clinical pharmacology of new and existing anticancer drugs. An integrated approach incorporating preclinical studies in in vitro and animal models as well as clinical trials and clinical pharmacokinetic studies is utilized in the development of new agents. An emphasis is placed on studying the central nervous system pharmacology of new agents and on the development of new treatment approaches for central nervous

system tumors. For new systemic anticancer drugs, analytical techniques to assay drug concentrations are developed and preclinical pharmacokinetic studies are performed in a nonhuman primate model, which is highly predictive of drug disposition in humans. Methods for measuring tissue and tumor drug concentrations using microdialysis are being investigated. Cytotoxicity studies in pediatric tumor cell lines are used to define therapeutic concentrations, schedule dependence, and the applicability and spectrum of activity of the new agent against pediatric tumors. These preclinical studies guide in the design of the initial phase I and II clinical trials and pharmacokinetic studies in children with refractory cancer. New agents studied in preclinical models and in clinical trials include standard cytotoxic agents as well as noncytotoxic agents such as molecularly targeted agents, differentiating agents, and agents that modulate anticancer drugs (e.g., p-glycoprotein inhibitors). Systemically administered drugs that penetrate the blood-brain barrier are identified and targeted for studies in patients with brain tumors. We are also evaluating agents that can alter blood-brain barrier permeability, such as lobradamil, and we have developed several new agents that can be administered intrathecally for the treatment of meningeal tumor.

Studies of the clinical pharmacology of anticancer drugs used in the treatment of children with cancer focus on the pharmacokinetics and pharmacodynamics of standard drugs. Drug concentrations are measured by a variety of techniques, including high-pressure liquid chromatography, gas chromatography, radioimmunoassays, and enzyme inhibition assays. Preclinical pharmacokinetic studies are performed in animal models, and clinical pharmacokinetic/pharmacodynamic studies are performed in children treated in NCI clinical trials of standard anticancer drugs. Mathematical models are developed to describe drug disposition and relate drug concentrations to measures of toxicity and response. Our section also collaborates with other CCR branches and has performed pharmacokinetic studies in adults treated with both investigational and standard agents. The studies of existing agents are designed to identify more rational approaches to their use by developing limited sampling strategies to simplify drug monitoring and defining pharmacodynamic correlates that relate drug concentrations to toxicity or efficacy of the drug. The latter approach should lead to more individualized, adaptive dosing methods that can improve efficacy and reduce the toxicity of anticancer and antiretroviral agents.

This section is also investigating fluorescent pteridine-based nucleoside analogs that can be site-specifically incorporated into DNA oligonucleotides with a DNA synthesizer. These novel fluorophores are highly fluorescent and have chemical structures that resemble purines. The fluorescent properties of a series of these compounds are being characterized and potential applications are being investigated. These new fluorophores are promising probes for the study of protein/DNA interactions because they are not attached externally and they do not appear to disrupt the tertiary structure of DNA. Applications for the fluorophores include an assay for akyltransferase activity and as hybridization probes where they have the potential to replace radioactive probes.

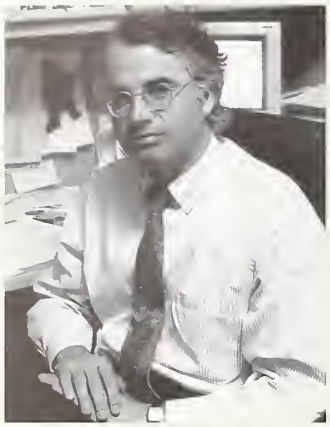
Recent Publications:

Hawkins ME, et al. *Nucleic Acids Res* 1995;23:2872–80.

Balis FM, et al. *Blood* 1998;92:3569–77.

Balis FM, et al. *Cancer Chemother Pharmacol* 2000;45:259–64.

Warren KE, et al. *J Clin Oncol* 2000;18:1020–6.



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Biography: *Dr. Chanock received his M.D. from Harvard Medical School and completed training in pediatrics, pediatric infectious diseases, and pediatric hematology/oncology at Boston Children's Hospital and the Dana-Farber Cancer Institute, Boston. Afterwards, he joined the NCI where he has an interest in the molecular, cellular, and clinical problems of infectious complications in patients with cancer and HIV infection.*

Pediatric Oncology Branch
Genetic Modifiers of Host Defense in Cancer and HIV Disease

Keywords:
functional genomics
genetic polymorphism
HIV
infection
NADPH-oxidase
phagocyte

Research: The primary goal of the Genomic Variation Section is to identify variants in genes of the immune system that might adversely affect disease outcome in patients with an immunodeficiency or cancer. We have focused our investigation on patient populations with one or more breaches in immune function based upon the *hypothesis* that in the setting of a major immune deficit, the significance of common variants of genes that play a role in immunity would be magnified. Since most common gene variants, known as single nucleotide polymorphisms (SNPs), possess minimal to no phenotypic effect in the normal population, it plausible that key SNPs might assume greater visibility when one or more other immune functions are disrupted. The laboratory is now focused on developing sets of SNPs, derived from pathways of known genes, so that it will be possible to investigate through genetic association studies the dynamic relationship between genes and their function in vivo. Understanding the role of modifying genes, namely SNPs or other rare variants, offers the potential for exploring risk factors for clinical outcomes, some of which could impact therapeutic decisions (known as pharmacogenomics).

Initially, we sought to identify variant genes (also known as modifying genes) that influence outcome in a rare primary immune deficiency that we have studied for nearly a decade, namely, chronic granulomatous disease (CGD). This is a rare genetic disorder of phagocytes characterized by recurrent infections and obstructive granulomas. Previously, we had cloned several of the genes of the phagocyte NADPH-oxidase and studied the biochemical and functional properties of the cytosolic factors of the multicomponent enzyme complex. The CGD phenotype results from a

mutation in one of four genes, each located on a different chromosome. The biochemical consequence is the failure to mount a respiratory burst, which is responsible for generating superoxide radicals, a critical component of host defense against intracellular pathogens. However, the wide variation in clinical outcomes in CGD cannot be explained by the primary mutation in a component of the NADPH oxidase alone. In search of additional contributing genetic factors, we investigated the role of common variants in other candidate genes that might influence the outcome in CGD. Specifically, we chose seven common variants in molecules of innate immunity other than the NADPH oxidase. We demonstrated that variant genotypes of two low affinity Fc gamma receptors (*FCGR2A* and *FCGR3B*), and myeloperoxidase, *MPO*, were associated with a greater likelihood of developing severe immune complications affecting the gastrointestinal tract that occur in approximately 40 percent of CGD patients. Similarly, we showed that variants of the *FCGR2A* and the mannose-binding lectin, *MBL2*, individually and in combination, were associated with development of other autoimmune complications of CGD. Our decision to study the genetic determinants of outcome in CGD was influenced by the fact that the primary disease process involves only the innate immune system, which is considerably less complex than the adaptive immune system. Furthermore, the number of suitable candidate genes for study, i.e., those genes for which sufficient a priori data was available to provide a rationale for investigation, was more limited for the innate immune system.

On the basis of this pilot study in CGD patients, we concluded that we had: (1) validated our conceptual approach of performing genetic association studies in a well-characterized population of immunodeficient patients; (2) identified modifying genes in CGD worthy of followup studies; and (3) yielded preliminary insights into defects of innate immunity other than those provided by mutation in the NADPH oxidase in CGD. Currently, we are conducting followup studies in additional cohorts. We will continue to focus on the investigation of variants in genes of the NADPH-oxidase, a model system we have studied and published on extensively in the past. We have had a longstanding interest in the cellular and molecular biology of the phagocyte NADPH-oxidase. Based upon our understanding of the molecular basis of subtypes of CGD, we have developed an interest in variants of p47-phox, p67-phox, and p40-phox (*NCF1*, *NCF2*, and *NCF4*). We will study the functional significance of two newly characterized common polymorphisms in the promoter region of p67-phox; for example, we will focus on in vitro characterization of the promoter region. This is of particular interest because it will aid us in determining whether p67-phox is a limiting factor in the phagocyte oxidase burst. Similarly, common variants in other components of the respiratory burst will be evaluated in cell-free and reconstitution systems to assess differences in biological function. These studies will proceed in parallel with genetic association studies utilizing SNPs in the genes of the NADPH-oxidase pathway to study infectious, immunological, and clinical outcomes in disease populations (e.g., BMT, CF, and SS).

Common variants of two genes, *FCGR3A* and *IL6* are associated with the development of Kaposi's sarcoma in HIV-infected men. For example, the *FCGR3A* genotype appears to exert a significant influence on susceptibility

to or protection from KS; the FF genotype confers protection and the VF genotype is associated with development of KS. In the future, the information derived from these studies and the informative SNPs that were identified will be incorporated into more complex studies designed to investigate not only the role of new candidate SNPs in disease but also their potential interactions with the previously well-characterized SNPs. This might allow us to gain new insights into pathogenesis at the same time we are identifying genetic markers for disease outcome.

We have broadened our investigations to apply known pathways of SNPs to other additional, well-characterized immunodeficient populations. We have focused specifically on three categories of patients at high risk for serious infectious complications. These include: (1) primary genetic immunodeficiencies (CGD, Wiskott-Aldrich syndrome [WAS], and hyper-IgM syndrome [HIGM]); (2) immunodeficiencies secondary to infection, malignancy, or ablative therapy (i.e, HIV–1 infection, childhood acute lymphoblastic leukemia [ALL], and bone marrow transplantation [BMT]); and (3) primary monogenetic disorders in which it has been suggested that perturbation(s) in innate immunity contribute to disease outcome (cystic fibrosis [CF], and sickle cell anemia [SS]). Recently, we have initiated a series of studies, investigating the role of immune SNPs in selected cancer cohorts. These studies include acute lymphoblastic leukemia, non-Hodgkin's lymphoma (in adults and children), and neuroblastoma. For each of these diseases, it is plausible that perturbations in immune genes could influence susceptibility to the cancer or outcomes within a similarly treated cohort. Furthermore, evaluating pathways of cytokine or adhesion molecule SNPs is supported by current models for disease pathogenesis.

To accomplish our goals, we have evolved a specialized program dedicated to identification and cataloging of candidate SNPs and analysis of their role in a spectrum of disease conditions in populations for which epidemiological endpoints are well defined. In the past, the genes we chose for study had to meet three requirements, namely: (1) the gene variants had to be common (>5 percent in a control population); (2) preliminary or promising information was available from in vitro laboratory studies or prior clinical gene association studies; and (3) there was a plausible biological mechanistic hypothesis for study in a particular population (e.g., investigation of genes of innate immunity in CGD). Initially, we limited our studies to genes of innate immunity; however, in the future, other more complex pathways will be investigated using the same criteria.

Because of the success of the Human Genome Project, the choice of genes for study is expanding rapidly. Two parallel systems for identifying candidate SNPs are being employed: (1) one focuses on SNPs that have been characterized and described in either the scientific literature or public databases, and (2) the second includes novel SNPs validated in our laboratory. We have developed a Web site to catalog known variants of immunologically important genes (**http://www-dcs.nci.nih.gov/branches/pedonc/ISNP/**). The rapid expansion in identification of new SNPs provides an opportunity to increase the choice of genes for study. New SNPs are

readily available from public databases such as the Cancer Genome Anatomy Project–Genetic Association Initiative, NCBI's db-SNP, and the SNP pipeline.

The laboratory participates in the Cancer Genome Anatomy Project—Genetic Annotation Initiative (CGAP–GAI). We have been the primary curators of the Cancer Gene List of 3,500 genes divided into 20 biologically driven categories (the Web site is **http://lpg.nci.nih.gov/html-cgap/cgl/**). The open reading frames of candidate genes are being resequenced in a reference population to look for common (>75 percent) SNPs. We have completed an analysis of 145 genes involved in apoptosis; this represents a first step in developing pathway-based collections of SNPs that will be utilized in future studies. Apoptosis is a high priority because the catalog of genes is restricted in number and because of the current interest in the role of apoptosis in both cancer and inflammation. We have also initiated a large-scale program known as the NCI–SNP500 to resequence in four diverse populations 500 SNPs of immediate importance to the molecular epidemiology community.

A substantial effort of the laboratory will continue to be devoted to introducing new technologies for SNP discovery and high-throughput analysis. We have participated in initial studies with an electronic dot-blot chip system, the NanoChip, a robust system for detection of candidate SNPs (gDNA or cDNA). Through control of the electric field, genetic samples are transported, concentrated, attached, and interrogated with fluorescent-labeled reporter probes on. The versatility and increased accuracy of this technology will be an asset and should shorten the time required for complex SNP analysis in large studies. Other technologies that we are investigating include single base extension system and real-time amplification of SNPs, also known as TaqMan. Studies are under way to define the parameters for pooling DNA in anticipation of screening larger cohorts.

Among our collaborators are Andrew Blauveldt, Ken Buetow, Jeffrey Cohen, Eric Green, Steven Holland, Konrad Huppi, Joseph Kovacs, Susan Leitman, Harry Malech, Joel Moss, Tom Nutman, Nat Rothman, Bob Strausberg, and Robert Yarchoan, NIH; John Curnutte, DNAX, Inc.; Gritta Janka-Schaub, University of Hamburg; Brigitta Mueller, Baylor College of Medicine; Hans Ochs, University of Washington; Peter Pappas, University of Alabama; and Jerry Winkelstein, Johns Hopkins University.

Recent Publications:

Gilles P, et al. *Nat Biotechnol* 1999;17:365–70.

Foster C, et al. *Blood* 2000;96:2562–7.

Lehrnbecher T, et al. *Blood* 2000;95:2386–90.

Foster C, et al. *Br J Hematol* 2001;113:596–9.

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Biography: *Dr. Khan obtained his bachelor's degree in 1984 and his master's degrees in 1989 in immunology and parasitology at England's University of Cambridge. He subsequently obtained his M.D. there and the postgraduate degree of MRCP (Membership of the Royal College of Physicians), equivalent to board examination in the United States. After clinical training in internal medicine and pediatrics as well as other specialties, he received a Leukemia Research Fellowship. In May 2001, Dr. Khan joined the Pediatric Branch, NCI, as a tenure track investigator. Dr. Khan and colleagues have published a new model for diagnosis of cancer using artificial neural networks (ANN), a form of artificial intelligence, and microarray technology. In April 2001, Dr. Khan was recognized by the American Association for Cancer Research for his work in tumor profiling by receiving a Scholar in Training Award.*

Pediatric Oncology Branch
Molecular Investigations of Cancer Using DNA Arrays

Keywords:
cDNA
EWS–FLI artificial neural networks
microarrays
molecular classification
molecular diagnostics
MYCN tumor profiling
oligonucleotide arrays
PAX3–FKHR
pediatric hematology
pediatric oncology
pediatric sarcomas
pediatrics

Research: Dr. Khan has demonstrated that cDNA microarray analysis can be used to identify genetic fingerprints of a specific type of muscle cancer, rhabdomyosarcoma (RMS), and is able to distinguish one type of cancer from another. He and his colleagues were the first to apply hierarchical clustering and visualization tools including multidimensional scaling to demonstrate the relationships between cancers based on gene expression profiling. Dr. Khan then went onto apply this technique to determine that the PAX3–FKHR fusion oncogene found in RMS activated a myogenic transcription program, which is a critical component to the oncogeneic process in these muscle cancers.

Molecular Taxonomy of Pediatric Cancers

Neuroblastomas are cancers of neural crest origin with variable prognoses depending on age at presentation, stage, histology, presence of MYCN amplification, chromosomal ploidy, and deletion status of 1p36. Very little is known of the molecular mechanisms that confer good or poor prognosis in this and other malignancies. We have recently demonstrated that cancers can be diagnosed on the basis of gene expression profiling using cDNA microarrays and sophisticated pattern recognition algorithms such as Artificial Neural Networks. The Oncogenomics Section will expand this concept further by profiling a series on neuroblastomas of different stages and prognosis. With this we hope to identify tumor-specific expression patterns, or "fingerprints," that uniquely identify a poor prognostic group, as well as those associated with specific genetic aberrations including MYCN amplification. By these methods, we may be able to classify expression profiles that correlate with prognosis and hence identify the genes that confer these biological properties.

We also plan to develop a database of a wide range of pediatric malignancies from patient tumors, including archival material and xenografts, as well as prospective samples from patients on treatment protocols at the Pediatric Oncology Branch (POB). By these methods we hope to narrow down the list

of genes that defines a particular cancer or diagnostic or prognostic group cluster to a minimum number, which can be used to make smaller microarrays for possible diagnostic purposes.

These studies will increase our knowledge of these malignancies by identifying genes that are significant to the biology of these cancers. In addition, by these methods we may identify secreted proteins that can be used for diagnosis (e.g., AFP for germ cell tumors), as well as following therapy including the monitoring of tumor regression or recurrence. We may also identify new targets for therapy, including immune therapy, or discover novel molecular targets such as death pathway genes, uniquely expressed in these cancers.

Identification of the Downstream Targets of Pax3–FKHR

The second area which we will address is characterizing the biological/biochemical properties of the t(2; 13)(q35; q14) that is found in ARMS. This translocation results in the fusion of PAX3, a developmental transcription factor required for limb myogenesis and neural crest development, with FKHR, a member of the forkhead family of transcription factors. The resultant PAX3–FKHR gene possesses transforming properties; however, the effects of this chimeric oncogene on gene expression are largely unknown. We have investigated the actions of these transcription factors by the introduction of both Pax3 and Pax3–FKHR into NIH–3T3 cells, and monitored the resultant gene expression changes with a murine cDNA microarray containing 2,225 elements. We found that PAX3–FKHR, but not PAX3, activated a myogenic transcription program. This included the induction of transcription factors MyoD, Myogenin, Six1, and Slug, as well as a battery of genes involved in several aspects of muscle function. Notable among the induced genes, were the growth factor gene Igf2, its binding protein Igfbp5, and the transforming growth factor-β2. Relevance of this model was suggested by verification that three of these genes (IGFBP5, HSIX1, and Slug) were also expressed in ARMS cell lines. This study demonstrated the profound myogenic properties of PAX3–FKHR and not PAX3 in NIH–3T3 cells. We postulate that the presence of the PAX3–FKHR gene in a progenitor cell triggers myogenesis, but these cells then fail to terminally differentiate and exit the cell cycle. This then allows the subsequent acquisition of secondary genetic alterations that would lead to the development of fully malignant ARMS. We have identified several candidate genes in microarray experiments that may prevent this terminal differentiation including the DNA-binding protein inhibitors ID–4 and ID–2 as well as SIX1 and FGFR4. We plan to further characterize these genes, both in terms of their temporal expression and their molecular effects. In addition, an exciting new develop-ment has been the production of a PAX3–FKHR monoclonal antibody. This antibody made to the fusion protein at the breakpoint does not recognize the native Pax3 or FKHR. It will prove to be an invaluable resource and one that we will use to identify the direct targets of PAX3–FKHR using a combination of chromatin immunoprecipitation and cDNA microarray technology.

Molecular Mechanisms of Drugs Using cDNA Microarrays

A third area which we will focus on is the monitoring of gene expression changes impacted by drugs. The choice and design of many chemotherapeutic agents currently used in cancer treatment has been

traditionally empirical in nature. The molecular mechanisms of their actions are not well understood, and their mode of action is often indiscriminate targeting, both of tumor and normal cells. As a model system, we have investigated the gene expression alterations during neural differentiation of the neuroblastoma (NB) cell line SMS–KCNR by retinoic acid (RA), using cDNA microarrays. Neuroblastoma is the most frequently occurring extracranial solid tumor of childhood and has the highest rate of spontaneous regression of any human cancer. RA is known to stimulate morphological neural differentiation of NB. It has been shown to enhance neurite extension, increase membrane excitability, induce neurotransmitter enzymes, and reduce tumorogenicity, as well as improve prognosis for high-stage disease. SMS–KCNR neuroblastoma cells (containing 1p del and N–Myc amplification), were treated with all-trans retinoic acid (ATRA), or the solvent ethanol (control). RNA was harvested at 0, 2, 6, 16, 30, 48, 80, and 96 hrs and 8, 12, 18, and 22 days following ATRA treatment. Gene expression profiles at these time points were compared with time point 0. By this method we are currently identifying pathways of critical genes in neuronal differentiation using the analysis tools mentioned above. In addition, we have developed a novel gene-clustering algorithm, based on predefined profile templates, to identify expression profiles that drive differentiation. This in vitro model is being translated to an in vivo model of neuroblastoma.

The combined approaches outlined in this proposed program will allow a comprehensive analysis of pediatric tumor genomes.

Collaborating with us are Timothy Cripe and Ola Mykelebost, University of Cincinnati; Lee Helman, Chand Khanna, Glenn Merlino, Kathy Pritchard-Jones, Carol Thiele, and Jon Wigginton, NIH; and J. Trent, National Human Genome Research Institute.

Recent Publications:

He YDD, et al. *Nat Med* 2001;7(6):658–9.

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Biography: *Dr. Mackall completed an accelerated 6-year B.S./M.D. program at the Northeastern Ohio Universities College of Medicine followed by a combined internal medicine/pediatrics residency in Akron, Ohio. She came to the NCI in 1989 as a Clinical Associate in pediatric oncology, completing her clinical fellowship in 1992 and her postdoctoral work in the Experimental Immunology Branch in 1996. Since that time she has served as an independent investigator in the Pediatric Oncology Branch. Dr. Mackall has received international recognition for her work on T cell immune reconstitution.*

Pediatric Oncology Branch
Directing T Cell Responses During Immune Reconstitution

Keywords:
AIDS
animal models
bone marrow transplantation
cancer immunotherapy
cellular immunity
childhood tumors
cytokines
Ewing's sarcoma family of tumors (ESFT)
HIV
immunoreconstitution
pediatric sarcomas
PNET
rhabdomyosarcoma
thymus
vaccine design

Research: The objectives of the Immunology Section's research are: (1) to elucidate the basic biological processes through which T cell populations are maintained and reconstituted following T cell depletion, and (2) to develop therapies that improve overall immune competence following T cell depletion and that allow the induction of antitumor immune responses in the setting of minimal residual neoplastic disease.

Using murine models, we previously identified and characterized the primary pathways of T cell regeneration. Subsequent studies in humans identified the critical importance of thymic-dependent pathways for restoration of host immune competence and provided readily accessible techniques for monitoring thymic-dependent progeny in clinical settings.

Ongoing current studies are focused on three primary projects. First, we are focused on the role of IL–7 as an immunorestorative agent. Because T cell-depleting chemotherapy induces prolonged T cell depletion in human beings due, in part, to inadequate thymic function, we utilize the athymic T cell-depleted mice to study immune reconstitution. Our goal is to induce and amplify stringent immune responses in T cell-depleted mice using model systems which mimic tumor antigens encountered in the clinical setting. These studies have shown that complete reconstitution of the T cell repertoire is not required for immune competence, even to a stringent antigen; rather, reconstitution of even 10 percent of the total repertoire appears adequate. Furthermore, with pharmacologic doses of IL–7, even 1 percent of the total T cell repertoire is adequate for response to nominal antigen. Thus, even in the absence of thymic function, the use of regenerative cytokines can directly enhance immune competence by improving the efficiency of the immune response. In addition, we have identified IL–7 as a potential endogenous regulator of T cell homeostasis by showing that in clinical settings associated with CD4 depletion, profound inverse relationships between circulating IL–7 and total CD4 counts are observed. This relationship appears unique to IL–7 and occurs in a variety of clinical settings associated with T cell depletion. Ongoing studies are

under way to clinically develop IL–7 as an immunorestorative agent and study the effects of IL–7 on allogeneic responses post-BMT.

In a second project, we are investigating the nature of immune responses to pediatric sarcomas by inducing cytolytic T cells against autologous tumors derived from patients presenting to the POB with primary or recurrent sarcoma. These studies have identified the existence of oligoclonal T cell populations circulating in patients with pediatric sarcomas which display potent cytolytic activity. The cytolytic activity is promiscuous, and sometimes non-MHC restricted due to the existence of NK receptors on these oligoclonal T cells. Furthermore, these cells have an unusual phenotype in that they lack the CD28 costimulatory molecule and instead express 4–1BB. Because Ewing's tumor expresses 4–1BB ligand, we are investigating whether these cells actually represent tumor-specific T cells that have developed this unique phenotype due to chronic antigenic stimulation. Future studies will use a murine xenograft model to further study potential antitumor effects of these cells in vivo.

In a third project, we are investigating programmed cell death in pediatric sarcomas. Previously we showed that Ewing's tumors universally express Fas, but are not universally susceptible to Fas-mediated cell death, suggesting the existence of intracellular inhibitors of programmed cell death. In this model, IFN-γ was potently able to restore Fas sensitivity in most, but not all cell lines. Furthermore, this work identified intracellular Fas ligand which was not expressed on the cell surface and, therefore, not capable of depleting Fas-sensitive immune effectors. More recent collaborative studies have shown that Ewing's sarcoma is even more susceptible to TRAIL mediated cell death compared to Fas and that even chemoresistant Ewing's sarcoma cell lines are universally susceptible to TRAIL. Ongoing trials are under way to investigate activity in vivo using a sarcoma xenograft model in immunodeficient mice.

Finally, we are also engaged in clinical trials which attempt to translate the principles gleaned from our studies of immune reconstitution to induce antitumor immune responses following intensive chemotherapy for pediatric sarcomas. Our current trial uses a combination of immune reconstitution/immunotherapy wherein patients with high-risk pediatric sarcomas undergo T cell harvest prior to standard, cytotoxic antineoplastic therapy. Following standard therapy, patients receive infusions of T cells, dendritic cell-based immunization, and rhIL–2. This trial targets the breakpoint region of the t(11;22) and t(2;13) found in pediatric sarcomas. The purpose of this trial is to study whether these approaches can enhance immune reconstitution and induce antitumor immune effects in the setting of minimal residual neoplastic disease.

Immune incompetence related to T cell depletion is a central problem limiting therapeutic progress both in HIV infection and in the setting of bone marrow transplantation. Furthermore, if T cell-based immune responses could be successfully induced toward a variety of tumors in the postchemotherapy setting, when the tumor burden is low, it is likely that significant therapeutic benefit would result. Hence, the development of successful approaches to enhance immune reconstitution in these clinical

settings would be expected to substantially improve the outcome in HIV infection, bone marrow transplantation, and potentially for cancer patients whose tumors can be targeted immunologically.

Recent Publications:

Kontny HU, et al. *Cell Death and Diff* 2001;8:506–14.

Merino ME, et al. *J Clin Oncol* 2001; in press.

Fry TJ, et al. *Blood* 2001;97:2983–90.

Fry TJ, et al. *Blood* 2001;97:1525–33.

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Biography: *Dr. Thiele received her Ph.D. in microbiology and immunology from the University of California, Los Angeles. She completed her postdoctoral research as a Cancer Research Institute and a Damon Runyon-Walter Winchell Fellow at the NCI. As the chief of the Cell and Molecular Biology Section in the Pediatric Oncology Branch and an associate professor of pediatrics at the Uniformed Services University of the Health Sciences, F. Edward Hébert School of Medicine, Dr. Thiele's scientific interest is in the field of cancer biology with special emphasis on pediatric neuroectodermal tumors and neuronal development.*

Pediatric Oncology Branch
Cell and Molecular Biology of Neuroectodermal Tumors

Keywords:
cDNA microarray
cell cycle
differentiation
neuroblastoma
retinoids

Research: The Cell and Molecular Biology Section studies the molecular mechanisms required to control growth, induce differentiation, and suppress tumorigenicity in pediatric neuroectodermal tumors. Studies are aimed at identifying signal transduction paths that can serve as targets for the development of novel therapies. Retinoic acid (RA)-induced differentiation of neuroblastoma tumors continues to be our paradigm for studies that define signal transduction pathways that mediate these processes. Delineation of these pathways will not only enable a more efficacious clinical utilization of retinoids and their congeners but also has the possibility of identifying novel molecules with therapeutic potential for the treatment of neuroectodermal tumors. Differential expression patterns and microarray analysis has been utilized to model the patterns of gene expression that occur during RA-induced differentiation in order to identify potential regulatory networks that control growth, suppress tumorigenicity, and express a differentiated phenotype.

Growth Control

Nmyc is amplified and overexpressed in NB tumors with the worst prognosis. As Nmyc is a HLH transcription factor that binds MAX and transactivates E-box containing DNA promoters, we have been studying how Nmyc disrupts transcription factor pools and competes with repressor

HLH transcription factors such as MNT and MAD. We have identified a number of putative Nmyc regulatory genes and have used CHP analysis to determine how Nmyc alters in vivo binding of HLH family members to target genes. Using EMSA and CHP analysis, we have detailed how general E box binding and specific Nmyc target genes are regulated during RA-induced differentiation. Overexpression of Nmyc in developing murine sympathetic nervous system tissue leads to a murine model of neuroblastoma. We have studied how Nmyc affects cell cycle progression and differentiation. We find that expression of Nmyc leads to a decrease in p27 levels and a functional inactivation of the RB tumor suppressor path. In studying the mechanism by which RA mediates cell cycle control, we find that RA decreases Nmyc levels that leads to an increase in p27. Current studies are aimed at determining how Nmyc alters cell cycle in NB cells.

Differentiation

We found that NB cells constitutively express the neurotrophic survival factor BDNF. Induction of differentiation increases TrkB levels and leads to the activation of an autocrine survival and differentiation path. In NB tumors, Trks serve as tumor markers: good prognosis tumors express TrkA while most poor prognosis tumors express BDNF and TrkB. We first established that neuroblastomas expressed TrkB from our finding that although cell lines express little TrkA or TrkB, retinoids induces TrkB in vitro. We have studied the functional consequences of activation of Trk signal transduction paths in a NB cell line from a poor prognosis patient that expresses TrkA or TrkB by gene transfection or selective stimulation. Activation of the BDNF–TrkB path stimulates cell survival, induces neurite extension, alters chemosensitivity, and increases cell migration (invasion). In contrast, activation of the NGF–TrkA path decreases cell growth, invasiveness, and tumorigenicity. Collaborative studies are in progress to determine intracellular signaling molecules that may account for the different functional consequences of Trk activation in NB cells. Our studies indicate that Trks not only serve as tumor markers but also impact on the biology of the tumor cell. If mechanisms can be identified that increase TrkA and decrease TrkB expression in NB cells, then it may be possible to alter the biologic behavior of the tumor cells in vivo and this may have clinical applicability. To effectively manipulate the level of Trk mRNA and ultimately protein in a cell, molecular mechanisms regulating gene transcription need to be defined. We have cloned the human TrkB and TrkA promoters. The functional and biochemical characterization of the TrkB gene promoter and its regulation by RA identified a novel RA-inducible transcription unit involving an INR element and a 3′ hormone responsive element.

Suppression of Tumorigenicity

NB tumors are marked by 1pLOH, amplification of Nmyc. We have identified two candidate tumor suppressor genes on chromosome 1p. One is involved in the metabolism of retinoids and the other is involved in neural differentiation. Ongoing studies will functionally define whether these genes are altered in NB tumors and how expression affects the biology of NB cells.

NB Animal Models

We have developed orthotopic NB animal models in which human tumors are implanted above the adrenal gland. In orthotopic models the tumors retain the invasive and angiogenic pathologic properties characteristic of the human tumors—this is not seen in the subcutaneous or intramuscular xenograft models. We have utilized these models as well as the Nmyc mouse to characterize the response of NB tumors to a variety of novel therapeutics. These include retinoids and HDAC inhibitors. Expression of c-kit and SCF creates an autocrine survival loop in NB cells. Inhibition of this survival path leads to increased apoptosis in vivo. Evaluation of drugs targeted to the c-kit kinase will be evaluated in NB and other neuro-ectodermal tumors that have SCF/c-kit survival paths.

Recent Publications:

Giannini G, et al. *J Biol Chem* 1997;42:26693–701.

Matsuo T, et al. *Oncogene* 1998;16:3337–43.

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Biography: *Dr. Walsh received his M.D. from the Johns Hopkins University School of Medicine. He completed training in infectious diseases and oncology at Johns Hopkins, the University of Maryland, and the NCI, where he also received additional training in pathology, pharmacology, immunology, and medical mycology. As chief of the Immunocompromised Host Section in the Pediatric Oncology Branch, Dr. Walsh has combined these areas of expertise in addressing the multidisciplinary problems of infections in immunocompromised patients with cancer and HIV infection.*

Pediatric Oncology Branch
Diagnosis and Treatment of Infections in Patients With Cancer and HIV Disease

Keywords:

AIDS
animal models
Aspergillus species
bone marrow transplantation
Candida species
cytokines
drug resistance
host defenses
infection
molecular diagnostics
mycoses
pharmacology

Research: Infections are an important cause of morbidity and mortality in patients receiving treatment for cancer and those with HIV infection. Among the different types of infections, invasive fungal infections have rapidly emerged as leading causes of attributable mortality and morbidity. Typically involving the respiratory tract, bloodstream, liver, spleen, and central nervous system with devastating consequences, invasive fungal infections are notoriously difficult to diagnose and often refractory to conventional therapy. The organisms most commonly causing these infections are *Aspergillus* spp., *Candida* spp., and several drug-resistant emerging pathogens including *Fusarium* spp. and *Trichosporon* spp. Current treatment is often toxic and ineffective.

Successful intervention against invasive mycoses requires a combined strategy of (1) early diagnosis, (2) augmentation of host defenses, and (3) antifungal chemotherapy. Accordingly, the Immunocompromised Host Section's laboratory and clinical research program is organized along these three interventional strategies. The overriding strategy of our program is the translation of bench observations into rationally designed and scientifically grounded clinical trials that will advance therapeutic and diagnostic strategies against infections in patients with neoplastic diseases.

A core element of our program is a series of unique experimental animal models, which have been predictive for understanding the pathogenesis, diagnosis, and treatment of invasive fungal infections complicating neutropenia, bone marrow transplantation, and other immunocompromised states. These in vivo models, which are closely integrated with in vitro molecular, immunological, and pharmacokinetic systems, include models of invasive pulmonary aspergillosis, hepatosplenic candidiasis, and vascular catheter-related candidemia; esophageal candidiasis, disseminated; and pulmonary fusariosis. These in vivo systems have been used extensively for the investigation of the pharmacokinetics, pharmacodynamics, and comparative efficacy of novel antifungal compounds, including antifungal triazoles and lipid formulations of amphotericin B. Results from these laboratory animal studies have provided the scientific foundation for fluconazole treatment and prevention of disseminated candidiasis, liposomal amphotericin B therapy against invasive pulmonary aspergillosis and refractory mycoses, and triazole therapy of disseminated trichosporonosis. These models serve as the pivotal translational platform for phase I–II clinical trials conducted within the CCR for novel antifungal strategies. The results of these studies have led to the successful recent completion of three stringently powered multicenter randomized clinical trials addressing the role of empirical antifungal therapy using antifungal triazoles and liposomal amphotericin B in neutropenic children and adults. Our current work in antifungal pharmacology is directed at developing novel cell-wall active agents alone and in combination with immunomodulators and other antifungal compounds.

The next arm of our program investigates the cellular and molecular basis of pulmonary and systemic host defense against pathogenic fungi. These studies have recently demonstrated new properties of recombinant cytokines and interferon, including reversal of corticosteroid-induced impairment of phagocytic cells against *Aspergillus fumigatus* by GCSF and interferon gamma, and amelioration of neutrophil and monocyte dysfunction against *A. fumigatus* in HIV-infected patients by GCSF. We are currently investigating the role of augmentation of monocytic and macrophage host defenses by MCSF and IL–15 as an adjunct to antifungal therapy during neutropenia. Extending these studies of systemic host defenses to the investigation of antimicrobial peptides in mucosal host defenses, our team is characterizing the antifungal effects and mechanisms of action of histatin and a newly identified family of mucosal peptides, adrenomedullins. We have recently found the presence of adrenomedullin and its gene-related peptides in high concentrations in mucosal surfaces and identified for the first time the

antimicrobial activity of this new class of antimicrobial peptides. We are currently focusing on augmenting the expression of these protective peptides on mucosal surfaces through gene therapy and cytokine regulation.

The third arm of our program entails the development of surrogate markers for diagnosis and therapeutic monitoring of invasive candidiasis, aspergillosis, and multidrug-resistant pathogens. These markers include enolase antigen and antibody, galactomannan, D-arabinitol, glucuronoxylomannan, D-mannitol, and ribosomal RNA nucleic acid fragments. Several large, ongoing, prospective clinical trials of antifungal compounds are employing these immunologic and molecular markers as nonculture surrogates of therapeutic response. Our focus is now to adapt these markers to high throughput systems for general clinical laboratory utilization. Further enhancing strategies for detection is an expanding new initiative in molecular epidemiology of nosocomial infections. The results from these studies may provide a rational approach to prevention of transmission of organisms to high-risk patients. In parallel with early detection is the recognition of high-risk patients through genetic markers; such studies are being pursued in collaboration with Dr. Stephen Chanock.

In summary, our laboratory program is meeting the challenges of emerging opportunistic fungal pathogens in our patients with cancer through translational research studies of antifungal pharmacology, augmentation of mucosal and systemic host defenses, and early molecular detection. Such studies provide the foundation for clinical trials and a model for approaching emerging infectious pathogens in patients with cancer.

Recent Publications:

Roilides E, et al. *J Infect Dis* 1998;178:589–92.

Groll AH, et al. *Antimicrob Agents Chemother* 1998;42:2700–5.

Roilides E, et al. *Infect Immun* 1999;67:3047–50.

Walsh TJ, et al. *N Engl J Med* 1999;340:764–71.

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Biography: *Dr. Wayne received his M.D. from Northwestern University and carried out his residency and fellowship training at Children's Hospital and the Dana-Farber Cancer Institute in Boston. Past positions have included associate director of the blood bank at Boston Children's, director of transfusion medicine in the Division of Allergy/Immunology/Bone Marrow Transplantation at the University of South Florida/All Children's Hospital, and director of pediatric bone marrow transplantation at the University of Miami. In July 1999, Dr. Wayne joined the NCI as the clinical director of the Pediatric Oncology Branch (POB) and as a clinical tenure track investigator. In addition to overseeing the clinical service, Dr. Wayne directs the Fellowship Training Program in Pediatric Hematology-Oncology, which became a joint program with Johns Hopkins University in 2000. Dr. Wayne is a diplomate of the American Board of Pediatrics and the Subboard of Pediatric Hematology-Oncology.*

Pediatric Oncology Branch
Experimental Therapeutics of Pediatric Hematologic Malignancies

Keywords:
cancer immunotherapy
chemotherapeutics
childhood tumors
leukemia
lymphoma
stem cell transplantation
targeted immunotherapy

Research: The primary objective of the Hematologic Diseases Section is to develop new treatment strategies for hematopoietic malignancies of childhood and adolescence. Although current frontline treatments are highly effective for pediatric patients with leukemias and lymphomas, new therapeutic approaches are needed to overcome resistance to and decrease toxicities associated with standard regimens. Current research efforts include clinical trials of novel, nonmyeloablative allogeneic stem cell transplant regimens designed to achieve complete donor chimerism with improved toxicity profiles. Biologic correlative studies conducted as part of these trials include valuation of chemotherapy-induced immunosuppression, immune recovery after allogeneic transplantation, and the pathophysiology of graft-versus-host disease. Additional clinical trial activities include phase I development of immunotoxins for refractory leukemias and lymphomas. In an effort to improve understanding of the molecular mechanisms of leukemogenesis and identify potential new therapeutic targets, collaborative investigations of gene expression profiles of pediatric leukemias are also being conducted.

Our collaborators include Michael Bishop, Daniel Fowler, Ronald Gress, Robert Kreitman, Ira Pastan, and Thomas Waldmann, NIH; Curt Civin, John's Hopkins University; and Paul Meltzer, NHGRI.

Recent Publications:

Weitzman S, et al. *Med Pediatr Oncol* 1999;33:476–81.

Walters MC, et al. *Blood* 2000;95:1918–24.

Wayne AS, et al. *Blood* 2000;96:2369–72.

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Biography: *After obtaining her M.D. from the medical school of the University of Cologne, Germany, in 1986, Dr. Widemann became board certified in pediatrics in 1992 and served the NCI in fellowship training until 1995. Since then, she has been a member of the Pediatric Oncology Branch and, since 1999, a tenure track investigator.*

Pediatric Oncology Branch
Clinical Pharmacology and Development of Novel Drugs for Children With Cancer and Neurofibromatosis Type 1

Keywords:
childhood tumors
drug development
molecular targets
neurofibromatosis type 1
pharmacokinetics
plexiform neurofibromas
surrogate markers

Research: Anticancer drug discovery and development are moving towards a more rational and targeted approach. The application of new molecularly targeted agents to the treatment of childhood cancers and neurofibromatosis type 1 (NF1) is a research objective of the Pharmacology and Experimental Therapeutics Section (PETS). In addition to studying the pharmacology, pharmacokinetics, pharmacodynamics, and toxicities of these novel agents, it is also a goal of the PETS to evaluate novel clinical trial designs and trial endpoints, which may be more applicable for molecularly targeted agents. The clinical development of farnesyltransferase inhibitors (FTI), which inhibit the posttranslational farnesylation required for the activity of wild-type and mutant ras proteins for patients with NF1 and refractory leukemias serves as an example for this approach. Pharmacodynamic endpoints that assess the effect of FTI on the target enzyme and farnesylation of cellular proteins are important endpoints of these trials. The clinical development of anti-metabolites, such as raltitrexed, and agents that modulate the effects of antimetabolites, such as the recombinant bacterial enzyme, carboxypeptidase-G2 (CPDG2), is another research focus. CPDG2 hydrolyzes methotrexate (MTX) to inactive metabolites. CPDG2 provides an alternative route of elimination for MTX for patients with high-dose MTX- induced renal dysfunction, and plasma MTX concentrations declined by >95 percent within minutes in all patients. The intrathecal administration of CPDG2 has also been successfully used as a rescue agent in patients who received accidental overdoses of intrathecal MTX. A new drug application for the use of CPDG2 in HDMTX-induced renal dysfunction will be filed based on our data.

We have collaborated with Robert Arceci, Johns Hopkins Oncology Center, Baltimore, MD; Frank Balis, T. Fojo, Elizabeth Fox, and Kathy Warren, NIH; Jean Belasco, Children's Hospital of Philadelphia; Susan Blaney, Baylor University; Stewart Goldman, Children's Memorial Hospital, Chicago, IL; David Gutmann, Washington University School of Medicine, St. Louis, MO; Douglas Hyder, Children's Hospital of Los Angeles; Regina Jakacki, Children's Hospital of Pittsburgh, PA; Mark Kieran, Dana-Farber Cancer Institute; Bruce Korf, Partners Center for Human Genetics, Boston, MA;

Roger Packer, Children's Hospital National Medical Center; and Gregory Reaman, Children's Oncology Group.

Recent Publications:

Widemann B, et al. *J Clin Oncol* 1997;15:2125–34.

Widemann B, et al. *Cancer Chemother Pharmacol* 1999;44:439–43.

Widemann B, et al. *JPET* 2000;294:894–901.

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Biography: *Dr. Wigginton received his M.D. in 1989 from the University of Michigan Medical School and completed a residency in pediatrics in 1992 at the Mott Children's Hospital of the University of Michigan Medical Center. He next completed fellowship training in pediatric hematology-oncology in 1995 at the Pediatric Oncology Branch, NCI, including laboratory studies with Dr. Robert Wiltrout, Laboratory of Experimental Immunology. Dr. Wigginton was recruited as a tenure-track investigator and head, NCI–CCR Translational Research Initiative, in 2001. He also holds an appointment as assistant professor of pediatrics and pediatric oncology, Johns Hopkins University School of Medicine. His laboratory, the Investigational Biologics Section, studies antitumor mechanisms engaged by biologically-targeted strategies for the treatment of neuroblastoma and renal cell carcinoma, and seeks to translate these new approaches into the clinical setting.*

Pediatric Oncology Branch
Biological Therapy and Modulation of the Tumor Microenvironment: New Strategies for the Treatment of Neuroblastoma and Renal Cell Carcinoma

Keywords:
angiogenesis
animal models
apoptosis
clinical trials
cytokines
immunotherapy
neuroblastoma
renal cell carcinoma
translational research

Research: In recent years, intensive effort has focused on the investigation of biological approaches to the treatment of cancer, including immunotherapy utilizing cytokines, antibodies and/or vaccines, and gene therapy, as well as inhibition of tumor angiogenesis. Although immunotherapeutic approaches such as systemic administration of interleukin 2 (IL–2) have provided meaningful benefit to some patients with metastatic renal cell carcinoma or melanoma, many questions remain regarding the best approach to maximize the potential efficacy of biological therapy. Given the complexity of signals engaged during the host antitumor immune response, and the intricate network of interactions within the tumor microenvironment, it appears that, much as has occurred with the clinical evolution of combination chemotherapy, the full potential of biological therapies for cancer will most likely be realized using rationally-designed combinations of agents with complementary mechanisms of action.

Laboratory studies: The Investigational Biologics Section investigates molecular mechanisms by which the host immune response may be engaged to induce alterations in the tumor microenvironment to effect disease

regression (i.e., modulation of tumor neovascularization and induction of tumor and/or endothelial apoptosis), and also use these observations to facilitate the design of novel biologically-targeted treatment strategies for neuroblastoma and/or renal cell carcinoma. In these studies, we utilize a range of molecular, cellular immunology, and histopathology techniques, as well as novel orthotopic models of transplantable murine neuroblastoma and renal cell carcinoma, an N-myc transgenic mouse model of spontaneous neuroblastoma, knock-out mice with targeted disruption of genes encoding key mediators of the host immune response, and unique in vivo angiogenesis assay systems we have established. Ultimately, where therapeutically-active regimens are identified, these approaches are assessed in primate toxicology testing and translated into phase I/II clinical investigations in adults with solid tumors such as renal cell carcinoma and/or children with neuroblastoma. In conjunction with these clinical trials, studies are performed to investigate the relevance of preclinical hypotheses utilizing novel tumor imaging technologies as well as cellular and molecular studies of leukocyte effector cell populations and/or tumor specimens obtained from treated patients. Much of our recent effort has focused on investigation of the antitumor activity of two cytokine-based combinations, IL–12/pulse IL–2 and IL–18/IL–2, and delineation of the respective mechanisms which mediate their therapeutic efficacy. In mice bearing well-established primary and/or metastatic neuroblastoma or renal cell carcinoma tumors, systemic administration of IL–12/pulse IL–2 can induce complete durable tumor regression in 80 percent or more of treated mice. Comparable responses are achieved after treatment with IL–18+/– IL–2. We have now defined several of the critical mechanisms by which these therapies can modulate the local tumor microenvironment to induce disease regression. Notably, IL–12/pulse IL–2 synergistically enhances IFN-γ production and induces the IFN-γ-dependent expression of both Fas and Fas-L genes within the local tumor site. In turn, IL–12/pulse IL–2 induces rapid vascular endothelial injury with tumor and/or endothelial apoptosis, and inhibits tumor neovascularization and mediates overall tumor regression via mechanisms which share a common dependency on IFN-γ and the Fas/ Fas-L apoptosis pathway. Collectively, these observations suggest a mechanism whereby CD8+ FAS–L+ T cells infiltrate the local tumor site and interact with Fas+ vascular endothelial and/or tumor cell populations to induce apoptosis, inhibition of angiogenesis, and, ultimately, overall tumor regression. Recent studies also have now shown that the antitumor activity of IL–18/IL–2 is critically dependent on CD8+ T cells, IFN-γ, and the FAS/FAS–L pathway, but not IL–12. To specifically address the role of tumor versus host cell (i.e., endothelial) responsiveness to biological therapies such as IL–12/pulse IL–2 or IL–18/IL–2, N-myc transgenic mice are being bred with relevant knock-out strains to establish N-myc transgenic–knock-out murine hosts and/or novel derivative neuroblastoma cell lines with targeted disruption of the genes encoding the IFN-γ receptor, and proapoptotic genes such as Fas. Future studies will compare the patterns of gene expression induced by IL–12 versus IL–18 and seek to identify novel molecular targets engaged by these cytokines in neuroblastoma-bearing mice using cDNA microarray analysis of specific leukocyte subsets and/or tumor cell populations.

In other studies, we are investigating the angiogenic phenotype and basic mechanisms governing the neovascularization of neuroblastoma tumors. We have demonstrated marked constitutive expression of vascular endothelial growth factor (VEGF) and FLT–1/FLK–1, angiopoietin-1, and TIE–2, as well as the matrix metalloproteinases MMP–2 and MMP–9, by both murine and human neuroblastoma tumors and cell lines. Further, although treatment of tumors with immune-based therapies such as IL–12/pulse IL–2 inhibits tumor neovascularization and induces local expression of antiangiogenic, IFN-γ inducible CXC chemokines including IP–10 and MIG, there is no apparent impact on the expression of various proangiogenic mediators and/or their receptors. These observations suggest that targeted antagonists of proangiogenic mediators such as VEGF could play a role in the treatment of neuroblastoma, and that the efficacy of antiangiogenic immunoregulatory cytokines such as IL–12 might be expanded by combined administration with targeted antagonists of proangiogenic mediators such as VEGF for the treatment of neuroblastoma, a proposed immunoangiostatic approach.

Clinical investigation: Based on the potent efficacy of IL–12/pulse IL–2 in preclinical tumor models, we subsequently designed and executed a primate toxicology evaluation of the safety of this combination in cynomolgus macaques, and have now initiated a phase I investigation of IL–12/pulse IL–2 in adults with advanced solid tumors. Guided by novel mechanisms we have identified in the preclinical setting, a set of hypothesis-driven translational studies have been incorporated into this clinical trial to investigate antitumor mechanisms engaged by the administration of IL–12/pulse IL–2 in humans. These include characterization of the functional immunoregulatory effects of IL–12/pulse IL–2 in vivo, prospective quantitative assessment of the antivascular activity of IL–12/pulse IL–2 utilizing dynamic-enhanced MRI (DEMRI) scans, and investigation of molecular mechanisms mediating the ability of IL–12/pulse IL–2 to inhibit tumor neovascularization and induce tumor and/or endothelial apoptosis via an analysis of gene expression in tumor biopsy specimens. Additional studies are now planned using IL–12/pulse IL–2 as well as IL–18-based approaches in children with neuroblastoma and adults with solid tumors such as renal cell carcinoma.

Our collaborators include Susan Bates, Peter Choyke, Lee Helman, John Janik, Javed Khan, Joseph Tomaszewski, Robert Wiltrout, NIH; Jared Gollob, Harvard Medical School; Zdenka Jonak, Glaxo-Smithkline; Paul Sondel, University of Wisconsin Medical School; Stanley Wolf, Genetics Institute; and Maurice Wolin, Chiron Corporation.

Recent Publications:

Wigginton JM, et al. *J Natl Cancer Inst* 1996;88:38–43.

Wigginton JM, et al. *J Clin Invest* 2001;108:51–62.

Wigginton JM, et al. *J Immunol* 2001;166:1156–68.

Clinical Trials:

Frank M. Balis

90–C–0095: A phase I study of intrathecal mafosfamide

98–C–0141: A phase I trial and pharmacokinetic study of the farnesyltransferase inhibitor R115777 in pediatric patients with refractory solid tumors

99–C–0039: A phase I trial and pharmacokinetic study of TLC D–99 in pediatric patients with refractory solid tumors

99–C–0161: Pharmacokinetics of etoposide in patients with breast cancer receiving high-dose etoposide

00–C–0070: A phase I trial and pharmacokinetic study of arsenic trioxide in pediatric patients with refractory leukemia or lymphoma

00–C–0092: A randomized trial of SD/01–filgrastim vs. filgrastim in newly diagnosed children and young adults with sarcoma treated with dose-intensive chemotherapy

00–C–0105: A phase I trial and pharmacokinetic study of temozolomide and O6–benzylguanine in childhood solid tumors

01–C–0091: A phase I trial and pharmacokinetic study of XR9576 ap-glycoprotein inhibitor, in combination w/Doxorubicin, Vinorelbine or Docetaxel in pediatric patients with refractory solid tumors including brain tumors

01–C–0123: A phase II study of intrathecal Topotecan (NSC#609699) in patients with refractory meningeal malignancies, a POG/CCG intergroup study

Steven Chanock

83–C–0022: Psychological benefits of a "normalized" camping experience for children with cancer and HIV infection

Lee J. Helman

98–C–0037: Evaluation, treatment, and natural history of children with cancer

99–C–0125: Osteosarcoma: Outcome of therapy based on histological response. A collaborative effort of the POB /NCI, Texas Children's Hospital, and the University of Oklahoma

Crystal L. Mackall

97–C–0050: A pilot study of tumor-specific peptide vaccination and IL–2 with or without autologous T cell transplantation in recurrent pediatric sarcomas. The trial is accruing patients at this time

97–C–0052: A pilot study of autologous T cell transplantation with vaccine-driven expansion of antitumor effectors after cytoreductive therapy in metastatic pediatric sarcomas. The trial is accruing patients at this time

Thomas J. Walsh

98–C–0063: A randomized open label, comparative, multicenter trial of Voriconazole versus AmBisome for empirical antifungal therapy in immunocompromised patients with persistent fever and neutropenia

98–C–0137: Evaluation of the association of polymorphisms in the innate immune system with the risk for *Cryptococcus neoformans* infection in patients not infected with HIV and complications associated with *Cryptococcus neoformans* infection

Clinical Trials (continued):

Thomas J. Walsh	**98–C–0138:** Evaluation of the association of polymorphisms in the innate immune system with the risk for *Blastomyces dermatitidis* infection in patients not infected with HIV and complications associated with *Blastomyces dermatitidis* infection
	99–C–0007: A phase I study of the safety, tolerance, and pharmacokinetics of FK463 in immunocompromised children with fever and neutropenia
	99–C–0094: An open label, noncomparative, multicenter trial of the efficacy, safety, and toleration of Voriconazole in the primary or secondary treatment of invasive fungal infections
	00–C–0014: A phase III randomized, double-blind, comparative trial of FK463 versus fluconazole for prophylaxis of fungal infections in patients undergoing a hematopoietic stem cell transplant
Alan S. Wayne	**01–C–0125:** Pilot study of nonmyeloablative, HLA-matched allogeneic stem cell transplantation for pediatric hematopoietic malignancies
Brigitte C. Widemann	**92–C–0137:** A trial of carboxypeptidase-G2 (CPDG2) for the management of patients with intrathecal methotrexate overdose
	98–C–0053: A pharmacokinetic and pharmacodynamic study of vincristine in children with leukemia
	01–C–0027: Natural history of plexiform neurofibromas in neurofibromatosis type 1 (NF1)
	01–C–0196: A phase I trial and pharmacokinetic study of RL15777 in pediatric patients with refractory leukemias
	01–C–0222: A phase II randomized, cross-over, double-blinded, placebo-controlled trial of the farnesyltransferase inhibitor RL15777 in pediatric patients with neurofibromatosis type 1 and progressive plexiform neurofibromas
Jon W. Wigginton	**00–C–0121:** A phase I investigation of IL–12/pulse IL–2 in adults with advanced solid tumors

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The Radiation Biology Branch research activities are focused on preclinical basic science research aimed at identifying and incorporating novel approaches to cancer treatment and evaluation. Emphasis is placed on gaining a better understanding of the mechanisms of cell killing and protection at the molecular, biochemical, cellular, and physiological levels for a variety of cancer treatment modalities including ionizing radiation, cytotoxic and noncytotoxic drugs, combinations of drugs and radiation, biological modifiers, sonosensitizers, and a variety of agents that impose oxidative stress. Additionally, the development of novel functional imaging devices has progressed to the point that we can now evaluate the efficacy of noninvasive in vivo oxygen concentration assessment and the potential application of tissue redox mapping.

The branch is divided into three sections: Tumor Biology, Molecular Mechanisms, and Biophysical Spectroscopy. The Tumor Biology Section employs a variety of experimental models (both in vitro and in vivo) with the aim of identifying ways to improve cancer treatment. A variety of cancer treatment modalities are evaluated both alone and in combination with the purpose of understanding chemical and biochemical modes of action. cDNA microarray technology is being used to interrogate gene expression profiles following treatment with a variety of oxidants. The Molecular Mechanisms Section continues to explore the utility of intracellular immunity and the inhibition of intracellular target molecules. In addition, the section provides molecular biology support/collaboration for the other sections. The focus of the Biophysical Spectroscopy Section is the development, testing, and refinement of low-frequency electron paramagnetic resonance (EPR) functional imaging devices suitable for in vivo imaging of paramagnetic species. Additionally, research is currently under way to define basic chemical mechanisms involved in sonodynamic therapy as well as to understand the mechanism of action of sonosensitizers.

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Biography: *Dr. Mitchell received his Ph.D. from Colorado State University in cellular radiation biology in 1978. He came to the NIH and the Radiation Oncology Branch of the NCI in 1979 and became an independent investigator in 1984. He served as chief of the Radiobiology Section and later as deputy branch chief of the Radiation Oncology Branch. In 1993, he was named chief of the Radiation Biology Branch.*

Radiation Biology Branch
Modification of the Radiation Response

Keywords:
free radicals
functional imaging
oxidative stress
radiation protection
radiation sensitization

Research: The term "oxidative stress" has emerged to encompass a broad variety of biological stresses, some of which have obvious implications for health care. Several modalities used in cancer treatment including x-rays, phototherapy, and some chemotherapy drugs exert their cytotoxicity by producing oxygen-related free radicals, which imposes an added burden of oxidative stress to normal cellular detoxification systems. Toxic oxygen-related species including superoxide, hydrogen peroxide, and hydroxyl radical are produced by diverse initiating agents and both chronic and acute diseases. When left unchecked, these redox active species undoubtedly damage cells and tissues. There is an obvious interest in discovering and implementing additional approaches, apart from inherent intracellular detoxification systems, to protect cells, tissues, animals, and humans against oxidative stress.

We have identified a class of compounds known as nitroxides (stable free radicals) as being protectors against oxidative stress resulting from hydrogen peroxide, ionizing radiation, and selected redox-cycling chemotherapy drugs. We are currently evaluating a number of nitroxides as radiation protectors of normal tissues. Selective protection of normal tissues in cancer patients receiving radiation treatment would be advantageous. If such selective protection of normal tissues were possible, higher radiation doses could be delivered to the tumor, accompanied possibly by higher local control rates. Recent studies using a murine model show that nitroxides protect normal tissues but do not protect tumor. We are currently attempting to define the mechanism of selective radioprotection. A possibility may be a differential rate of reduction of the nitroxide in normal versus tumor cells. Using magnetic resonance imaging techniques being developed in the branch, free radical distribution in tissue can be assessed. This may allow for noninvasive determination of redox status and oxygen levels in three dimensions in normal and tumor tissue. Other more mechanistic studies are presently being directed on determining what impact radioprotectors (such as the nitroxides) may have on radiation-induced early response genes and signal transduction pathways. Our selection of nitroxide structures may be of particular advantage since we have experience with nitroxides that freely diffuse into cells, others that do not go into cells and would remain extracellular, and finally others that partition selectively into lipid environments. The ability to

"place" nitroxides in different locations with respect to the cell could give information about the location of targets involved in radiation-induced transduction pathways. We have recently shown that nitroxides protect against cytotoxicity and mutation induction mediated by specific estrogen metabolites. We are currently exploring the chemical/biochemical mechanism of these observations using electron paramagnetic resonance technology. We have begun preliminary studies using cDNA microarray technology to further explore and define the complex molecular events imposed by oxidative stress to better understand the relationship between gene expression and cellular response following treatment with various oxidants including nitroxides, hydrogen peroxide, nitric oxide, and ionizing radiation.

Another way to modify the radiation response that may have utility in cancer treatment is the use of radiation sensitizers. The overall objective here is to obtain a therapeutic gain by providing selective radiosensitization in tumors as compared to normal tissues. Admittedly, this has been and continues to be a difficult goal to achieve. We are presently evaluating in human tumor cell lines the cytotoxicity, cell cycle distribution, and radiosensitization profiles of such drugs as MGI–114, PS–341(proteasome inhibitor), halifuginone (TGFβ inhibitor), and UCN–01.

Recent Publications:

Mitchell JB, et al. *Ann NY Acad Sci* 2000;899:28–43.

Bernhard EJ, et al. *Cancer Res* 2000;60:86–91.

Rak R, et al. *J Neurosurg* 2000;92:646–51.

Samuni Y, et al. *Radiat Res* 2001;155:304–10.

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Biography: *Dr. Krishna obtained his Ph.D. in physics from the Indian Institute of Technology, Madras, India, in 1984 and joined the NCI the same year. He became an independent investigator in 1993. His research interests include studies of free radical processes in causing oxidative injury and antioxidant defense. More recently, he has been involved in developing functional imaging tools to study tumor physiology.*

Radiation Biology Branch
Magnetic Resonance Imaging of Tumor Physiology

Keywords:
EPR imaging
free radicals
functional imaging
oxygen mapping

Research: Characterizing tumors and related pathological conditions on a physiologic basis by noninvasive radiological techniques is a potentially useful capability for diagnosis as well as for devising appropriate cancer treatment approaches. Magnetic resonance imaging (MRI) is a commonly used clinical imaging procedure that provides images of soft tissue anatomy with excellent detail. Recent advances have made it possible to provide

functional information such as brain function using functional MRI (fMRI) techniques. To obtain physiological information such as oxygen or tissue redox stats in pathological situations such as solid tumors or ischemic/hypoxic tissue, quantitatively and noninvasively, we are developing imaging techniques based on electron paramagnetic resonance (EPR). EPR is similar to nuclear magnetic resonance (NMR) in many ways. NMR probes nuclei such as 1H, 13C, and 31P, while EPR detects paramagnetic species. Paramagnetic species by definition contain unpaired orbital electrons. Examples of paramagnetic species are free radicals and transition metal complexes such as iron and copper. While EPR spectroscopy is used to detect metal complexes and free radicals for medical imaging applications, stable free radicals with simple EPR spectra are desirable. Examples of free radicals, which can be used at nontoxic levels for in vivo imaging, are nitroxides and trityl radicals. These species have simple EPR spectra and have long in vivo pharmacological half-lives. The intrinsic EPR spectral property of the free radical, such as the spectral bandwidth (full width of the absorption band at half maximum) is directly related to oxygen concentration. Imaging modalities, which can map the spatial distribution of the free radical concentration and extract the spectral information on a pixel-by-pixel basis, will therefore provide oxygen maps of the object being imaged. Based on this theme, we have developed instrumentation for in vivo detection and imaging of free radical contrast agents that can be detected and imaged in experimental animals such as mice and rats. Three separate imaging configurations for the detection and imaging of free radicals are being developed and evaluated for medical noninvasive imaging applications:

- FT EPR imaging: With this method, the free radicals are imaged by collecting their responses after pulsed excitation under static gradient magnetic fields. Using this technique, we are able to obtain pO_2 images within 2 minutes.
- Continuous wave EPR imaging: Using free radical contrast agents, which participate in redox reactions in tissue, it is possible to perform pharmacokinetic imaging experiments in which the free radical tracer is converted to a nonmagnetic species by intracellular redox processes. Using this imaging technique, it will be possible to examine differences in redox status between normal and pathological conditions noninvasively.
- Overhauser enhanced MRI (OMRI): This is a hybrid technique, which uses the inherent sensitivity of EPR spectroscopy to enhance the intensity of images obtained by conventional MRI. This technique therefore requires a free radical contrast agent like in EPR imaging. The object to be imaged is infused with the free radical contrast agent and placed in a coil, which is tuned to the resonant frequency of the electron and proton. The MRI sequence is preceded by RF irradiation specific to the free radical. The extent of image enhancement is inversely dependent on the concentration of oxygen. Using this technique, it is possible to obtain anatomically coregistered pO_2 maps in less than 10 minutes. Our studies in tumor bearing mice show that tumors of ~1 cm in diameter contain regions of significantly hypoxic regions as well as normoxic regions.

We are assessing these three techniques as potential functional imaging modalities, which can be hopefully used in humans to provide important physiologic information.

Recent Publications:

Devasahayam D, et al. *J Magn Reson* 2000;142:168–76.

Afeworki M, et al. *Magn Reson Med* 2000;43:375–82.

Koscielniak J, et al. *Rev Sci Instrum* 2000;71:4273–81.

Krishna MC, et al. *Semin Radiat Oncol* 2001;11:58–69.

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Biography: *Before joining the National Cancer Institute as a research chemist in 1958, Dr. Riesz was a research associate in the chemistry departments at the Argonne National Laboratory, Brookhaven National Laboratory, and Pennsylvania State University. He obtained his Ph.D. in physical chemistry at Columbia University in New York in 1953 and his B.A. and B.Sc. degrees at Oxford University in England in 1947.*

Radiation Biology Branch
Electron Paramagnetic Resonance (EPR) Studies of Chemical and Biological Effects of Ultrasound, Light, and Ionizing Radiation

Keywords:
electron paramagnetic resonance (EPR)
sonochemistry
sonodynamic therapy
spin trapping

Research: Recently it has been shown that the combination of ultrasound and certain drugs (sonosensitizers) is a promising modality for cancer treatment. The effectiveness of sonodynamic therapy has been demonstrated in cell studies and in tumor-bearing animals. Our studies indicate that the mechanism of this drug-dependent sonosensitization involves the formation of free radicals from the sonosensitizer that react with oxygen to form peroxyl and alkoxyl radicals that have a higher probability of reaching critical cellular sites than hydroxyl radicals and hydrogen atoms, which are initially formed by ultrasound in the cavitation bubbles. Our laboratory is focused on the detection and identification of free radical intermediates in sonochemistry (related to sonodynamic therapy) and radiation chemistry (related to radiation therapy). Some recent results and ongoing projects are summarized below:

- Using the temperature dependence of the kinetic deuterium isotope effect, we have estimated that the effective temperatures in collapsing cavitation bubbles in aqueous solutions are in the region of 2,000–4,000K.
- An EPR spin trapping investigation of the sonochemistry of neutral aqueous solutions has shown that hydrated electrons are not a significant intermediate. This is in agreement with the hot spot theory of sonochemistry and provides evidence against the electrical discharge theories.
- Low concentrations of gallium porphyrin ATX–70 significantly enhanced cellular toxicity in human leukemia HL–525 cells exposed to 50 kHz ultrasound. Extracellular localization of ATX–70 molecules was found to be effective for sonosensitization.

- Combination of focused ultrasound, which can penetrate deeply into tissue with sonodynamic sensitizers, may prove to be a useful approach for locally intensive chemotherapy.
- The effect of gas-containing microspheres and echo-contrast agents on free radical formation by ultrasound was studied. Possible deleterious consequences of the formation of sonochemical intermediates may have to be assessed, particularly since some of the contrast agents lower the cavitation threshold of diagnostic ultrasound.
- The free radical intermediates formed during the ultrasound exposure of cell culture media were identified by EPR spin trapping. The dominant free radicals were derived from the hydrophobic amino acids Trp, Phe, Tyr, Leu, Val, and Met consistent with the accumulation of hydrophobic solutes at the liquid-gas interface of collapsing cavitation bubbles.
- The effects of cysteamine and cystamine on the sonochemical accumulation of hydrogen peroxide has been investigated. The protective effect of cysteamine and the lack of protection of cystamine on mammalian cells exposed to ultrasound is due to their differential abilities to lower hydrogen peroxide yields without the necessity of invoking intracellular cavitation.

Recent Publications:

Kondo T, et al. *Free Radic Biol Med* 1998;25:605–11.

Misik V, et al. *Free Radic Biol Med* 1999;26:936–43.

Misik V, et al. *Free Radic Biol Med* 1999;26:961–7.

Misik V, et al. *Ann NY Acad Sci* 2000;899:335–48.

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Photo not available

Biography: *Dr. Russo received his Ph.D. in bio-organic chemistry in 1973 and his M.D. in 1978 from Louisiana State University. He came to the National Cancer Institute in 1980 as a clinical fellow in medical oncology after finishing an internal medicine residency at Alton Ochsner Medical Foundation. He became an independent investigator in 1985. Before becoming the chief of the Molecular Mechanisms Section within the Radiation Biology Branch, Dr. Russo was an associate in the Radiation Oncology Branch working on basic research issues related to radiation and medical oncology.*

Radiation Biology Branch
Development of Protein Arrays for Binding of Intracellular and Surface Proteins

Keywords:
oxidative stress
phage library
sentinel node

Research: Over the last several years, binding proteins have been molecularly engineered by randomly generating strings of consecutive mutations within one or more of the hypervariable complimentary determining regions within the heavy-chain variable immunoglobulin framework. The variable regions of

the reengineered single peptide have been displayed as fusion proteins in a phage library cassette. The size of the library depends on the number of nucleotides mutated within the complimentary determining region, and the phage libraries we have created range from 107 to 109 binding proteins. Specific binding proteins that have been shown to bind to targets of interest are reengineered for placement into eukaryotic shuttle vectors for expression in eukaryotic cells, and the effect of such expression is being assessed. Likewise, the aleatoric library has been altered for inducible expression as well as intracellular compartment trafficking. The aim is to develop de facto intracellular immunity that will allow inactivation of targets within the cell. The research should provide a clearer understanding of the function of targeted proteins. In addition, binding proteins can be discovered that function by interacting with transducing or transactivating proteins or specific nucleotide motifs and thereby act themselves as inhibitors of regulators molecules. Examples of projects being studied are listed here.

- In a murine cell model the effects of inactivating the intracellular target glucose-6-phosphate dehydrogenase (G6PD) have been examined and our results show that G6PD can be inactivated. After induction of expression of the binding protein has been stopped, full G6PD activity is returned. The loss of G6PD activity is dependent on calpain I-sensitive pathways. Currently, binding proteins from the array that bind but do not inactivate G6PD in the screens are being studied to determine if cytoplasmic binding results in inactivation of G6PD. If that is the case, then the method may have a wide range of uses to inactivate unique proteins within the cell without knowing their function.

- In a human breast tumor cell model, the binding protein library is being screened to determine if a specific binding protein can be found that recognizes unique surface antigens on cancer cells. The potential of the method is that without having to screen mRNA, one may be able to find differentially translated proteins. The natural consequence would be a capability to manipulate the function/activity of proteins' targets of previously unknown function, as well as potential to provide unique targets for therapeutic intervention.

- The binding protein library is being screened for unique proteins that bind to intranuclear proteins. As a target, the Ku complex is being used to demonstrate that its function can be inactivated. If the project is successful, the possibility of inactivating or suppressing transactivator or regulator proteins or repair enzymes should be established. Moreover, there is the potential to design studies to revert cells from malignant to normal phenotype.

Recent Publication:

Mulligan-Kehoe MJ, Russo A. *J Mol Biol* 1999;289:41–55.

Herscher LL, et al. *Oncology* 1999;13:11–22.

Offer T, et al. *FASEB J* 2000;14:1215–23.

Mitchell JB, et al. *Ann NY Acad Sci* 2000;899:28–43.

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Biography: *Dr. David A. Wink received his Ph.D. in chemistry at the University of California, Santa Barbara. Following a postdoctoral fellowship in biochemistry as a National Research Service Award recipient at the Massachusetts Institute of Technology, he joined the Laboratory of Comparative Carcinogenesis at the NCI Frederick Cancer Research and Development Center (FCRDC) as a staff fellow. He then joined the Radiation Biology Branch at the NCI in 1995, where he received tenure in November 1999.*

Radiation Biology Branch
The Role of Nitric Oxide in Cancer Treatment

Keywords:
chemotherapeutics
radiation

Research: Over the last decade, the role of free radicals and oxidative stress has been shown to be important in a vast number of biological processes. The diatomic molecule nitric oxide (NO) has been shown to participate in a large number of physiological processes ranging from cardiovascular and neurologic to playing essential roles in a variety of immunological responses. This broad range of biologic effects has created one of the fastest growing fields in biomedical science. With this in mind, we have sought to determine if there are roles for this diatomic radical in cancer treatment. Nitric oxide participates in various processes associated with cancer biology. Though NO possesses tumoricidal properties, it also can promote tumor growth. Our research is aimed at understanding the role of NO with different processes in tumor biology at the chemical, biochemical, cellular, and physiological levels to explore potential new strategies.

The major determinant for NO effects in vivo is its chemical properties. The chemistry of NO in biological systems is complex, and each reaction has potential deleterious or beneficial effects. To decipher the chemical reactions that may account for the paradoxical effects of NO, we have developed a discussion referred to as the "chemical biology of NO," which is a guide to the chemistry of NO that can take place in vivo. This compilation of chemical reactions provides the fundamental information needed to translate the chemistry of NO into methods for improving modalities of cancer treatment. There are currently three main projects:

- **Chemistry of NO in biology.** This research is dedicated to understanding the chemical, biochemical, and cellular mechanisms of NO, research that provides methods for detection as well as delivery (modulation) of NO chemistry. Other aspects involve what effect these chemical/biochemical reactions have on cytokine function and DNA repair. We are also starting to chart endogenous chemistry mediated by nitric oxide synthase in vitro and in vivo using various analytical techniques.
- **The chemical biology of NO and associated toxicological mechanisms.** This research is devoted to understanding what effect the chemical reactions of NO have on cytotoxicity and tissue damage.

- **NO and cancer therapy.** This research is dedicated to applying the basic chemical knowledge of NO to improve existing modalities of cancer treatment. We are exploring how NO donors and inhibitors may modulate chemotherapeutic agents and radiation response.

The investigation of the basic chemistry of NO as it relates to physiological, cellular, and signal transduction, coupled with the development of methods for the detection and delivery of NO in vivo, may be readily translated to strategies for improved treatment of cancer as well as other diseases.

Recent Publications:

Espey MG, et al. J Biol Chem 2000;275:11341–7.

Espey MG, et al. Ann NY Acad Sci 2000;288:209–21.

Miranda KM, et al. J Biol Chem 2001;276:1720–7.

Miranda KM, et al. J Nitro Oxide 2001;5:62–71.

Radiation Oncology Branch

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The Radiation Oncology Branch (ROB) is one of the three components of the recently configured Radiation Oncology Sciences Program (ROSP), which also includes the Radiation Biology Branch (RBB) and Radiation Research Program (RRP) within the Division of Cancer Treatment and Diagnosis (DCTD). The purpose of the ROSP is to assemble a critical mass of talent and expertise within the broad field of radiation oncology, which ranges from molecular and cellular biology through radiation biology and preclinical research, through imaging and clinical trials, to health policy, communications, and outreach to the community. Other investigators associated with ROSP are within the newly formed Center for Cancer Research (CCR) of the NCI intramural program. Technological expertise in patient imaging, treatment planning, and delivery are essential so that innovative molecular and biological therapy is built upon a superb technical platform. The ROB is in the process of upgrading its equipment so that we now have the capability for radiosurgery, intensity-modulated 3–D conformal radiotherapy, and real-time dose measurement. CT simulation and MR image fusion are now a routine part of treatment planning. A brachytherapy program is planned in the context of an intraoperative MR-guided surgical suite. Close collaboration is planned with the Imaging Sciences Program of the Clinical Center and diagnostic imaging experts in the Biomedical Imaging Program, DCTD. Described in the Molecular Therapeutics Section is the ROSP plan to link molecular imaging, signatures, and therapy, areas of emphasis among the NCI Director's Extraordinary Opportunities.

Accreditation Council on Graduate Medical Education: The Accredited Residency Training Program

The ROB and our collaborators, the National Naval Medical Center and Walter Reed Army Medical Center, are committed to training radiation oncologists with the knowledge and skills necessary so that the role of radiation therapy is optimized for the treatment of patients in the "post–human genome" era. The program has recently been broadened with the addition of pediatric oncology patients from DC Children's Hospital and from collaborative programs with Portsmouth Naval Hospital and Johns Hopkins University. For individuals interested in an academic career, there is the possibility of obtaining a Ph.D. degree as part of a joint program among the University of Maryland, Johns Hopkins, and the NCI. A recently established ASTRO–NCI fellowship helps support 2 postgraduate years of research. An ASTRO sabbatical program will begin in 2002 and bring in senior

investigators to ROSP. For information regarding the Training Program contact: Rosemary Altemus, M.D., Ph.D., ROB, B3B69, NIH, Bethesda, MD 20892–1002; phone 301–496–5457, Fax 301–480–5439.

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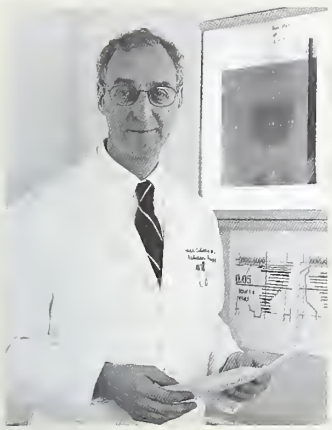

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Biography: *Dr. Coleman graduated from the University of Vermont with a B.A. in theoretical mathematics, then graduated from Yale University School of Medicine in 1970. He completed his internship and residency in internal medicine at the University of California in San Francisco and at the NCI. Board certified in internal medicine and radiation oncology, Dr. Coleman was a staff member at the Stanford University School of Medicine before joining Harvard Medical School in 1985. In 1999, he came to the NCI and became director of the new Radiation Oncology Sciences Program that he created to coordinate all radiation oncology activities there. He also serves the NCI as chief of the Radiation Oncology Branch, deputy director of the Center for Cancer Research, associate director of the Radiation Research Program, and special advisor to the director of the NCI. He has written extensively in his field and has won numerous awards.*

Radiation Oncology Branch
Experimental Therapeutics

Keywords:
animal models
apoptosis
brain tumor
cell cycle
cell proliferation
cell signaling
chemotherapeutics
clinical trials
cyclo-oxgenase
cytokines
drug development
genomics
head and neck cancer
hypoxia
inflammation
lipoxygenase
molecular imaging
monoclonal antibodies
NFκB
NSAIDs
pancreatic cancer
pharmacology
prostate cancer
proteomics
radiation
radioimmunotherapy
signal transduction

Research: The research program within the Radiation Oncology Branch (ROB) includes technical development, clinical investigation, combined modality therapy, imaging, and molecular therapeutics. An overarching theme of the ROB, in conjunction with the Radiation Biology Branch (RBB), will be a program closely linking molecular and functional imaging, molecular signatures and profiling, and molecular therapeutics, three major concepts in the NCI Director's Extraordinary Opportunities. The concept is to have a detailed investigation of a limited number of patients, that is, a "dense study," of imaging—including MR imaging and MR spectroscopy, PET, CT, and novel techniques such as EPR and optical imaging; molecular signatures—including genomics and proteomics as well as immunohistochemistry; and molecular therapeutics—with radiation, chemotherapy, immunotherapy, and biological therapies. A closely related theme is that radiation is "focused biology" in that it can create molecular changes within the radiation field. These changes may be useful in cell killing in conjunction with novel therapeutic approaches that by themselves are cytostatic—for example, antiangiogenesis treatment or radiation might be used to activate cellular processes that may become targets for molecular therapeutics. The concept of radiation dose may be redefined by molecular events produced rather than dose in an ionization chamber. The breadth of radiation oncology, including expertise in imaging, treatment, and basic science, lends itself well to being a linker among imaging, signatures, and therapeutics.

The research of the physics and chemistry groups and the new tenure track investigators are described herein. Clinical trials are being conducted and developed in conjunction with medical, pediatric, surgical, immunological, and pharmacological expertise at the NCI. Combined modality clinical trials are ongoing in head and neck cancer, breast cancer, prostate cancer, pancreas cancer, and brain tumors. The molecular therapeutics programs will undergo

rapid expansion with the recruitment of Drs. Tofilon, Gius, and Camphausen. Dr. Coleman is currently pursuing the role of nonsteroidal anti-inflammatory agents (NSAIDs) as radiation modifiers, including the COX-2 inhibitors. The potential mechanism of action includes a wide range of targets including the COX-2 enzyme, NFkB, antiangiogenesis (VEGF) and apoptosis-modifying effects. Collaborative studies with the Basic Research Laboratory (Dr. Amundsen and Fornace) and the Radiation Research Program (RRP) (Dr. Eric Chuang, ATC) will define novel molecular targets for radiation sensitization and protection of normal tissue from treatment-induced injury. The new Molecular Therapeutics Branch (Dr. Tofilon, RRP) serves as a national resource for studying novel radiation modifiers and will help bring new agents to clinical trials within the field of radiation oncology, including the RTOG, NCI, other research institutions and industry.

An important theme of Radiation Oncology Sciences Program (ROSP) is to serve as a national resource for the entire field of radiation oncology. This includes specific programs such as the Molecular Therapeutics program; training of residents, fellows, and senior faculty including a sabbatical program; conducting workshops to stimulate new ideas and collaborations as conducted by the RRP; and serving as a locus for the technological, imaging, and pharmaceutical industries to interact in a highly focused environment. Through the Telesynergy telemedicine program, the ROB is involved in bringing research opportunities to partners within our region and, in particular, with areas in which health disparities are significant problems. With the first wave of recruitment now completed, the next few years promise to bring a new level of energy, enthusiasm, and accomplishment to ROSP and to a central role for radiation oncology in the era of "molecular medicine."

We collaborated in this work with Sally Amundson, Al Fornace, Elaine Ron, Jeff Struewing, and Peggy Tucker, NIH.

Recent Publications:

Coleman CN. *Int J Radiat Oncol Biol Phys* 2001;49:301–9.

Palayoor ST, et al. *Oncogene* 1999;18:7389–94.

Stevenson MA, et al. *J Immunol* 1999;163:5608–16.

Stone HB, et al. *Radiat Res* 1998;150(2):134–47.

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Biography: *Dr. Brechbiel joined the NCI in 1983 and received his Ph.D. from American University in 1988. He has a broad range of interests that includes the synthesis of macrocyclic polyamine molecules, the synthesis of chelating agents for the sequestration of radioactive metal ions, and the synthesis of novel chelating agents for the creation of new chemotherapeutics. Dr. Brechbiel became the acting section chief of the Radioimmune and Inorganic Chemistry Section in 1997.*

Radiation Oncology Branch
Radiolabeled Monoclonal Antibodies for Diagnosis and Therapy

Keywords:
metal chelates
monoclonal antibodies
MRI contrast agents
radioimmunotherapy

Research: Tumor-associated monoclonal antibodies (mAbs), related immunoproteins, and their respective engineered fragments are useful therapeutic or diagnostic agents when used as selective carriers of cytotoxic or imaging elements. The Chemistry Section develops the chemical and physical science necessary to test and implement this concept by linking cytocidal or image-producing radionuclides to targeted moieties for treatment and diagnosis of malignancies in animal model systems. The Chemistry Section then creates the radiochemical protocols for preparation of pharmaceuticals for clinical trials.

Cytocidal agents employed include α- and β-emitting radionuclides. Chelation chemistries required to link radiometals such as ^{66,67}Ga, ^{90}Y, ^{111}In, ^{212,203}Pb, ^{212,213}Bi, ^{177}Lu, and ^{225}Ac are developed for collaborative biological studies to evaluate new chelation technology, to obtain scintigraphic image of tumors, and to measure therapeutic efficacy of mAb radioconjugates. Based upon these studies, treatment of patients has been initiated and continues in collaboration with the Metabolism Branch, the Laboratory of Molecular Biology, the Department of Nuclear Medicine, and Memorial Sloan-Kettering Cancer Center.

Recent numerous preclinical results in the ongoing development of novel bifunctional chelating agents and linkers for targeted radiotherapy with the α-emitting radionuclides ^{213}Bi, ^{225}Ac, and ^{211}At have been reported. In the case of ^{213}Bi, a specific potential clinical application of treating prostate cancer with an antibody that targets $PSMA_{ext}$ demonstrated significant delay in tumor onset, extended life expectancy, and decreased levels of PSA in a murine model with single doses of radioimmunoconjugate. Studies addressing the possibility of using ^{225}Ac as a therapeutic radionuclide in targeted radiotherapy applications using the most stable in vivo chelating agent to date, HEHA, were performed in two different murine models, a vasculature targeting model and a solid tumor model. Both studies independently indicated there to be significant unacceptable toxicity originating from the decay product daughters. While this radionuclide may still be of value in a

limited setting of rapid targeting and internalization, this condition in conjunction with the challenges associated with coordination chemistry eliminate this radionuclide from clinical contention. Studies with ^{211}At have yielded a novel protein modification reagent wherein the linking moiety has been removed from being the traditional aryl carboxylate active ester and placed several atoms away from the aryl astatine bond. Preclinical studies with this novel reagent termed SAPS conjugated to humanized monoclonal antibody anti-Tac indicate this agent to be stable in vivo and equivalent to the indirectly radioiodinated protein in every regard. In the case of both ^{213}Bi and ^{211}At, preclinical studies continue to evaluate potential therapeutic applications that may be translated into clinical protocols. With the recent revival in availability of both ^{212}Bi and ^{212}Pb, preclinical evaluation of ^{213}Bi, ^{212}Bi, and ^{212}Pb for the treatment of disseminated intraperitoneal diseases such as ovarian and pancreatic cancer have been initiated. Preliminary results have indicated that substantial increases in median life expectancy in murine models are possible with single doses of these isotopes conjugated to clinically relevant antibodies such as CC49 or Herceptin™.

Chelating agents produced for studies with ^{90}Y have also proven useful for the sequestration of paramagnetic ions such as Gd(III). The creation of potentially useful dendrimeric polymer-based contrast agents for MRI applications has followed. Polymeric dendrimers, such as the PAMAM type, allow for the precise, controlled, and reproducible chemical modification of a discrete chemical species, unlike polylysine.

Evaluation of macromolecular chelate conjugated dendrimer-based Gd(III) MR contrast agents based on the PAMAM or DAB classes of dendrimers has revealed that these agents can be tuned for various applications by virtue of choosing generation size, core elements, conjugation with elements of polyethylene glycol, and by adjusting clearance rates with coadministration of lysine. To this end, use of the PAMAM-based agents has demonstrated the ability to image tumor vasculature accurately at the 200 μm scale. The DAB class of agents has remarkably selective properties wherein reverse contrast images of metastatic liver tumors could be imaged at as small a scale as 0.3 mm. The PAMAM agents have also been used to develop an imaging technique to complement gene therapy methodology and provide not only an accurate accounting of the pharmacokinetics of the nucleotide but also to then image the targeted tissues. Lastly, we have demonstrated that these agents can be selectively targeted, not only by conjugation to antibodies but to other vectors, to deliver exceptionally high levels of Gd(III) into disseminated intraperitoneal ovarian cancer tumor for treatment by neutron capture therapy.

Complementary to the MR contrast agents, ESR spin-label agents have been prepared and are undergoing evaluation. In addition to these two types of imaging technology, protein-based and dendrimer-based CT contrast agents have been prepared and are also being evaluated in the appropriate model systems.

In sum, the ongoing research of the Radioimmune and Inorganic Chemistry Section develops for human medicine purposes the necessary chemistry to allow the biomedical sciences to design diagnostic imaging protocols and

rational therapies for malignancies using monoclonal antibody-mediated diagnosis and selective targeting of radiation to tumors and metastases.

Collaborating on this work are Jorge Carrasquillo, William Eckelman, Ira Pastan, Jeff Schlom, and Thomas A. Waldmann, NIH; Steve J. Kennell and Saed Mirzadeh, Oak Ridge National Laboratories; Roy Planalp, University of New Hampshire; David Scheinberg, Memorial Sloan-Kettering Cancer Center; and Suzy Torti and Frank Torti, Wake Forest University.

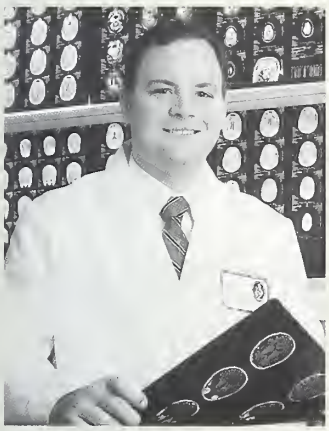
Recent Publications:

Hassfjell S, et al. *Chem Rev* 2001;101:2016–36.

Milenic DE, et al. *Cancer Biother Radiopharm* 2001;16:133–46.

Kobayashi H, et al. *Cancer Res* 2001;61:4966–70.

McDevitt MR, et al. *Cancer Res* 2000;60:6095–100.



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Biography: *Dr. Camphausen received his M.D. from Georgetown University in 1996. He completed his internship at Georgetown in 1997, followed by a residency in radiation oncology at the Joint Center for Radiation Therapy at Harvard Medical School in 2001. Dr. Camphausen has spent the last 2 years working in the laboratory of Dr. Judah Folkman studying the interaction of angiogenesis inhibitors and radiotherapy. He joined the NCI in July of 2001.*

Radiation Oncology Branch
Imaging and Molecular Therapeutics

Keywords:
angiogenesis
angiostatin
endostatin
molecular imaging
radiation

Research: The conventional explanation of the efficacy of radiotherapy in treating cancer is that tumor cells are the principal target of ionizing radiation that kills them through direct DNA damage. Similarly, radiation damage of normal tissues is thought to be a direct effect of DNA damage of normal cells. However, an alternate target of radiotherapy might be the tumor endothelium. If, for example, the microvascular endothelial cell is the principal target, damage to the endothelial cells may lead to the death of the tumor cells they support. This could explain the synergistic effect that has been observed with combinations of angiogenesis inhibitors plus radiotherapy. Even if the endothelial cell response is only a component of the tumor response to radiation, attacking both compartments is a logical strategy to be pursued. It may be possible to modify the radiosensitivity of a tumor by changing the levels of circulating endothelial inhibitors or stimulators, thereby making the tumor microvasculature more radiosensitive. Therefore, a large component of my research is examining the interaction of

inhibitors of angiogenesis and radiotherapy both in vitro and in vivo at the molecular level utilizing gene array technology.

The second large project involves the rapidly proliferating endothelium as a target for imaging techniques. New imaging technologies using fluorescent probes that emit in the near-infrared (NIR) spectrum are ideal for the laboratory setting. One compound, 4–(N–(S-glutathionylacetyl)amino)phenylarsenoxide (GSAO), which is a hydrophilic trivalent arsenical that senses redox events in cell-surface proteins and binds selectively to proliferating endothelial cells, was labeled with a NIR fluorochrome, Cy5.5, and successfully imaged murine and human tumors in vivo. This work will be expanded with molecular correlation using gene array technology.

Our collaborators are William Figg and Steven Libutti, NIH; Judah Folkman and Marsha Moses, Harvard University; Phillip Hogg, University of New Castle; and Michael O'Reilly, MD Anderson Cancer Center.

Recent Publications:

Camphausen K, et al. *Cancer Res* 2001;61:2207–11.

Folkman J, et al. *Science* 2001;293:227–8.

Joussen A, et al. *Int J Radiat Oncol Biol Phys* 2001;49(3):817–25.

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Biography: *Dr. Gius graduated from the University of Illinois with a B.S in chemistry in 1983, finished his Ph.D. thesis work from the University of Chicago in 1989, and graduated from Loyola Medical School in 1992. He completed an internship year at the University of Chicago and his radiation oncology residency at Washington University School of Medicine, the Mallinckrodt Institute of Radiology. Dr. Gius received an ASTRO Fellowship Award during his postdoctoral training at the Howard Hughes Medical Institute. In addition, he was a faculty member for 4 years in the Section of Cancer Biology and the Radiation Oncology Department at the Mallinckrodt Institute of Radiology prior to his current position at the NCI.*

Radiation Oncology Branch
Molecular Radiation Oncology Section

Keywords:
biochemistry
cancer preventing genes
carcinogenesis
cDNA microarray

Continued on page 255

Research: The laboratory has several areas of research interests with a central theme involving the signaling mechanism that tumor cells use to respond to the damaging and/or cytotoxic effect of exogenous agents that induce oxidative stress. This work is built on the general theme that eukaryotic cells have evolved adaptive responses to multiple forms of environmental stress by initiating genetically preprogrammed signaling pathways. These adaptive responses include the activation of cellular machinery involved in DNA

Keywords (continued):

cell cycle regulation
cervical cancer
CRE and AP-1 crosstalk
cyclo-oxygenase
DNA binding domain
DNA methylation
gene expression
heat shock
human papilloma virus (HPV)
ionizing radiation
methyltransferase
multidrug resistance
oxidation/reduction
oxidative stress
quantitative real time RT/PCR
reactive oxygen species
signaling molecules
thioredoxin reductase
transcription factors

repair, cell cycle arrest, apoptosis, gene induction, and lethality. In response to certain environmental stresses, tumor cells activate a class of proto-oncogenes referred to as early response or immediate early genes. These genes, originally characterized in quiescent cells stimulated by the addition of high concentrations of fetal calf serum, encode nuclear transcription factors. Such factors are involved in the transmission of inter- and intracellular information through multiple cellular signal transduction pathways. It is well established that several specific early response genes are activated in response to exogenous agents that induce intracellular stress including several therapeutic modalities such as chemotherapeutic agents, heat, and ionizing radiation (IR). In this regard, these gene products may function in coupled short-term changes in cellular phenotype by modulating the expression of specific target genes involved in cellular defenses to the damaging effects of IR. Hence, activation of these transcription factors and the subsequent expression of their target genes provide an ideal model system to study the cellular molecular and biochemical events of how tumor and normal eukaryotic cells respond to therapeutic agents including IR.

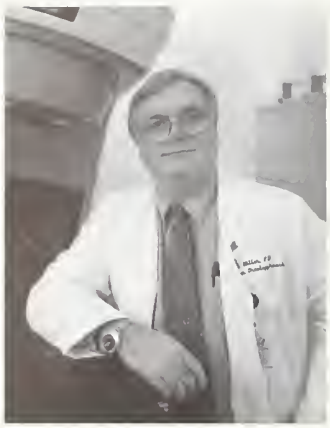
The overall direction of the laboratory is to investigate the signaling factors/pathways and the downstream transcription factors that are activated in response to IR and determine the physiological consequences of these processes. This is of importance since a greater understanding of the cellular mechanism protecting tumor cells from the cytotoxic effects of IR is a necessary starting point to developing new modalities and agents that could potentially inhibit these protective responses. The laboratory has recently shown that IR and several other cytotoxic agents that are used as radiation sensitizing agents activate several of the same signal transduction pathways and downstream transcription factors through stress-induced alternations in oxidation/reduction (redox) status of these factors. These redox-sensitive signaling proteins that are activated by various other agents that induce cellular oxidative stress appear to transduce signals via critical cysteine residues located in the enzymatic regions of these protein(s). As such, these redox-sensitive signaling cascades may represent a common mechanism that tumor cells use to initiate preprogrammed protective responses to the damaging effects of oxidative agents, including heat and/or IR. Thus, these redox-sensitive signaling cascades may provide a starting point to investigate the protective cellular mechanisms responding to the damaging effects of cytotoxic agents and/or IR.

Recent Publications:

Curry HA, et al. *J Biol Chem* 1999;274:23061–7.

Wei J, et al. *Cancer Res* 2000;60:6688–95.

Bradbury CM, et al. *Cancer Res* 2001;61:3486–92.



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Biography: *Dr. Miller received his undergraduate degree in physics in 1971 and his M.S. in radiation health in 1974, both from the University of Pittsburgh. He received his Ph.D. in radiological sciences from the George Washington University in 1998. He joined the NCI in 1981 and has board certification in therapeutic radiological physics. Dr. Miller has been the acting chief of the CCR's Radiation Physics and Computer Automation Section since 1997.*

Radiation Oncology Branch
Radiation Physics, Dosimetry, and Imaging Section

Keywords:
fiberoptic dosimetry
functional radiotherapy
image directed radiotherapy
radiolabeled antibodies
treatment planning

Research: The technology available to deliver radiation therapy has advanced to the point that it is currently possible with external beams to deliver a radiation intensity distribution of any desired shape to a selected point in space with a spatial accuracy of ±1 mm. The remaining problems from a physics standpoint are of target identification and tracking—that is, to properly define the target, to position it correctly with respect to the treatment fields, and to continually monitor its position during treatment delivery, correcting the treatment field in real-time to ensure proper target coverage.

Currently, target definition is anatomically based, using either CT or MR to define a macroscopic target volume based on either changes in effective atomic number (CT) or in hydrogen content (MR) that are believed to be caused by the presence of carcinoma. The next stage in radiotherapy, however, will be to image a target area based on specific functionality, in addition to anatomical structure, using tools such as functional MRI, MRS, SPECT, PET, or EPRI. This ability will depend on very sophisticated image fusion technology to register and stack the differing imaging methodologies so that a physician can combine selected studies in order to design a treatment plan that selectively targets those regions of the tumor that are most critical to control.

The next level of sophistication will involve real-time image and dose gating. This will involve the use of anatomical information acquired in real-time during radiotherapy, using either ultrasound or electronic portal imaging to directly determine target motion. Image comparison can be used to gate the treatment and determine the direction of target motion, generating signals to reposition either the treatment field (by shifting leaf positions of a multileaf collimator) or the patient (by shifting the treatment table). Once a correction is applied, an imaging dose is generated to verify correct target position prior to continuing treatment.

The tools necessary to accomplish this include (1) a sophisticated image fusion program necessary for registering the results of various imaging modalities, (2) a treatment planning system which displays image stacks in the desired viewing plane and for rapid recalculation of the dose distribution from real-time image data, and (3) small fiberoptic dosimeter probes capable of being used in vivo and multiplexed into a readout system that measures dose in real-time. These will be interfaced with a state-of-the-art linear accelerator equipped with dynamic multileaf collimation, amorphous silicon imaging technology, and a gated-dose control mechanism capable of millisecond response, in order to achieve image-controlled radiotherapy.

The sophistication of rendering multiple image volumes will also be necessary to ensure accurate treatment delivery and tissue sampling for MR-guided prostate brachytherapy using high dose-rate (HDR) remote controlled afterloader technology. The Physics Section will have a primary role in developing MR compatible treatment accessories and will provide the computer technology necessary for accurate tissue biopsy under MRI/MRS guidance, for calculation of the radiation dose at biopsy sites as well as the source locations and residence times required for the optimum dose distribution, and for real-time, in-vivo dose verification of treatment delivery

Novel treatment strategies must also be explored when they promise an increased dose to the target with reduced normal tissue exposure. One such avenue is the use of radiolabeled antibodies, either as an adjunct to conventional external beam radiotherapy or as a potential replacement. The Physics Section will be responsible for source selection and for the imaging required to determine biodistribution and organ distribution, and retention of the selected antibody.

The Physics Section also is engaged in a partnership with the Naval Research Laboratory to develop Optically Stimulated Luminescent (OSL) dosimeters for dose verification. Single fiber point dosimeters small enough for implantation or insertion in a catheter have already been developed and are undergoing clinical trials. These dosimeters will supplant existing detector methodology such as TLDs and diodes for routine clinical measurement. Additionally, a two-dimensional OSL film is being developed to supplant photographic and radiochromic film for dosimetry QA purposes.

Our collaborators include Marie Ann Descalle, Christine Hartman-Siantor and Rodemary Walling, Lawrence Livermore National Laboratory; Michael Green and James Vucich, NIH; and Alan Huston and Brian Justus, Naval Research Laboratory.

Recent Publications:

Huston AL, et al. *Nucl Instr Met Phys B* 2001;184:55–67.

Huston AL, et al. *Radiat Prot Dosimetry* 2001; in press.

Clinical Trials:

Rosemary Altemus	**79-C-0111:** The treatment of stage I and II carcinoma of the breast with mastectomy and axillary dissection vs. excisional biopsy, axillary dissection, and definitive irradiation
	95-C-0003: Total body irradiation for bone marrow transplants: collaborative efforts
	97-C-0129: Clinical evaluation of a laser heated, fiberoptic-coupled radiation dose verification system
	00-C-0074: Evaluation of late effects and natural history of disease in patients treated with radiotherapy
	xx-C-xxxx (pending): A pilot study of noninvasive monitoring of exhaled nitric oxide levels as an indicator of radiation lung damage
	xx-C-xxxx (pending): A pilot study of total body irradiation for pediatric populations (CNMC collaboration)
C. Norman Coleman	**00-C-0181:** Evaluation for NCI Radiation Oncology Branch Research Protocols xx-C-xxxx (pending): Amifostine as a rectal protector during external beam radiotherapy for prostate cancer: A pilot study assessing radiation dose-response and the effect of a radioprotector
	xx-C-xxxx (pending): A study of radiation induced changes in peripheral blood lymphocytes in patients undergoing radiation therapy
Brian G. Fuller	**95-C-0069:** A phase I study of combined radiation response modifiers employing pentoxifylline and hydroxyurea for treatment of glioblastoma
	95-C-0092: A phase I study of 2-chlorodeoxyadenosine and radiation for the treatment of high-grade glioma
	99-C-0011: A pilot study of stereotactic radiosurgery for intracranial neoplasms oxygen level assessment in brain tumors: A pilot study
Laurie Herscher	**95-C-0162:** A pilot study of paclitaxel with radiation therapy for locally advanced head and neck cancer
	00-C-0218 (chairperson): A phase II trial of combined intraperitoneal gemcitabine, intravenous gemcitabine, radiotherapy, and surgery for advanced adenocarcinoma of the pancreas
	01-C-0143: A pilot study of pirfenidone for the treatment of radiation-induced fibrosis xx-C-xxxx (pending): Proteomics of the therapeutically irradiated patient

Surgery Branch

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The Surgery Branch of the National Cancer Institute has a dual function at the NIH. Investigators in the branch are involved in the conduct of laboratory and clinical research aimed at improving the management of patients with cancer. In addition, the Surgery Branch has a major commitment to providing surgical consultative care for patients throughout the Clinical Center and is responsible for clinical care in most surgical subspecialties including thoracic surgery, gynecological surgery, endocrinology surgery, vascular surgery, and vascular access. Laboratory research efforts emphasize studies of tumor immunology and immunotherapy, surgical metabolism, and molecular genetics. In these studies, an emphasis is placed on the translation of laboratory findings into the development of clinical protocols. Clinical efforts emphasize the development of new approaches to the immunotherapy of cancer, as well as the development of innovative surgical approaches to the treatment of patients with primary and metastatic cancer.

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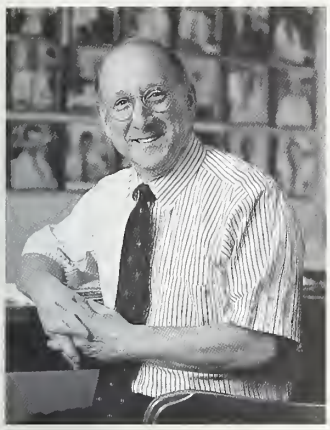

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Biography: *Dr. Rosenberg received his B.A. and M.D. from the Johns Hopkins University, and he received a Ph.D. in biophysics from Harvard University. Following the completion of his surgical residency at the Peter Bent Brigham Hospital in Boston, MA, Dr. Rosenberg became chief of the Surgery Branch in July 1974, a position he continues to hold now. His research interests have focused on studies of tumor immunology and the development of effective immunotherapies for the treatment of patients with cancer.*

Surgery Branch
Studies of Tumor Immunology and the Development of Approaches to the Immunotherapy of Patients With Cancer

Keywords:
antigen presentation
autoimmunity
cancer
cancer antigens
cancer immunotherapy
cellular immunity
cytokines
dendritic cells
gene therapy
IL–12
immunotherapy
interleukin 2
kidney cancer
lymphokines
melanoma
T lymphocytes
target antigens
tumor antigen
vaccines

Research: The goal of this research effort is the development of new approaches to the immunotherapy of patients with cancer based on an understanding of the molecular aspects of the tumor-host interaction. Early studies of the cellular immune reaction of mice and humans to growing cancers led to extensive studies of interleukin 2 (IL–2) and its ability to activate immune responses against tumor antigens. The development of IL–2 and adoptive cell transfer therapies in experimental animals led to clinical trials of these approaches and the ultimate approval of IL–2 as an effective immunotherapy for the treatment of selected patients with metastatic melanoma or kidney cancer. Attempts to generate lymphoid cells with specific antitumor reactivity led to the description of tumor-infiltrating lymphocytes (TIL) which are lymphoid cells that could be grown from the stroma of solid tumors. TIL with specific antitumor reactivity could be generated from a variety of murine tumors, and adoptive transfer studies demonstrated their therapeutic effectiveness, which led to the evaluation of TIL administration to patients with metastatic melanoma. Pilot clinical trials in humans demonstrated that 34 percent of patients with metastatic melanoma experienced objective regressions when receiving treatment with the adoptive transfer of TIL plus IL–2. Subsequent studies demonstrated that TIL traffic to tumor deposits following adoptive transfer, and these studies led to the first clinical trials of the adoptive transfer of gene-modified cells in humans. TILs transduced with the gene for neomycin phosphotransferase were used to study the long-term survival and distribution of TIL in vivo, and in subsequent studies TILs transduced with the gene for tumor necrosis factor were administered to patients in an attempt to increase antitumor efficacy of the TIL. TIL that recognized tumor antigens in a class I MHC-restricted fashion were used in studies to identify the genes encoding melanoma-specific antigens. In this strategy, TIL cells that were associated with tumor regression when adoptively transferred in vivo were used to clone the genes that encoded the antigens recognized by those TIL. Many

genes have been identified that encode melanoma regression antigens restricted by HLA–A2, A24, and A31. Extensive characterization of these genes and gene products and their role in human antitumor immune responses are under way.

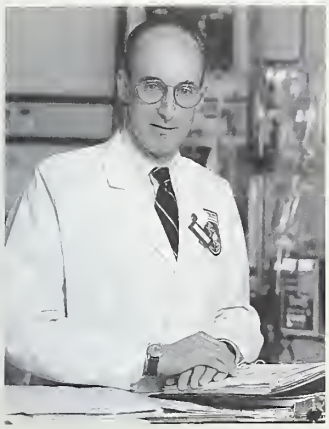
Several general approaches to the immunotherapy of human cancer are being investigated based on these findings. Using the specific immunogenic epitopes identified in melanoma tumor antigens, techniques are being developed to generate specific antitumor reactive lymphocytes in vitro for use in adoptive transfer. The genes encoding the T cell receptors capable of recognizing specific melanoma antigenic epitopes have been identified and cloned, and these genes are being transduced into alternative effector cells and into bone marrow stem cells for possible use in the treatment of patients with malignancy. In addition, the melanoma antigens are being used for direct immunization of cancer patients as "cancer vaccines." The genes encoding these melanoma antigens have been incorporated into recombinant fowlpox, vaccinia virus, and adenovirus for use in the development of immunization strategies. Amino acid modifications have been made in the immunodominant peptide epitopes to increase their binding to MHC molecules, and these synthetic peptides have proven to be effective immunogens in humans as well. Extensive immunologic assays studying the impact of these immunization strategies in patients are being performed.

Recent Publications:

Rosenberg SA. *Immunity* 1999;10:281–7.

Rosenberg SA, et al. *Nat Med* 1998;4:321–7.

Wang RF, et al. *Science* 1999;284:1351–4.



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Biography: *Dr. Alexander received his B.A. from the University of Colorado and M.D. from Georgetown University School of Medicine* (cum laude). *He completed a residency in general surgery at the National Naval Medical Center in Bethesda, MD, and a fellowship in surgical oncology at the Memorial Sloan-Kettering Cancer Center in New York. He joined the Surgical Metabolism Section of the Surgery Branch in 1989 and became chief of the section in 1995. Dr. Alexander is a Fellow of the American College of Surgeons, the American Surgical Association, and the Society of Surgical Oncology. He is a member of the Society of University Surgeons, the Association of Academic Surgery, the American Association of Cancer Research, and the American Association of Endocrine Surgeons. He is an internationally recognized expert in endocrine and oncologic surgery and provides surgical consultation and treatment for patients with disorders of the thyroid, parathyroid, and adrenal glands, and for endocrine tumors arising in the pancreas. He has made significant contributions in the development of regional and isolation perfusion in the treatment of cancer.*

Surgery Branch
Development of New Cancer Treatments and Imaging Techniques Using Regional Perfusion or Targeted Therapies Against Tumor or Its Neovasculature

Keywords:
colon cancer
endocrine surgery
isolation perfusion
regional therapy

Research: The Surgical Metabolism Section of the Surgery Branch conducts clinical trials evaluating regional cancer treatments for patients with a variety of solid tumor malignancies and provides surgical consultation and treatment for patients within the NIH who have endocrine disorders including parathyroid, thyroid, endocrine pancreas, and adrenal neoplasms. Investigators in the section have considerable expertise in advanced therapeutic laparoscopy including procedures such as laparoscopic partial pancreatectomy, liver resection, and adrenalectomy. In collaboration with others at the NIH, they are actively evaluating and developing new imaging techniques for patients with solid tumor malignancies.

The clinical trials conducted by the section are evaluating novel cancer treatments administered via isolation perfusion of the limb or liver and regional peritoneal perfusion using biological agents, chemotherapy, and hyperthermia. Initial results with isolated hepatic or limb perfusion have established that this treatment results in substantial and durable regression of locally advanced cancers such as unresectable extremity sarcoma or unresectable primary or metastatic cancers of the liver. A major thrust of the clinical trials has been to determine if tumor necrosis factor (TNF), a protein with impressive antitumor effects in experimental models but which is too toxic for systemic administration in humans, has significant antitumor activity when used in isolation perfusion, which eliminates systemic exposure and toxicity. In patients with advanced refractory unresectable cancers confined to liver, isolated hepatic perfusion (IHP) with TNF and melphalan results in significant regression (>50 percent reduction in size) of tumors in 75 percent of patients. In patients with colorectal cancer metastatic to liver,

the combination of IHP with melphalan followed by monthly hepatic arterial infusion of FUDR and leucovorin results in a 75 percent response rate with a median duration of response of 18 months.

Laboratory research falls into several broad areas that complement and advance the clinical research effort. Adeno- and vaccinia virus constructs with reporter or therapeutic suicide or antiangiogenic genes are being developed to target tumors in experimental murine, rodent, and lapine models of liver metastases, peritoneal carcinomatosis, and pulmonary metastases. Vaccinia viral (VV) constructs containing either a reporter gene (luciferase) or suicide gene (cytosine deaminase, CD) under the control of a synthetic early/late promoter have been shown to selectively replicate and express the gene of interest in high titer in tumor after intravenous administration. VV with CD followed by prodrug treatment with 5–FC results in a significant survival benefit in mice with established liver metastases with long-term (>99 days) cures observed in about 25 percent of treated animals.

The unique phenotype of tumor neovasculature is being characterized and explored as an important potential target for novel cancer therapies. The laboratory has shown that a tumor-derived cytokine, endothelial monocyte-activating polypeptide (EMAP), will cause procoagulant effects in tumor microvasculature. EMAP expression in tumors correlates with TNF sensitivity in vivo and retroviral transduction of TNF-resistant tumors, so that they constitutively express high titers of EMAP, rendering them sensitive to TNF in experimental models. In addition, using a rat aorta assay of angiogenesis, the laboratory has shown that EMAP has potent antiangiogenic effects. The section has developed adenoviral vectors engineered to secrete potent antiangiogenic proteins as a novel method of delivering this new form of therapy.

In order to better understand the mechanisms of antitumor activity in isolated organ perfusion, we have been characterizing the effects of melphalan, hyperthermia, and TNF on endothelial cell viability and permeability in vitro and on tumor microvascular permeability in experimental models and in patients undergoing isolation perfusion. Data show that there is a marked increase in capillary permeability in tumor vasculature during isolation perfusion independent of TNF. The major goal of these studies is to develop isolation perfusion as a treatment option for patients with regionally confined cancers.

Recent Publications:

Alexander HR, et al. *Cancer: Prin Prac Oncol* 2001;15:1–16.

Bartlett DL, et al. *Surgery* 2000;129:176–87.

Lans TE, et al. *Clin Cancer Res* 2001;7:784–90.

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Biography: *Dr. Bartlett received his M.D. from the University of Texas Medical School at Houston. He completed his clinical surgical training at the Hospital of the University of Pennsylvania and a fellowship in surgical oncology at the Memorial Sloan-Kettering Cancer Center in New York. He has been a member of the Surgery Branch of the NCI since July 1995. His expertise is in the regional delivery of chemotherapeutics via isolated perfusion systems including liver perfusion, peritoneal perfusion, and limb perfusion. In addition, he acts as a surgical consultant to the endocrine services at the NIH, and he has nationally recognized expertise in upper gastrointestinal and hepatobiliary surgery.*

Surgery Branch
Tumor-Specific Replicating Viruses for In Vivo Gene Delivery

Keyword:
vaccinia virus

Research: As we become more knowledgeable about the genetic triggers that transform a normal cell into a cancer cell, it becomes feasible to replace genes or alter the genetic expression within a cancer cell in order to reverse it back into a normal cell or destroy it. Gene therapy for cancer may therefore be possible, as has been shown repeatedly in experiments in the laboratory, but many obstacles to effective gene therapy in patients exist. The primary obstacle is achieving tumor-specific in vivo gene delivery in a safe way to cancer patients.

We have focused on the development of tumor-specific replicating viruses as a means of efficient gene delivery to tumors. We have studied a melanoma-specific promoter and enhancer combination which allows for high levels of gene expression within melanoma cells. We are in the process of using this transcriptional specificity to develop an adenovirus which specifically replicates in melanoma cells. The hope is that this method of transcriptional targeting will improve both the efficiency and safety of viral mediated gene delivery to tumors. We have also explored vaccinia virus as a vector for tumor-directed gene delivery. We have found that vaccinia virus is able to target tumor tissue in vivo when delivered intravenously to different animal models. This targeting can lead to an effective antitumor response. We have developed a vaccinia virus which is deleted for both the thymidine kinase gene and the vaccinia growth factor gene which markedly attenuates viral replication in normal cells but allows for efficient replication in dividing tumor cells. This results in an efficient targeted vector for gene delivery to tumors. The use of a suicide gene has been shown to mediate an antitumor response when using the vaccinia virus vector. We have also developed an alternative replicating pox virus vector for in vivo gene delivery. The yaba-like disease virus efficiently expresses genes in tumor cells but does not have immune cross reactivity with vaccinia virus, making it potentially more efficient in patients who have been immunized for smallpox. We are

continuing to explore ways to make both vaccinia and the yaba-like disease virus more efficient and specific for tumor cells.

Because of our clinical expertise in the regional delivery of therapeutics, we are applying this strategy to deliver these viruses for gene therapy. Many examples of regionally confined primary and metastatic cancers exist, such as intransit melanoma of the extremity, unresected isolated hepatic metastases from colon cancer, and isolated peritoneal carcinomatosis. We are studying the regional delivery of viral vectors using perfusion techniques in animal models of tumors. Our ultimate plan is to explore clinical trials using the vectors that we have developed in a regional setting.

Dr. Bartlett is now a principal investigator on clinical studies exploring the use of chemotherapeutics delivered into the peritoneal cavity for patients with peritoneal carcinomatosis. This involves a hyperthermic peritoneal perfusion using cisplatin followed by intraperitoneal dwell chemotherapy with paclitaxel and 5–FU. We have defined the maximum tolerated dose of these agents and are beginning phase II trials for tumor response assessment. Dr. Bartlett is also an active coinvestigator in protocols for isolated perfusion of the liver and imaging studies for recurrent colorectal cancer.

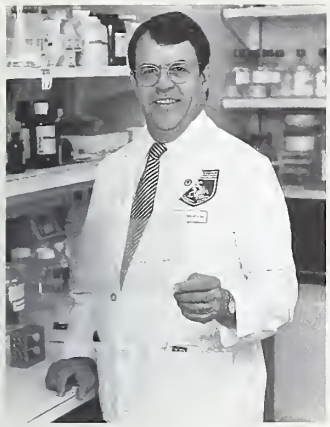
Recent Publications:

Park BJ, et al. *Hum Gene Ther* 1999;10:889–98.

Puhlmann M, et al. *Hum Gene Ther* 1998;10:649–57.

Gnant MFX, et al. *J Natl Cancer Inst* 2000; in press.

Park BJ, et al. *Ann Surg Oncol* 1999;16:582–90.



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Surgery Branch
Hormonal Regulation of Normal Mammary and Breast Cancer Cell Growth, Metabolism, and Apoptosis

Keywords:
antiestrogen
apoptosis
breast cancer
breast epithelium
chemoprevention
retinoids

Research: Tamoxifen and retinoids (vitamin A-related compounds) are important agents that are active against breast cancer and have significant potential both for chemoprevention of breast cancer and for treatment of established breast cancer. Studies are being conducted to develop an effective in vitro system to examine the effects of retinoids on high-risk normal mammary epithelial cells as a model for chemoprevention trials. Studies are also in progress to examine and characterize the additive effects of retinoids in combination with other agents, including tamoxifen, γ-interferon, and Fas antibody, as a means of enhancing inhibition of cell growth, metabolism, and stimulation of programmed cell death (apoptosis) of human breast cancer cells. The gene expression profile of these high-risk cells is being examined to identify early changes in the carcinogenic pathway and to identify retinoid responsive genes that can be used for development of criteria for selection of retinoids active against breast epithelial cells for chemoprevention studies. These studies complement clinical trials (both therapeutic and chemo-preventive) and should allow identification of new targets and the development of new treatment strategies for breast cancer.

A method has been developed in this laboratory for establishing epithelial cell lines from normal breast tissue, as well as solid breast cancer, including primary tumor, cutaneous lesions, and axillary lymph node metastases. Normal or malignant epithelial cells from patients have been characterized cytologically, are cytokeratin positive, and express DF3 antigen. Cells from malignant tumors have been passaged in nude mice and reestablished in culture. Matched normal and malignant cell lines from the same patient are available for comparative purposes. Early passages are frozen and stored, and a cell line bank developed for future studies. This concept is being further developed in a recent clinical trial initiated from this laboratory entitled "Establishment of Breast Epithelial Cell Lines from Women at High Risk for Breast Cancer." A bank of cell lines will be established from solid breast tissue from women at high risk for breast cancer, frozen for future studies, and examined for phenotypic growth and metabolic characteristics as

well as genetic studies including regulation of BRCA1 and BRCA2 expression.

Important recent studies have shown that the antiestrogen drug tamoxifen acts additively with all-trans retinoic acid and 9-cis retinoic acid to inhibit growth and to stimulate programmed cell death (apoptosis) of breast cancer cells. The apoptotic regulatory gene bcl-2 is downregulated by each of these agents and may mediate their additive effect on apoptosis. Downregulation of both gene expression and protein expression of bcl-2 has been demonstrated, suggesting an important target for gene therapy studies. These breast cancer cells also secrete the negative growth factor TGFβ. Studies have examined its role in mediating the synergism between AT and TAM. These studies have demonstrated that TGFβ secreted by these cells acts synergistically with AT to downregulate bcl-2 protein, stimulate apoptosis, and inhibit growth. This novel interaction of AT and TGF plays an important role in the synergism of AT+TAM, and represents an important autocrine mechanism for regulation of breast epithelial cell growth. In vivo studies of this interaction are planned.

The cell surface antigen FAS is an important mediator of apoptosis in a variety of cell types. Expression of FAS-antigen can be induced on breast cancer cells by γ-interferon and all-*trans* retinoic acid, and subsequent exposure to anti-FAS antibody markedly enhances breast cancer cell death. The mechanism and regulation of FAS-antigen expression are being actively studied, including possible mediation by bcl-2. Importantly, these studies have identified a means for inducing a major target (Fas-antigen) for enhancing cell death, and identified an important additive effect of retinoids in combination with other agents (γ-interferon and Fas-antibody) for this process that may apply to normal, as well as malignant, breast epithelial cells. This may allow for new preventive and therapeutic treatment strategies for breast cancer.

Recent Publications:

Danforth DN Jr, et al. *Cancer Res* 1993; 53:1538–45.

Sgagias MK, et al. *J Immunother* 1995; 17:88–97.

Danforth DN Jr, et al. *Clin Cancer Res* 1996; 2:827–35.

Sgagias MK, et al. *Cancer Bioth* 1996;11:177–85.

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Biography: *Graduating* summa cum laude *in 1983 from Lehigh University's 6-year B.A./M.D. program, Dr. Hwu attended the Medical College of Pennsylvania from 1983 to 1987. After graduation, he became house officer in internal medicine at the Johns Hopkins Hospital in Baltimore, MD, followed in 1989 by his appointment as a clinical associate in medical oncology and immunotherapy at the NCI. Board-certified in internal medicine and medical oncology, Dr. Hwu became a senior investigator in the Surgery Branch of the NCI in 1993.*

Surgery Branch
The Genetic Manipulation of the Immune Response Against Cancer

Keywords:
cancer
dendritic cells
gene therapy
immunotherapy
melanoma
ovarian cancer
renal cell carcinoma

Research: Our goals are to develop improved methods to utilize the immune system against cancer and to broaden the use of immunotherapy for common cancers, such as ovarian cancer, breast cancer, and colon cancer. Currently, immunotherapies have been developed that are effective in some patients with melanoma or renal cancer. However, the design of therapies which utilizes the cellular immune response against other, more common cancers has been hampered by the difficulty in growing T cells against these cancers and the absence of known antigens on these tumors that are recognized by T cells. Many monoclonal antibodies (mAb) have been described, however, which are relatively specific for certain kinds of cancer, such as ovarian, breast, or colon cancer. Therefore, in order to combine the tumor-recognition capabilities of antibodies with the potent effector functions of T cells, we have constructed chimeric receptor genes consisting of the variable regions from mAb joined to T cell receptor signaling chains, such as the Fc receptor-associated γ chain.

Previously, we demonstrated that T cells transduced with chimeric receptor genes are redirected to recognize new targets, using both in vitro and in vivo models. Current work is focused on the transduction of hematopoietic stem cells with chimeric antibody/T cell receptor genes in order to provide a constant supply of differentiated cells expressing the chimeric receptor in vivo. Because T cell receptor and Fc receptor signaling chains share common activation motifs, this method might provide a continuous supply of transduced, functional monocytes, NK cells, and granulocytes, as well as lymphocytes directed against the tumor. To study this, mice were lethally irradiated and reconstituted with bone marrow that was either nontransduced or transduced with a chimeric T cell receptor gene against ovarian cancer (Mov-γ). Growth of a control tumor was similar in mice reconstituted with nontransduced bone marrow or Mov-γ-transduced bone marrow. However, growth of a tumor expressing the ovarian cancer antigen recognized by the Mov-γ receptor was significantly reduced in mice reconstituted with Mov-γ-transduced bone marrow compared to mice

reconstituted with normal bone marrow. Activated splenic T cells from Mov-γ-reconstituted mice specifically secreted mIFN-γ when cocultured with ovarian tumor cells. This suggests that the Mov-γ chimeric receptor is functional in immune cells derived from transduced hematopoietic stem cells. In vivo depletion of T cells in reconstituted mice by treatment with anti-murine CD4 and CD8 antibodies did not affect antitumor activity, suggesting that other immune cells, such as natural killer (NK) cells, macrophages, and neutrophils, may play an important role in tumor rejection in this system. Current efforts are focused on further characterizing the immune effector cells responsible for the antitumor activity.

By utilizing gene transfer into hematopoietic cells, we have also developed a novel way to gene modify dendritic cells, potent antigen-presenting cells that may be able to stimulate a strong antitumor immune response. Although primary, mature dendritic cells are difficult to gene modify, we reasoned that hematopoietic stem cells might be gene modified followed by in vitro differentiation into dendritic cells. We have demonstrated that this novel technique can generate dendritic cells expressing foreign genes. We have shown that the MART–1 melanoma antigen can be expressed by dendritic cells and that these cells could stimulate MART-specific T cells as well as the generation of specific T cells from resting lymphocytes. This technique may allow us to raise specific T cells against common tumors and may also provide a novel way to actively immunize patients against known tumor antigens by utilizing dendritic cells transduced with antigen or cytokine genes.

Because dendritic cells are rare cell types in vivo and difficult to grow in large numbers, we are studying methods of in vivo priming with Flt3L, a recently discovered cytokine that has been shown to increase the number of circulating dendritic cells in murine models as well as normal volunteers. We are currently conducting a clinical trial using Flt3L in combination with melanoma peptide vaccination. We have found that subcutaneous administration of Flt3L increased circulating cells with a dendritic cell phenotype capable of stimulating T cells, presumably through the increased expression of costimulatory molecules. This ability to mobilize cells with a DC phenotype that are capable of stimulating greater T cell proliferation may translate into a stronger in vivo antitumor response.

The activation state of dendritic cells may be critical in determining their ability to effectively stimulate T cells. Therefore, we are studying methods to fully activate dendritic cells, such as upregulating costimulatory molecules with CD40L stimulation to determine if activated dendritic cells can more effectively generate an antitumor response.

Finally, other cell types, such as activated B cells, may also be capable of generating antitumor responses in vivo. We have found that human and murine B cells stimulated with CD40L and cultured in IL–4 express large amounts of B7–1 and B7–2 and are capable of stimulating quiescent lymphocytes in a mixed leukocyte reaction. We are currently studying whether activated B cells can elicit an antitumor response in murine models.

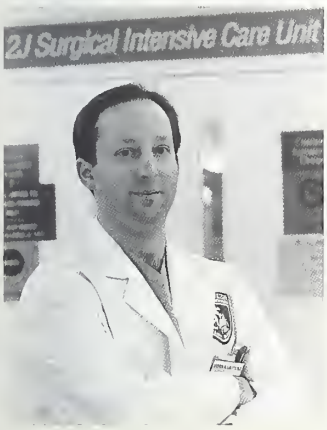
Recent Publications:

Wang G, et al. *Nat Med* 1998;4:168–72.

Specht J, et al. *J Exp Med* 1997;186:1213–21.

Reeves M, et al. *Cancer Res* 1996;56:5672–7.

Rosenberg S, et al. *Nat Med* 1998;4:321–7.



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Biography: *Dr. Libutti received his A.B. from Harvard University and his M.D. from the College of Physicians and Surgeons of Columbia University. He completed his surgical residency at the Presbyterian Hospital in New York and a fellowship in surgical oncology in the Surgery Branch of the National Cancer Institute, where he presently is a senior investigator as well as an assistant professor of surgery at the Uniformed Services University of the Health Sciences. He is a fellow of the American College of Surgeons, a fellow of the Society of Surgical Oncology, a member of the American Association for Cancer Research, a member of the American Association of Endocrine Surgeons, and a member of the Association for Academic Surgery. Dr. Libutti is studying tumor neovascular formation and the interaction between tumor cells and endothelial cells. His clinical expertise is in the management of malignancies of the liver, pancreas, and GI tract, in the management of endocrine tumors, and in applying laparoscopic surgery to managing patients with malignancies.*

Surgery Branch
Tumor Angiogenesis and Endothelial Cell Gene Expression Profiling

Keywords:
angiogenesis
colon cancer
endocrine surgery
gene expression profiling
gene therapy
imaging
pancreatic cancer

Research: The interaction of a tumor and its vasculature is critical for both tumor growth and the spread of tumor cells to distant organs. The process of new vessel development within the tumor is termed angiogenesis and is required for tumors to grow larger than a few millimeters. In order to better understand the relationship between the tumor and its blood supply, our research is focused on the interaction between tumor-derived factors and endothelial cells developing in the tumor microenvironment. By understanding this interaction we hope to be able to design novel treatment strategies to inhibit both the growth and the spread of tumors. We are currently studying a variety of tumor-derived factors with effects on tumor-associated vasculature. These include vascular endothelial growth factor (VEGF) and endothelial cell monocyte-activating polypeptide II (EMAP–II). These cytokines appear to be produced in varying amounts by tumors and have direct effects on the tumor neovasculature. Our approach to the study of these interactions has been through the utilization of a variety of in vitro and in vivo model systems. We are using gene expression profiling to understand the changes that occur in endothelial cells exposed to tumor-derived factors. The laboratory is developing techniques, which allow us to isolate

endothelial cells from tumor tissue. This will result in our ability to study tumor-derived endothelial cells directly. We are also using noninvasive imaging techniques, including dynamic MRI and PET, to map changes in tumor blood flow within tumors both in animal models and in our patients on clinical trials. A variety of inhibitors of tumor angiogenesis are being actively studied. These include both recombinant proteins derived from naturally occurring substances as well as small molecules designed to act on specific pathways. Various methods of delivering these agents, including gene therapy approaches, are being pursued. Our overall goal is to translate a better understanding of tumor cell-endothelial cell interactions into better therapies for our patients with cancer.

Recent Publications:

Wu PC, et al. *Cancer Res* 1999;59:205–12.

Gnant MF, et al. *Cancer Res* 1999;59:4668–74.

Libutti SI, et al. *Cancer J Sci Am* 1999;5:252–6.

Feldman AL, et al. *J Natl Cancer Inst* 2001;93:1014–20.

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Biography: *Dr. Marincola received his M.D. from the University of Milan, Italy. He completed his training in general surgery at Stanford University in California and in surgical oncology at the National Cancer Institute. He is a senior investigator at the NCI, associate director for science of the HLA Laboratory, NIH, and has faculty appointments at the Uniformed Services University of the Health Sciences and at New York Medical College. He is associate editor of the* Journal of Immunotherapy. *Dr. Marincola's scientific interest focuses on the field of tumor immunogenetics.*

Surgery Branch
Tumor-Host Immunogenetic Interactions

Keywords:
HLA
immunogenetics
microarray
tumor immunology

Research: The limited success of immunologic approaches to the treatment of advanced cancer is related to the poor understanding of the biology of tumor-host interactions. The identification of the algorithm responsible for tumor regression may test the practical feasibility of this type of approach and/or lead to a more focused application of immunotherapy and/or gene therapy strategies. Such algorithm may include:

- **Factors related to genetic variability of patients, such as the genetic polymorphism of the human leukocyte antigen (HLA).** HLA is the most polymorphic gene in the human genome. Knowledge about HLA polymorphism in relation to the melanoma population is important for the understanding of the basic physiology of T cell recognition. For example, an analysis of 412 consecutive melanoma patients showed that HLA class II alleles are linked to individual tolerance to systemic administration of

interleukin 2 with potentially limiting effects on their treatment. Furthermore, North American Caucasian (NAC) melanoma patients are genetically different from the rest of the Northern American Caucasian population and closer to the Northern European populations. This finding corresponds to the observation that patients of the same "race" (Caucasoid), but of different subracial ancestry, are genetically and functionally different. Further studies have subsequently shown that these genetic variabilities have a significant impact on predicting the immunological response of individual patients to the same antigen. Recent work has demonstrated that antigenic immunodominance is dependent upon stringent epitope/HLA allele requirements, and even single amino acid variations in HLA molecules may totally alter the immunogenic potential of a given molecule. These findings have very important implications for the development of vaccination strategies broadly applicable to heterogeneous patient populations.

- **Factors related to individual variability in natural or vaccine-induced immunocompetence against melanoma-associated antigens (MAA).** By repetitive in vitro stimulation of peripheral blood monocytes with an immunodominant MAA-derived peptide cytotoxic T cell (CTL), reactivity against the same MAA was compared in melanoma patients and normal individuals; it was possible to induce CTL reactivity against MART–1 more frequently and more rapidly in melanoma patients, suggesting that quantitative and/or qualitative differences in exposure to self antigens have a direct effect on CTL reactivity, and differences in T cell activation among different individuals could be detected by in vitro assays. When the same analysis was used to detect differences in T cell reactivity within the same individual after epitope-specific vaccination, it was found that immunization with MAA-derived peptide could enhance specific T cell reactivity, although this enhancement was not sufficient to induce tumor regression in the majority of patients. The reasons for this paradoxical ineffectiveness of tumor-specific CTL in vivo are not known. We have therefore devised a strategy to evaluate the function of CTL in vivo at tumor site by serial fine needle aspiration of metastatic lesions and direct analysis of the material obtained by a variety of techniques including immunohistochemistry, in vitro expansion of tumor and lymphocyte pairs, and finally establishment of a cDNA library for analysis of gene expression. These efforts are presently ongoing.

- **Factors related to variability of tumors.** Tumors may escape immune recognition by competent T cells by loss of expression of MAA antigens or HLA class I molecules necessary for presentation of these antigens to T cells. HLA–B and C locus downregulation occurs in approximately 50 percent of metastatic melanomas, loss of HLA-haplotype in 10 percent of melanoma cell lines. Other less frequent mechanisms of loss of HLA expression are allelic-specific loss or downregulation and total loss of HLA class I due to loss of functional b2-microglobulin. Among the tumors that do express HLA–A alleles (believed to be dominant for CTL recognition), the level of their expression is quite variable. This variability has significant functional implications as a strict correlation exists between surface expression of HLA and recognition by CTL. Tumors are also variable in the amount of expression of MAA, and their decreased expression has important effects on CTL recognition in situations of low expression of the relevant HLA class I allele. We have also recently reported that tumors can be quite different in the level of MAA expression even in the same patient at the same time and that approximately 30 percent of metastatic lesions in patients with advanced

melanoma have completely different expression of MAA and HLA class I antigens. These findings are of extreme importance because they underscore the fact that each lesion in a patient with advanced cancer develops and behaves differently from other lesions. Therefore, attempts to correlate variables relevant to the immune biology of cancer to clinical outcomes are most likely to succeed if distinct lesions are followed serially—for example by the use of repeated fine needle aspirations.

In summary, T cell-mediated recognition of tumors occurs through complex interactions related to patients' genetic makeup, individual exposure to immunogen, and instability of tumor cells during the neoplastic process. Knowledge of these and of other not-yet-identified variables is necessary for the understanding of tumor immunology and the definition of rational therapeutics.

Recent Publications:

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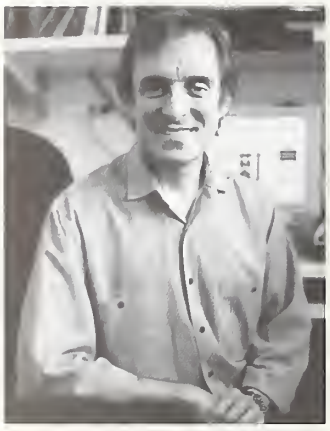
Lee K–H, et al. *J Immunol* 1999;163:6292–300.

Kammula U, et al. *J Immunol* 1999;163:6867–79.

Marincola FM, et al. *Adv Immunol* 2000;74:181–273.



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Biography: *Dr. Restifo, a 1983 honors graduate from Johns Hopkins University, obtained his M.D. in 1987 from New York University. He held fellowships at the Memorial Sloan-Kettering Cancer Center and the NCI before becoming a principal investigator in 1993. He won the NIH Technology Transfer Award in 1995 and 1998 for his work on the design of recombinant and synthetic anticancer vaccines. He is a research preceptor for the Howard Hughes Medical Institute and an associate editor at the* Journal of Immunotherapy *and the* Journal of Immunology.

Surgery Branch
Designing Recombinant and Synthetic Vaccines for Cancer

Keywords:
autoimmunity
cancer immunotherapy
cellular immunity
vaccine design

Research: The essence of our research efforts is the design of recombinant and synthetic vaccines for the treatment of patients with cancer. At the heart of our efforts are mouse models for human cancer. Our goal is to translate findings into new treatment strategies for patients with cancer.

Vaccines are traditionally thought of as preventing infectious diseases, but they may have new uses in the treatment of malignancies. In the case of cancer, it is now clear that the immune system can recognize and destroy even large quantities of established tumor. Evidence for this immune-

mediated destruction comes primarily from clinical trials using interleukin 2 (IL–2), which is now an FDA-approved treatment for melanoma and renal cell carcinoma. New immunotherapies based on vaccines designed to treat cancer have now been used in the clinic. These therapeutic vaccines have been clearly demonstrated to elicit antitumor immune responses. In a number of cases, these vaccines have also been reported to mediate the destruction of established cancer.

How does one go about choosing an antigen appropriate for use in the design of a cancer vaccine? The molecular identification of the antigens present on cancers that are recognized by the immune system is central to the development of recombinant and synthetic vaccines. Because of the difficulty in predicting what peptides will be present on the cell surface, one of the most successful approaches to identifying tumor-associated antigens suitable for the development of cancer vaccines starts with the antitumor immune response. T cells with antitumor reactivity are then used to screen cDNA libraries made from melanoma cell lines.

Many of the tumor antigens that have been identified are tissue differentiation antigens in melanocytes and include gp100, MART–1/MelanA, tyrosinase, and tyrosinase-related proteins (TRP)–1/gp75 and TRP–2. Interestingly, these antigens are involved in the synthesis of melanin and give both melanocytes and deposits of melanoma tumor their dark pigment.

The fact that differentiation antigens are nonmutated in most tumors has two important implications. First, expression of these tissue differentiation enzymes is shared by the great majority of melanoma nodules from the great majority of patients, and thus an off-the-shelf vaccine strategy targeting these antigens is possible (a strategy that targets a mutated antigen may have to be individualized for every mutation). Second, the nonmutated nature of these antigens suggests that immunotherapies that target these antigens could elicit autoreactivity.

One consequence of this autoreactivity may be vitiligo, the patchy and permanent loss of pigment from the skin and hair thought to result from the autoimmune destruction of pigment cells. Vitiligo generally heralds a positive response to the intervention and has been correlated with objective shrinkage of deposits of metastatic melanoma in patients receiving high dose interleukin 2 (IL–2), a cytokine known to activate and expand T lymphocytes. Thus, there is evidence that vitiligo can be coupled with tumor regression, and that adoptive transfer of antitumor T cells recognizing differentiation antigens is associated with objective shrinkage of melanoma deposits.

As immunogens we have used peptides and proteins made so that their sequences correspond to sequences from tumor antigens. We have also used viruses like vaccinia, influenza A, fowlpox, and adenoviruses to immunize patients with cancer. We have adapted these viruses to treat cancer by making them recombinant—that is, capable of mediating the expression of proteins that are not normally encoded by the virus. We have not yet been able to demonstrate that these recombinant viruses have clinical efficacy. However, we have shown recently that immunization of mice with a

recombinant vaccinia virus encoding a melanocyte differentiation antigen (TRP–1) can both induce vitiligo and protect mice from a challenge with an experimental melanoma.

One particularly exciting strategy designed to treat patients with cancer involves the use of naked nucleic acid vaccines. We have recently significantly enhanced the immunogenicity of a nucleic acid vaccine by making it self-replicating. This was accomplished by using a gene encoding an RNA replicase polyprotein derived from the Semliki Forest Virus (SFV) used in combination with a model antigen. A single intramuscular injection of a self-replicating RNA immunogen elicited antigen-specific antibody and CD8+ T cell responses at doses as low as 0.1 mg. Preimmunization with a self-replicating RNA vector protected mice from tumor challenge and, more importantly, therapeutic immunization prolonged the survival of mice bearing established tumor.

Interestingly, the self-replicating RNA vectors did not mediate the production of significantly greater quantities of the model antigen when compared with a conventional DNA vaccine in vitro. However, the enhanced efficacy in vivo correlated with a caspase-dependent apoptotic death in transfected cells. This death facilitated the uptake of apoptotic cells by dendritic cells, providing a potential mechanism for enhanced immunogenicity. Naked, noninfectious, self-replicating RNA may be an excellent candidate for the development of novel cancer vaccines.

Among our collaborators are Jack Bennink, Bernard Moss, and Jonathan Yewdell, NIH; Michael De Veer, Robert Silverman, and Bryan Williams, Cleveland Clinic Foundation; Linda Gritz and Dennis Panicalli, Therion Corp.; and Martin Sanda, University of Michigan.

Recent Publications:

Ying H, et al. *Nat Med* 1999;5(7):823–7.

Overwijk WW, et al. *Proc Natl Acad Sci USA* 1999;96(6):2982–7.

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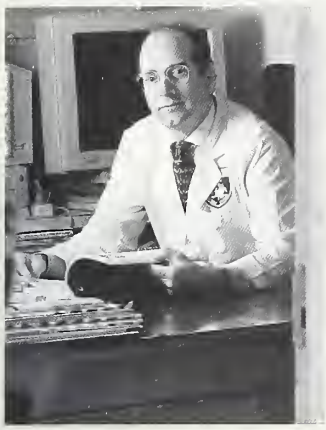

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Biography: *David S. Schrump graduated from the University of Connecticut School of Medicine and completed his general surgery residency at the University of Chicago as well as a 3-year research fellowship in Human Cancer Immunology at Memorial Sloan-Kettering Cancer Center. Following completion of his thoracic surgery residency at the University of Michigan in 1993, Dr. Schrump was appointed assistant professor of thoracic surgery at the M.D. Anderson Cancer Center, where he gained expertise in the molecular biology and clinical management of thoracic malignancies. In 1997, he was appointed head of thoracic oncology.*

Surgery Branch
Molecular Intervention in Thoracic Malignancies

Keywords:
antigen presentation
antisense
cancer antigens
cancer immunotherapy
cell cycle
clinical trial
esophageal cancer
gene regulation
gene therapy
lung cancer
tumor antigen
vaccines

Research: The inability of current treatment modalities to alter the natural history of cancers involving the lungs, esophagus, or pleura, together with the prevalence of these malignancies, underscores the need for more fundamental appreciation of molecular mechanisms pertaining to malignant transformation in the aerodigestive tract and pleural space. Mutations that disrupt G1 Restriction Point control occur early during multistep carcinogenesis, and in published studies we have utilized recombinant viral vectors to examine the significance of cyclin D1, p16, and p53 mutations in lung and esophageal cancer cells, as well as SV40 T antigen expression in pleural mesotheliomas.

Although gene therapy is a major focus of our laboratory work, recent efforts have been devoted to the evaluation of pharmacologic agents that might achieve molecular endpoints in clinical settings without the complexities and limitations of gene therapy mediated by viral vectors. In conjunction with Dr. Dao Nguyen, Senior Investigator, Thoracic Oncology Section, we have published data indicating that the synthetic cdk inhibitor flavopiridol induces profound cell cycle arrest and apoptosis in esophageal cancer cells irrespective of histology or tumor suppressor gene status, and markedly enhances their sensitivity to paclitaxel. In addition, we have observed that flavopiridol induces cell cycle arrest and apoptosis in pleural mesothelioma cells, irrespective of SV40 T antigen expression. These data have provided the rationale for the evaluation of flavopiridol in conjunction with paclitaxel in esophageal cancer patients, and indicate that flavopiridol may be a novel agent for the treatment of mesotheliomas that typically are refractory to conventional chemotherapeutics.

Approximately 30 percent of lung and esophageal cancers overexpress the erbB–2 oncogene that mediates resistance to chemotherapeutic agents such as cisplatin or paclitaxel. Recently, we have observed rapid, dose-dependent depletion of p185 protein in lung and esophageal cancer cells treated with the ansamycin analog 17-allylamino geldanamycin (17–AAGA). Reduction of p185 expression by 17–AAGA markedly enhances paclitaxel-mediated

cytotoxicity in these cells, and inhibits their metastatic phenotype evidenced by enhanced expression of E-cadherin, decreased expression of MMP–9 and VEGF, and reduced invasion into extracellular matrix. These preclinical data support the evaluation of 17–AAGA in combination with paclitaxel in patients with thoracic malignancies.

In additional studies we have utilized the demethylating agent 5 Aza-2′ deoxycytidine (DAC) and the histone deacetylase inhibitor FR901228 (depsipeptide) to induce expression of tumor suppressor genes such as p16 and ARF that are frequently inactivated by promoter hypermethylation in thoracic neoplasms. Under conditions potentially achievable in clinical settings, DAC mediates pronounced inhibition of cancer cell proliferation in part via induction of p16 expression. In parallel studies we have demonstrated induction of NY–ESO–1 and MAGE–3 cancer testis antigen expression in lung and esophageal cancer as well as pleural mesothelioma cells by 5 Aza-2′ deoxycytidine. Our studies have indicated that cancer testis antigen expression is more readily induced in tumor cells relative to normal human bronchial epithelial cells, fibroblasts, or EBV-transformed lymphocytes. Exposure of lung and esophageal cancer as well as pleural mesothelioma cells to 5 Aza-2′ deoxycytidine facilitates their recognition by cytolytic T cells specific for NY–ESO–1. Additional studies have shown that depsipeptide exhibits only modest activity regarding target gene induction; however, this agent mediates pronounced cytotoxicity in lung cancer cells. Furthermore, depsipeptide synergistically augments gene induction and apoptosis mediated by DAC in lung and esophageal cancer, and in pleural mesothelioma cells. Thus, sequential decitabine/depsipeptide treatment may be a novel strategy to simultaneously induce growth arrest and apoptosis in solid tumors and augment antitumor immunity in patients with these neoplasms. Current protocols are under way to examine the feasibility of tumor-specific gene induction in patients with thoracic malignancies.

Recently we have sought to define the molecular mechanisms conferring preferential sensitivity to apoptosis in lung cancer cells relative to normal human bronchial epithelial cells following sequential decitabine/depsipeptide treatment. Using cDNA microarray techniques, we have identified a variety of cell cycle-related genes that are either induced or repressed following decitabine, depsipeptide, or sequential drug exposure. Two genes that are induced in lung cancer cells by drug treatment have been implicated in G2/M checkpoint control and apoptosis in normal cells; studies are in progress to define their roles in lung cancer, which as of yet have not been elucidated. Further work is in progress to expand this analysis in cultured lung cancer cells and to refine techniques for microarray analysis of gene expression in biopsy specimens obtained from lung cancer patients receiving decitabine or depsipeptide in clinical trials conducted by the Thoracic Oncology Section.

Nearly 20 percent of all cancer patients succumb to isolated pulmonary metastases, and additional efforts in our section have been directed toward the evaluation of regional drug delivery to the lungs. A sheep model of isolated lung perfusion (ILuP) has been established, and pharmacokinetics of paclitaxel administered by ILuP have been characterized. Additional studies have shown that moderate hyperthermia (39.5 °C) markedly enhances

paclitaxel-mediated toxicity in cancer cells, but not in normal bronchial epithelial cells. These preclinical experiments have provided the rationale for analysis of isolated lung perfusion utilizing paclitaxel and moderate hyperthermia in patients with unresectable pulmonary malignancies. In conjunction with Battelle Memorial Institute, additional clinical efforts have been devoted to the evaluation of chemotherapeutic agents administered by novel inhalation techniques that may enable highly efficient drug uptake in the lungs with negligible systemic toxicity in patients with unresectable pulmonary neoplasms.

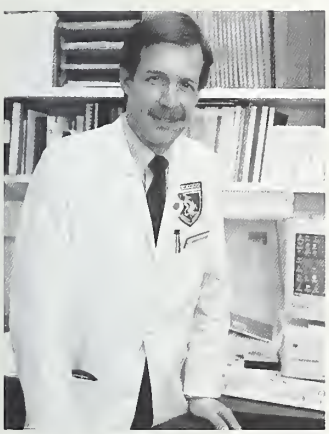
Recent Publications:

Schrump DS, et al. *Clin Cancer Res* 1998;4:2885–90.

Waheed I, et al. *Cancer Res* 1999;59:6068–73.

Weiser T, et al. *J Immunother* 2001;24:151–61.

Schrump DS, et al. *Semin Cancer Biol* 2001;11:73–80.



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Biography: *Dr. Schwartzentruber received his M.D. in 1982 and general surgery training at Indiana University School of Medicine. He completed immunotherapy and surgical oncology fellowships at the NCI and was then appointed senior investigator in the Surgery Branch of the NCI. He is also a clinical associate professor of surgery at the University of Maryland School of Medicine. Dr. Schwartzentruber's interests are in tumor immunology, primarily as it pertains to breast cancer, melanoma, and renal cell carcinoma.*

Surgery Branch
Identification of Tumor-Reactive Lymphocytes in Breast Cancer

Keywords:
breast cancer
immunotherapy
lymphocytes

Research: Immunotherapy of patients with metastatic malignant melanoma using tumor-infiltrating lymphocytes (TIL) and interleukin 2 has resulted in significant clinical regressions. Subsequent studies have led to the identification of the tumor antigens recognized by melanoma TIL. Our studies are focused on lymphocytes infiltrating breast carcinomas. The goal of this work is to identify breast cancer-specific TIL and to characterize the tumor antigens they recognize. We expect to translate these findings into therapies for patients with breast cancer by creating vaccines which utilize the antigens recognized by TIL.

We have cultured and studied TIL from 54 breast cancer patients (primary or metastatic sites). No culture has shown specific tumor cytolysis. However, three CD4+ cultures have shown specific cytokine secretion when stimulated with fresh autologous tumor, suggesting that they recognize unique tumor

antigens. TILs have also been cultured in limiting dilution conditions, and CD8+ cytolytic lymphocytes (CTL) with specific tumor reactivity have not been generated. We are studying culture conditions and means of in vitro T cell activation that will result in specific CTL generation (which at present is common only in melanoma).

The long-term culture of fresh breast cancer cells derived from patients has been largely unsuccessful in our lab and many other labs. The short supply of tumor cells is a limitation to our studies. Hence, our recent approach has been to immortalize short-term tumor cultures with the retroviral vector CRIP E6E7. In parallel, we have developed fibroblast and B cell lines, all of which are necessary for lymphocyte cloning and testing.

In summary, we are studying biologic reagents that we anticipate will enhance our understanding of immune-mediated cancer regression, and allow us to develop novel immunotherapies for patients with metastatic cancer.

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Biography: *Dr. Topalian received her M.D. from Tufts University School of Medicine. She is a board-certified general surgeon, having completed her residency training at the Thomas Jefferson University Hospital in Philadelphia. Her research training includes fellowships at the Children's Hospital of Philadelphia and subsequently in the Surgery Branch, NCI. Dr. Topalian's clinical and basic research efforts focus on the immunology and immunotherapy of human cancers.*

Surgery Branch
Specific Immune Recognition of Tumor-Associated Antigens by Human T Cells

Keywords:
immunotherapy
melanoma
prostate cancer
vaccines

Research: The focus of this laboratory is to characterize human immune responses to tumor-associated antigens (Ag), to identify the recognized Ag, and to use this knowledge to develop novel immunotherapies for cancer. We have previously demonstrated that both CD8+ and CD4+ lymphocytes derived from cancer patients can manifest MHC-restricted recognition of tumor-associated Ag. This has been shown for patients with melanoma, colon carcinoma, breast carcinoma, and lymphoma. Current projects focus on identifying melanoma-associated proteins and peptides recognized by CD4+ T cells, as well as extending this work to prostate cancer. These studies are directly relevant to clinical efforts to develop cancer vaccines.

Presently, specific immunotherapies for cancer, such as vaccines, emphasize MHC class I-restricted Ag recognized by CD8+ T cells. While CD8+ cells are important effectors in antitumor immunity, animal models of immunity

against viral Ag, alloantigens, and tumor Ag demonstrate the critical role of the helper arm of the immune response in initiating, amplifying, and maintaining Ag-specific immunity. Thus, since 1991 this laboratory has emphasized studies of specific CD4-mediated antitumor immune responses. By screening melanoma patients whose CD8+ tumor infiltrating lymphocytes (TIL) manifested specific recognition of autologous tumor, we identified several patients whose CD4+ TIL also demonstrated antitumor specificity. A new system was developed for assessing T cell recognition of shared MHC class II-restricted tumor Ag using autologous EBV–B cells as antigen-presenting cells (APC) for tumor lysates. Using this system, we have shown that tyrosinase, a protein with expression linked to the melanocytic lineage, is recognized by melanoma-reactive CD4+ T cells from two of nine patients tested. This was the first identification of a shared human melanoma-associated protein recognized by CD4+ T cells. Importantly, since tyrosinase is commonly expressed by melanoma tumors and is recognized by CD8+ as well as CD4+ T cells, our results suggest that this protein could be an optimal immunogen for melanoma vaccines. Clinical protocol 99–C–0095 will test the effects of vaccination against tyrosinase in treating patients with metastatic melanoma.

While tyrosinase-specific CD4+ T cells recognize a commonly expressed melanoma-associated Ag, melanoma-specific CD4+ T cells derived from seven other patients seem to recognize Ag unique to autologous tumor cells that are likely the result of somatic mutations. The nature of these Ags is beginning to be defined. By applying sequential biochemical purifications to tumor lysates, coupled with mass spectrometric sequencing, a mutated enzyme triosephosphate isomerase was identified as the specific Ag in one system. In collaboration with other Surgery Branch investigators, a different approach applied a novel molecular cloning strategy to identify a mutation in the cell cycle-related protein CDC27, which generated a neoepitope recognized by one patient's T cells. Identification of proteins other than tyrosinase that can serve as targets for CD4-mediated immune responses will enhance our knowledge of how the human immune system responds to cancer and may ultimately lead to improved strategies for cancer immunotherapy.

Additional efforts are directed toward applying information gained from melanoma studies to a much more common form of cancer, prostate cancer. Studies of antitumor immunity in prostate cancer are rare, in part owing to difficulties in obtaining adequate amounts of tumor tissue for testing. However, there is a critical need to develop new methods of diagnosis and treatment for this disease. To generate reagents for immunologic studies, we recently developed a highly successful method for establishing long-term prostate epithelial cell lines from fresh operative specimens, involving immortalization with the HPV–16 E6 and E7 proteins. Using completely autologous systems consisting of lymphocytes and cultured tumor cells derived from prostate cancer patients treated at the NCI, we have developed in vitro stimulation techniques to demonstrate prostate cancer-reactive human T cells. From one patient, CD8+ T cells recognized a protein expressed widely by many prostate cancers as well as by colon cancers, through a nonclassical restriction mechanism that is still under study. Since nonclassical

restriction molecules are typically nonpolymorphic, identification of tumor Ag presented by these molecules holds promise for the development of "universal" cancer vaccines.

Recent Publications:

Wang R–F, et al. *Science* 1999;284:1351–4.

Pieper R, et al. *J Exp Med* 1999;189:757–65.

Pardoll DM, et al. *Curr Opin Immunol* 1998;10:588–94.

Cormier JN, et al. *J Immunother* 1998;21:27–31.

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Biography: *Dr. Yang graduated from the Massachusetts Institute of Technology with a B.S. in biology. He then completed his M.D. and surgical internship and residency at the University of California, San Diego. In 1984, he came to the Surgery Branch, NCI, as a medical staff fellow. He subsequently became a senior staff fellow, then a senior investigator in 1987. Dr. Yang has been involved in studies of the immune response to tumor-associated antigens and has been a principal investigator of clinical trials in renal cell carcinoma and soft tissue sarcoma.*

Surgery Branch
Tumor Antigens in Murine Models and Human Renal Cancer

Keywords:
angiogenesis
immune response
renal cell carcinoma
tumor antigen

Research: The laboratory's major interest is in elucidating mechanisms of immune tumor rejection using murine models and studying human renal cancer. In the last decade, it has been established that the immune system can cause the complete and durable rejection of certain metastatic cancers in some individuals. Our understanding of this process has been greatly enhanced by the discovery of several dozen tumor-associated antigens expressed by human melanoma, which can be recognized by tumor-reactive T cells. At this point, the two major new challenges are to develop ways to manipulate and enhance the reactivity to known antigens to increase the frequency of tumor rejection, and to identify similar antigens from tumors other than melanoma. Towards the first goal, we have been working to identify the dominant native antigens in realistic murine tumor models so that therapeutic strategies can be developed using accurate preclinical systems. We described tyrosinase-related protein-2 (TRP–2) as an antigen for the B16 melanoma and identified the specific epitope processed and recognized by T cells. Another tumor-expressed antigen, p15E, an endogenous murine retroviral envelope protein, was identified as an immune target on the murine MC–38 colon cancer as well as on other syngeneic tumors. The T cell response to these antigens and the parameters which quantitate the response in immune and nonimmune

mice were also clarified. We have recently identified two other antigens expressed by the MC–38 tumor and are in the process of characterizing them. Future work will test strategies for enhancing the immune response and therapeutic potential of these immune responses by modifying the antigens and adding vaccine and cytokine adjuncts.

In pursuing the second major goal, we have concentrated on human renal cell cancer (RCC). This malignancy is one that is responsive to immunotherapy, showing some durable complete responses to interleukin 2. We are conducting a randomized trial of 400 patients to determine the effect of IL–2 dose on response rate and overall survival of patients with metastatic RCC. In parallel, we have been developing methods for cloning T cells from patients with RCC that recognize their cancer. This has been successfully accomplished and these cells have been used to identify two antigens from a renal cancer which serve as immune targets. Both of these antigens are unmutated and one is expressed on multiple renal cancers as well as on some samples of prostate and bladder cancer. Current work directed at developing ways to immunize with these antigens may be of utility against a variety of cancers.

Another new clinical initiative in RCC, begun in collaboration with other investigators, has been a clinical trial of an antiangiogenic agent. We are conducting a blinded, randomized, placebo-controlled trial of an antibody which neutralizes vascular endothelial growth factor (VEGF). VEGF is a hormone involved in the growth of cells lining new blood vessels. It is overproduced by nearly all RCCs and this overproduction seems related to the mutation in the von Hippel-Lindau tumor suppressor gene that is found in nearly all clear-cell RCCs. Therefore it is thought that VEGF is important in the growth of the abundance of tumor vessels seen in RCC and represents a favorable target for antiangiogenic therapy. This trial is examining response rate, time-to-progression, and survival in patients with metastatic RCC. In addition, surrogate endpoints for antiangiogenic trials are being sought using experimental imaging techniques in coordination with clinical evaluation.

Recent Publications:

Bloom MB, et al. *J Exp Med* 1997;185:453–9.

Zeh HJ, et al. *J Immunol* 1999;162:989–94.

Yang JC, et al. *J Immunother* 2000; in press.

Hanada K, et al. *Cancer Res* 2001;61:5511–6.

Clinical Trials:

H. Richard Alexander	**99–C–0093:** A phase II trial of isolated hepatic perfusion with melphalan followed by intra-arterial FUDR and leucovorin for metastatic colorectal cancer
	99–C–0123: A phase II study of isolated hepatic perfusion (IHP) with melphalan for metastatic unresectable cancers of the liver
	99–C–0145: A two-stage randomized phase II trial of isolated limb perfusion (ILP) with melphalan with or without tumor necrosis factor for patients with high-grade unresectable extremity sarcoma
David L. Bartlett	**97–C–0072:** A phase I trial of continuous hyperthermic peritoneal perfusion (CHPP) with cisplatin plus early postoperative intraperitoneal paclitaxel and 5–FU for peritoneal carcinomatosis
	00–C–0069: A phase II trial of continuous hyperthermic peritoneal perfusion (CHPP) with cisplatin plus early postoperative intraperitoneal paclitaxel and 5–FU for peritoneal carcinomatosis
David N. Danforth	**90–C–0044:** Effects of preoperative chemotherapy on axillary lymph node metastases in stage II breast cancer: a prospective randomized trial
	94–C–0151: Positron emission tomography in patients with breast cancer
	96–C–0026: Establishment of normal breast epithelial cell lines from patients at high risk for breast cancer
Patrick Hwu	**96–C–0011:** Treatment of patients with advanced epithelial ovarian cancer using anti-CD3-stimulated peripheral blood lymphocytes transduced with a gene encoding a chimeric T cell receptor reactive with folate binding protein
	98–C–0040: A phase II protocol of FLT3 ligand in patients with metastatic melanoma and renal cancer
	99–C–0132: A randomized comparison between adjuvant CD34-derived and peripheral monocyte-derived dendritic cells in patients with high-risk stage III or completely resected metastatic melanoma
Steven K. Libutti	**97–C–0068:** A phase II study of the role of anti-CEA antibody immunoscintigraphy and positron emission tomography in the localization of recurrent colorectal carcinoma in patients with rising serum CEA levels in the absence of imageable disease by conventional modalities
	98–C–0163: The use of positron emission tomography (PET) and magnetic resonance imaging (MRI) to assess the effects of antineoplastic therapy on tumor-associated vasculature
	OH99–C–N008: A pilot study to analyze gene expression by microarray technique in human breast cancer samples
	99–C–0025: The use of radiofrequency ablation to treat hepatic neoplasms
	99–C–0102: A phase II trial of oral thalidomide as an adjuvant agent following metastasectomy in patients with recurrent colorectal cancer
Francesco M. Marincola	**97–C–0019:** A phase I study in patients with metastatic melanoma of immunization with dendritic cells presenting epitopes derived from the melanoma-associated antigens MART–1 and gp100

Clinical Trials (continued):

Steven A. Rosenberg

96–C–0121: A phase I trial in patients with metastatic melanoma of immunization with a recombinant fowlpox virus encoding the gp100 melanoma antigen

98–C–0022: Immunization of patients with metastatic melanoma using immunodominant peptides from the tyrosinase protein or tyrosinase-related protein-1 (TRP–1) or gp100 protein

98–C–0086: Immunization of patients with metastatic melanoma using DNA encoding the gp100 melanoma antigen

98–C–0095: Treatment of patients with metastatic melanoma using cloned peripheral blood lymphocytes sensitized in vitro to the gp209–2M immunodominant peptide

98–C–0142: Immunization of patients with metastatic melanoma using the gp100 peptide preceded by an endoplasmic reticulum insertion signal sequence

99–C–0044: Immunization of patients with metastatic melanoma using a recombinant fowlpox virus encoding a gp100 peptide preceded by an endoplasmic reticulum insertion signal sequence

99–C–0092: Immunization of patients with metastatic melanoma using MART–1 and gp100 peptides modified to increase binding to HLA–0201

99–C–0128: Evaluation for NCI Surgery Branch clinical research protocols

99–C–0158: Treatment of patients with metastatic melanoma using cloned lymphocytes following the administration of a nonmyeloablative but lymphocyte depleting protocol

99–C–0159: Immunization of patients with metastatic melanoma using a class II restricted peptide from the gp100 antigen and class I restricted peptides from the gp100 and MART–1 antigen

00–C–0098: A phase I trial of a live, genetically modified *Salmonella typhimurium* (VNP20009) for the treatment of cancer by intravenous administration

00–C–0216: A randomized comparison of three schedules of peptide immunization in patients at high risk for recurrence of melanoma

01–C–0032: Immunization of HLA–A *0201 patients with metastatic cancer using a modified epitope from the ESO–1 antigen

01–C–0176: Immunization of HLA–A *0201 patients with metastatic cancer using a peptide epitope from the telomerase antigen

Pending: Immunization of HLA–0201-positive patients with metastatic melanoma using a peptide from tyrosinases-related protein 2 (TRP–2)

David S. Schrump

99–C–0129: A phase I study of decitabine-mediated induction of tumor antigen and tumor suppressor gene expression in patients with cancers involving the lung, esophagus, or pleura

00–C–0019: A phase I study of isolated lung perfusion with paclitaxel and moderate hyperthermia in patients with unresectable pulmonary malignancies

Clinical Trials (continued):

David S. Schrump	**00–C–0088:** A phase I and clinical pharmacologic study of inhaled doxorubicin in adults with advanced solid tumors affecting the lungs
	00–C –0123: A phase II study of gene induction mediated by 4-hr intravenous depsipeptide (FR901228/NSC 630176) infusion in lung cancer patients
Suzanne L. Topalian	**99–C–0095:** Immunization of patients with metastatic melanoma using recombinant vaccinia and fowlpox viruses encoding the tyrosinase antigen
James C. Yang	**91–C–0094:** A randomized three-arm study of the treatment of patients with metastatic renal cell carcinoma using low-dose IL–2 or high-dose IL–2
	98–C–0033: Trafficking of indium-III-labeled cultured immune cells in patients undergoing immunotherapy for advanced cancer
	98–C–0159: Treatment of patients with metastatic renal cell carcinoma with neutralizing antibody to vascular endothelial growth factor (VEGF)

Urologic Oncology Branch

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The Urologic Oncology Branch conducts clinical and basic research designed to develop better methods for detection, prevention, and therapy of patients with genitourinary malignancies. The primary focus of the Urologic Oncology Branch is the study of the genes associated with initiation and progression of kidney and prostate cancers.

In the Urologic Oncology Branch, patients with hereditary forms of genitourinary malignancies, von Hippel-Lindau (VHL) disease, hereditary papillary renal carcinoma (HPRC), and hereditary prostate carcinoma are evaluated and treated by a multidisciplinary team. In the laboratory, studies are under way to determine the molecular mechanisms of kidney cancer and prostate cancer. The kidney cancer disease genes, the VHL gene, and the HPRC genes are studied. Studies of molecular genetics of renal carcinoma, including the role of the recently identified VHL tumor suppressor gene—the disruption of whose processes causes acquired or hereditary cancer—are under investigation. Studies of the subcellular localization and identification, both of the associated proteins and of the function of the VHL protein, are in progress, as well as the genomic and cytogenetic changes associated with the initiation of urologic malignancies. The role of tumor suppressor genes in prostate cancer is also under investigation. Study of the molecular biological changes associated with hereditary and sporadic prostate cancer is being carried out. This branch utilizes the findings of the molecular genetics of genitourinary malignancies to develop better methods for early diagnosis of both the hereditary and the nonhereditary (sporadic) forms of urologic malignancies.

Urologic Oncology Branch Staff

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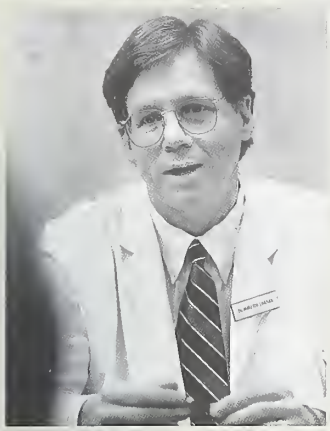

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Biography: *Dr. Linehan came to the NIH after completing his training in urologic surgery at Duke University Medical Center in Durham, NC. He has held positions of increasing responsibility at the NCI and was part of the team responsible for identification of the von Hippel-Lindau (VHL) and hereditary papillary renal cell carcinoma (HPRC) kidney cancer genes. He has a wide range of scientific interests and accomplishments spanning molecular genetics of urologic malignancies and evaluation and treatment of patients with hereditary and sporadic forms of genitourinary cancers.*

Urologic Oncology Branch
Molecular Genetic Analysis of Genetic Events Associated With Initiation and Progression of Urologic Malignancies

Keywords:
kidney cancer
prostate cancer

Research:

Characterization of the VHL Suppressor Gene Product

The VHL tumor suppressor gene is the gene for both von Hippel-Lindau disease as well as sporadic, clear-cell renal carcinoma. Germline mutations in the VHL gene predispose individuals to a variety of tumors, including renal carcinoma, CNS hemangioblastoma, islet cell tumors of the pancreas, and pheochromocytoma. The cellular transcription factor Elongin (SIII) has been identified as a functional target of the VHL protein. The VHL protein has been shown to bind tightly and specifically to the Elongin B and C subunits and to inhibit Elongin (SIII) transcriptional activity in vitro. The VHL protein is found both in the nucleus and in the cytosol of transiently transfected cells. There is a tightly regulated, cell density-dependent transport of VHL into and/or out of the nucleus. A putative nuclear localization signal, a nuclear export signal, and a cytoplasmic retention locus have been identified in the VHL protein. These findings provide the initial indication of a novel cell density-dependent pathway that is responsible for the regulation of VHL cellular localization. In order to further study the function of the VHL gene, additional studies of VHL-associated proteins have been carried out. Recently, Hs–CUL–2, a member of the recently identified multigene family, the cullins, has been shown to specifically associate with the trimeric pVHL-elongin B–C (VBC) complex in vitro and in vivo. Nearly 70 percent of naturally occurring cancer-predisposing mutations of VHL disrupt this interaction. Immunofluorescence studies show Hs–CUL–2 to be a cytosolic protein that can be translocated to the nucleus by pVHL. Hs–CUL–2 may be required for VHL function and, therefore, may be a candidate human tumor suppressor gene. We have recently developed an improved method of detecting germline mutations in the von Hippel-Lindau disease tumor suppressor gene. We currently can detect mutations in a high percentage of kindreds with this hereditary cancer syndrome (nearly 100 percent). We have also recently identified a new phenotype associated with complete deletion of the VHL gene and are studying the somatic events associated with the

development of cancer in this syndrome. We hope that understanding the molecular mechanism leading to cancer will lead to the development of new strategies for early detection, prevention, and treatment.

Characterization of the Hereditary Papillary Renal Cell Carcinoma Gene
A new hereditary cancer syndrome, hereditary papillary renal cell carcinoma (HPRC), has been described. Affected individuals with this hereditary form of renal cell carcinoma are at risk of developing multifocal, bilateral hereditary papillary renal cell carcinoma. Families with HPRC have been evaluated and linkage analysis has localized the HPRC to a locus on chromosome 7. The c-Met oncogene has been determined to be the HPRC gene. We have detected germline and somatic mutations in the tyrosine kinase domain of the Met proto-oncogene in papillary renal carcinomas and developed a strategy for predictive testing in this hereditary cancer syndrome. We have characterized the nonrandom duplication of the chromosome bearing the mutated Met in HPRC and demonstrated that this implicates this event in tumorigenesis.

Molecular Genetic Events Associated With Initiation and Progression of Prostate Cancer
The molecular genetic events associated with the initiation and progression of prostate cancer are poorly understood. Using a novel technique to microdissect tissue under direct microscopic visualization, pure populations of prostate carcinoma have been procured from prostate specimens. This microdissection has allowed the accurate examination of both DNA content and RNA expression in prostate carcinoma. Loss of heterozygosity at a locus on chromosome 8 in a high percentage of tumors from patients with prostate carcinoma has been detected and the area of minimal deletion on chromosome 8p12–21 determined. The identical genetic loss has been shown to be associated with prostate intraepithelial neoplasia (PIN), a precursor lesion in prostate cancer. In collaboration with scientists in the Laboratory of Pathology, a technique for recovering and analyzing RNA from microdissected prostate cancer and by differential gene expression analysis has been developed, and a novel zinc finger gene upregulated in prostate cancer has been identified. cDNA libraries have been developed from normal, low-grade PIN, high-grade PIN, and prostate cancer. These libraries will be used in the microarray analysis to evaluate differentially expressed genes involved in initiation and progression of prostate cancer.

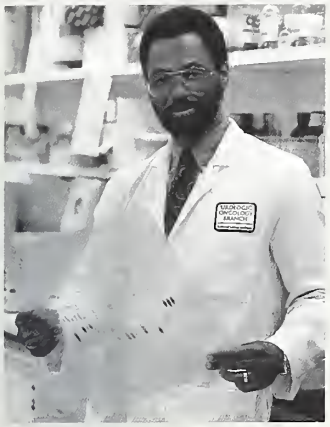
Recent Publications:

Schmidt L, et al. *Oncogene* 1999;18:2343–50.

Toro J, et al. *Arch Dermatol* 1999;135:1195–202.

Koochekpour S, et al. *Mol Cell Biol* 1999;19:5902–12.

Childs R, et al. *N Engl J Med* 2000;343:750–8.



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Biography: *Dr. Leach received his Ph.D. and M.D. from Stanford Medical School in 1990, and subsequently became first a surgical resident, then a postdoctoral fellow with the Oncology Center at Johns Hopkins Hospital. After completing his urology residency at the University of Texas Southwestern Medical Center in 1999, he joined the Urologic Oncology Branch of the NCI.*

Urologic Oncology Branch
Molecular Genetics of Urologic Malignancies

Keywords:
bladder cancer
hMSH2
mismatch repair

Research: One of our research aims is to investigate the importance of mismatch repair genes in the pathogenesis of urothelial malignancies. The human mismatch repair gene, hMSH2, is one of several genes which predisposes to the development of hereditary cancer(s)when mutated. We have recently shown overexpression of this gene product in bladder cancer and sporadic colon cancers using immunohistochemical analysis. Based on these data, an assay using reverse transcriptase polymerase chain reaction (RT–PCR) amplification of bladder washes has been developed. Using this assay, we hope to identify both those individuals with primary bladder cancers and recurrences in which the hMSH2 gene is actively expressed as well as those individuals with bladder cancers that do not express the hMSH2 gene product. Tumors from these individuals may be important in understanding the molecular events leading to development, recurrence, and progression of urothelial malignancies.

A further goal is to investigate the molecular pathogenesis of bladder cancer invasion and progression. Many individuals present with superficial bladder cancer that progresses to muscle invasive disease.

Finally, we are focusing our efforts on the development of novel approaches to the treatment of advanced invasive bladder cancer.

Recent Publications:

Leach FS, et al. *Cancer Res* 1996;56:235–40.

Leach FS, et al. *Cell* 1993;75:1215–25.

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Biography: *Dr. Walther received his B.S. in chemical engineering and M.D. from Louisiana State University. He finished his training in urologic surgery at Emory University in 1986 and was an attending there for 2 years. He joined the staff in the Urologic Oncology Branch in November 1988. His expertise is the area of urologic oncology, including the management of hereditary kidney and adrenal tumors, and complex laparoscopic operations. He acts as urologic consultant and has interests in urologic oncology including renal surgery, laparoscopy, and natural history and management of hereditary cancer syndromes.*

Urologic Oncology Branch
Natural History and Treatment of Hereditary Kidney Cancer Syndromes

Keywords:
adrenal tumors
genetics
hereditary disease
linkage studies
metastasis
renal cell carcinoma

Research: Our group screens families with hereditary forms of renal cancer to identify affected members and perform linkage analysis. As part of this study, we see patients with hereditary and sporadic adrenal tumors. The goal of our research is the identification of genes important to the development of urologic malignancies. The molecular mechanisms of carcinogenesis are just now beginning to be examined and understood. The study of families affected with hereditary forms of cancer provides two avenues of research. First, study of inheritance patterns allows the powerful tool of linkage analysis to localize a disease gene to a chromosomal location. Further study can lead to identification of the disease gene. Second, the study of different germline mutations gives insight into how loss or altered function of a gene gives rise to different clinical findings in humans.

Identification of hereditary mutations should describe the earliest genetic changes that lead to malignant transformation. The study of somatic mutations in tumors from patients with sporadic forms of cancer can corroborate the familial studies and give further insight into mechanisms of inactivation.

Collaborating with us are Peter Choyke, Graeme Eisenhoffer, John Gill, Gladys Glenn, Irina Lubensky, Karel Pacek, Steven Rosenberg, David Venzon, James Yang, and Berton Zbar, NIH; Louis Kavoussi, Johns Hopkins University; and Cathy Stolle, University of Pennsylvania.

Recent Publications:

Walther MM, et al. *Endocrinology* 1992; 131:2263–70.

Walther MM, et al. *Urology* 1997;50:199–206.

Walther MM, et al. *J Urol* 1999;161:1475–9.

Walther MM, et al. *Urology* 1999;53:496–501.

Clinical Trials:

W. Marston Linehan	**89–C–0086:** Clinical manifestations and molecular bases of heritable urologic malignancies
	94–C–0041: Familial prostate cancer
	OH94–C–0042: Prostate cancer in men under age 55: family history and collection of blood samples
	97–C–0147: Collection of serum and tissue samples from patients with biopsy-proved or suspected malignant diseases
	99–C–0101: Genetic analysis of inherited urologic malignant disorders: collection of samples
McClellan M. Walther	**94–C–0098:** A phase I trial using suramin to treat superficial transitional cell carcinoma of the bladder
	98–C–0054: A phase II study to evaluate a cautery device for the treatment of renal cancer
	99–C–0028: A phase II evaluation of telesurgery systems
	99–C–0170: A phase II study to evaluate radiofrequency ablation of renal cancer

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The CCR Basic Research Laboratory plans and conducts research on the cellular, molecular, genetic, biochemical, and immunological mechanisms affecting the progression, diagnosis, and treatment of cancer. Many of the researchers in the BRL actively collaborate with scientists on other research programs within both the NCI and the NIH.

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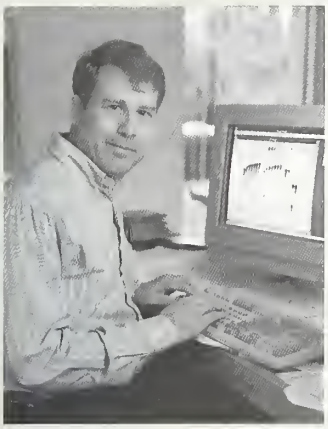

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Biography: *Dr. Baker studied adenovirus transcription with Dr. Edward Ziff at the Rockefeller University where he received his Ph.D. in molecular cell biology in 1981. He obtained his M.D. from Cornell University Medical College in 1982, followed by training in anatomic pathology in the Laboratory of Pathology, NCI, where he has remained on the clinical staff. He also trained in viral oncology with Dr. Peter Howley in the NCI's Laboratory of Tumor Virus Biology where he became a principal investigator and also served as acting chief from 1993 to 1996. He is now chief of the Cellular Regulation and Transformation Section for the Basic Research Laboratory. In addition, he is cochair of the NIH RNA Club.*

Basic Research Laboratory–Bethesda
Regulation of Papillomavirus Gene Expression

Keywords:
cervical cancer
gene expression
papillomaviruses
polyadenylation
posttranscriptional regulation
splicing

Research: The papillomaviruses are epitheliotropic viruses that induce benign and malignant lesions in a variety of squamous epithelia. The human papillomaviruses are etiologic agents of several human cancers and have been found in >95 percent of cervical cancers, >50 percent of other anogenital cancers, and 20 percent of oral, laryngeal, and nasal cancers. One interesting feature of these viruses is that their life cycle is intimately linked with the differentiation state of the squamous epithelium that they infect. The primary goals of our research are (1) to elucidate the regulatory mechanisms that control papillomavirus gene expression during keratinocyte differentiation, and (2) to study the genetic changes and cellular and viral gene expression changes that occur during malignant progression of papillomavirus lesions.

Regulation of papillomavirus gene expression occurs at both transcriptional and posttranscriptional levels. A major focus of the laboratory has been the posttranscriptional regulation of bovine papillomavirus (BPV–1) late gene expression. Previously we have used in situ hybridization to demonstrate that alternative splicing of BPV–1 late pre-mRNAs is regulated in a differentiation-dependent manner. We have identified several cis-elements that regulate splice site choice. Immediately downstream of the first of two alternative 3′ splice sites (the early 3′ splice site) is a bipartite splicing regulatory element consisting of a purine-rich exonic splicing enhancer (SE1) and a pyrimidine-rich exonic splicing suppressor (ESS1) with an essential GGCUCCCCC motif. A second enhancer element (SE2) is located a short distance upstream of the second alternative 3′ splice site (the late 3′ splice site). Exonic splicing enhancers (ESEs) function by recruiting essential splicing factors at early stages of assembly of the spliceosome while ESS elements block early steps in spliceosomal assembly. Surprisingly, we now have data indicating that ESEs can also play a role in the second step of splicing. We have used both in vitro and in vivo splicing assays to demonstrate that all three elements are essential for proper regulation of alternative splicing. All three elements require suboptimal 3′ splice sites for

proper function. SE2 is required to overcome the inhibitory effects of ESS1 at early stages of the life cycle and selects use of the early 3′ splice site. This function of SE2 is due to its ability to act as a strong ESE, even at a distance of more than 250 nt from the 3′ splice site. SE2 does not appear to block use of the downstream late 3′ splice site. Both ESE elements bind the same set of SR splicing factors. We have begun to investigate cis splicing elements in the exon downstream of the late 3′ splice site and have identified a second bipartite splicing regulatory element consisting of an AC-rich ESE (SE4) and a novel ESS (ESS2). The role of these elements in the early-to-late switch in splicing is currently being investigated. The early-to-late switch in splicing during keratinocyte differentiation is presumably due to changes in the activity of splicing factors. We are currently developing RNA affinity purification techniques to identify additional factors that bind to the papillomavirus RNA regulatory elements and will use these techniques to determine how these factors change during keratinocyte differentiation.

To study differentiation-dependent RNA processing, we have generated replication-defective recombinant adenovirus vectors that efficiently infect primary keratinocytes and express BPV–1 late pre-mRNAs from a CMV promoter. We have also become interested in parallels between the regulation of adenovirus and BPV–1 RNA processing. The early-to-late shift in adenovirus RNA processing also involves the switch from a promoter-proximal to promoter-distal 3′ splice site and from a promoter-proximal to promoter-distal poly(A) site. This switch in processing is due at least in part to virus-induced changes in the phosphorylation of SR proteins and changes in the activity of the general polyadenylation factor CstF. The chimeric BPV–1/adenovirus vectors can go through a complete adenovirus life cycle in 293 and 911 cells that complement the replication defect. Interestingly, the BPV–1 late pre-mRNA undergoes the correct early-to-late shift in both splicing and polyadenylation in parallel with similar changes in adenovirus splicing and polyadenylation. This suggests that both viruses regulate their RNA processing through the same splicing and polyadenylation factors.

We are currently expanding our splicing studies to human papillomavirus type 16 (HPV–16). In preparation for these studies, we are currently investigating differentiation-dependent promoter activity and splicing patterns in W12 cells.

Finally, we have initiated a new project to identify changes in cellular and papillomaviral gene expression as a function of both differentiation and malignant progression of HPV lesions of the cervix. Cervical cancers are associated with a high-risk subset of HPVs of which HPV–16 is the most common. The goal of this project is to identify cellular genes that regulate progression of the viral life cycle as well as genes involved in malignant progression. Laser capture microdissection is being used to collect clusters of similar cells in heterogeneous lesions. We are currently generating cDNA libraries to identify novel cervix-specific genes and to give a preliminary expression profile for cervical cancers. Future analyses will utilize microarray technology to profile gene expression. In addition, we will use quantitative real-time PCR to look at changes in alternative splicing during cervical carcinogenesis.

Our initial studies of cervical cancers have involved analysis of the Fragile Histidine Triad (FHIT) gene which is a candidate tumor suppressor gene located at 3p14.2, a locus that shows loss of heterozygosity (LOH) in cervical cancers. In addition, FHIT expression is absent in many epithelial malignancies including cervical cancer. This is frequently associated with the presence of aberrant FHIT transcripts. However, normal FHIT transcripts are sometimes found in cancer tissue and aberrant transcripts in normal tissue, raising some doubts about FHIT's role as a tumor suppressor gene. It is not known if tumor cells express both normal and aberrant transcripts or if the normal transcripts are expressed in noncancer cells in the tumor tissue. We have used nested RT/PCR to analyze FHIT expression in normal cervical epithelia and invasive cervical cancers. Normal FHIT transcript was found in two normal cervical specimens and one myometrial specimen. The majority of cancers showed both normal and aberrant transcripts, although a few tumors had only normal or aberrant transcripts. We are currently using manual and laser capture microdissection to determine if the normal transcripts in the cancers are expressed only in the contaminating stroma and normal epithelium. Five aberrant transcripts have been cloned and sequenced. All contain deletions of whole exons, including exon 5 which contains the translation initiation codon. Two transcripts also contain additional alternative exons that are flanked in the genome by 3′ and 5′ splice sites. It is not known if the aberrant transcripts arise through alternative splicing resulting from changes in splicing factors or are due to mutations in splice sites. We are currently investigating this.

Collaborators on this research include Hans-Ulrich Bernard, National University of Singapore, Republic of Singapore; Lance Liotta, Alison McBride, Shyh-Han Tan, NIH; and S.–Y. Park, Korea Cancer Center Hospital, Seoul, Korea.

Recent Publications:

Zheng ZM, et al. *Proc Natl Acad Sci USA* 1998;95:14088–93.

Zheng ZM, et al. *J Virol* 1999;73:29–36.

McBride AA, et al. *Proc Natl Acad Sci USA* 2000;97:5534–9.

Zheng ZM, et al. *J Virol* 2000;74(13):5902–10.

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Biography: *Dr. Brady joined the NCI in 1984, where he is presently chief of the Virus Tumor Biology Section. Under the guidance of Dr. Richard Consigli, he obtained his Ph.D. from Kansas State University studying the molecular structure of polyoma virus. Dr. Brady later studied SV40 transcription regulation as a staff fellow with Dr. Norman Salzman, National Institute of Allergy and Infectious Diseases, and Dr. George Khoury, NCI. His current research interests center on eukaryotic gene regulation, tumor suppressor proteins, and viral transformation.*

Basic Research Laboratory–Bethesda
Molecular Biology of Human Retroviruses

Keywords:
AIDS
cell cycle
gene transcription
HIV–1
HTLV–1
leukemia
p300

Research: Research is focused on the human retroviruses—human T cell lymphotropic virus type 1 (HTLV–1) and human immunodeficiency virus (HIV). HTLV–1 is closely associated with human cancer adult T cell leukemia (ATL) and the neurologic disease HTLV–1-associated myelopathy (HAM/TSP). HIV is the etiologic agent for acquired immunodeficiency syndrome (AIDS). The laboratory is interested in interactions between the virus and cell that influence viral gene regulation, viral pathogenesis, and oncogenic transformation. Highlights from recent research projects are given below.

Phosphorylation of p53: A Novel Pathway for p53 Inactivation in Human T Cell Lymphotropic Virus Type 1-Transformed Cells

Inhibition of p53 function, through either mutation or interaction with viral or cellular transforming proteins, correlates strongly with the oncogenic potential. Only a small percentage of human T cell lymphotropic virus type 1 (HTLV–1)-transformed cells carry p53 mutations, and mutated p53 genes have been found in only one-fourth of adult T cell leukemia cases. In previous studies, we demonstrated that wild-type p53 is stabilized and transcriptionally inactive in HTLV–1-transformed cells. Further, the viral transcriptional activator Tax plays a role in both the stabilization and inactivation of p53 through a mechanism involving the first 52 amino acids of p53. Phosphorylation of p53 inactivates p53 by blocking its interaction with basal transcription factors. Using two-dimensional peptide mapping, it was demonstrated that peptides corresponding to amino acids 1 to 19 and 387 to 393 are hyperphosphorylated in HTLV–1-transformed cells. Moreover, using antibodies specific for phosphorylated Ser15 and Ser392, an increased phosphorylation of these amino acids is shown. Since HTLV–1 p53 binds DNA in a sequence-specific manner but fails to interact with TFIID, experiments were done to test whether phosphorylation of the N terminus of p53 affected p53–TFIID interaction. Using biotinylated peptides, it is shown that phosphorylation of Ser15 alone inhibits p53–TFIID interaction. In contrast, phosphorylation at Ser15 and Ser37

restores TFIID binding and blocks MDM2 binding. These studies provide evidence that HTLV–1 utilizes the posttranslational modification of p53 in vivo to inactivate function of the tumor suppressor protein.

Phosphorylation of p53 Serine 15 Increases Interaction with CBP
P53 exerts its cell cycle regulatory effects through its ability to function as a sequence-specific DNA-binding transcription factor. CREB-binding protein (CBP)/p300, through its interaction with the N terminus of p53, acts as a coactivator for p53 and increases the sequence-specific DNA-binding activity of p53 by acetylating its C terminus. The same N terminal domain of p53 has recently been shown to be phosphorylated at Ser15 in response to gamma irradiation. Remarkably, we now demonstrate that phosphorylation of p53 at Ser15 increases its ability to recruit CBP/p300. The increase in CBP/p300 binding was followed by an increase in the overall level of acetylation of the C terminus of p53. These results provide a mechanism for the activation of p53-regulated genes following DNA damage, through a signaling pathway linking p53 N terminal kinase and C terminal acetyltransferase activities.

PCAF Interacts With Tax and Stimulates Tax Transactivation in a HAT-Independent Manner
Recent studies have shown that the p300/CBP binding protein-associated factor (PCAF) is involved in transcriptional activation. PCAF activity has been shown strongly associated with histone acetyltransferase (HAT) activity. In this report, evidence is presented for a HAT-independent transcription function that is activated in the presence of the HTLV–1 Tax protein. In vitro and in vivo, GST–Tax pull-down and coimmunoprecipitation experiments demonstrate that there is a direct interaction between Tax and PCAF, independent of p300/CBP. PCAF can be recruited to the HTLV–1 TRE site in the presence of Tax, and PCAF cooperates with Tax in vivo to activate transcription from the HTLV–1 LTR more than 10-fold. Point mutations at Tax amino acid 318 (TaxS318A) or 319 to 320 (Tax M47), which have decreased or no activity on HTLV–1 promoter, are defective for PCAF binding. Strikingly, the ability of PCAF to stimulate Tax transactivation is not solely dependent upon the PCAF HAT domain. Two independent PCAF HAT mutants, which knock out acetyltransferase enzyme activity, activate Tax transactivation to approximately the same level as wild-type PCAF. In contrast, p300 stimulation of Tax transactivation is HAT-dependent. These studies provide experimental evidence that PCAF contains a coactivator transcription function independent of the HAT activity on the viral LTR.

Cell Cycle-Regulated Transcription by the HIV–1 Tat Transactivator
Cyclin-dependent kinases are required for the Tat-dependent transition from abortive to productive elongation. Further, the HIV–1 Vpr protein prevents proliferation of infected cells by arresting them in the G2 phase of the cell cycle. These findings suggest the life cycle of the virus may be integrally related to the cell cycle. We now demonstrate by in vitro transcription analysis that Tat-dependent transcription takes place in a cell cycle-dependent manner. Remarkably, Tat activates gene expression in two distinct stages of the cell cycle. Tat-dependent LTR activation is observed in G1. This activation is TAR-dependent and requires a functional Sp1-binding

site. A second phase of transactivation by Tat is observed in G2, which is TAR-independent. This later phase of transcription is enhanced by a natural cell cycle blocker of HIV–1, vpr, which arrests infected cells at the G2/M boundary. These studies link the HIV–1 Tat protein to cell cycle-specific biological functions.

Collaborators include Ettore Appella and Yoshihiro Nakatani, NIH; Ajit Kumar, George Washington University; and Ramin Shiekhattar, Wistar Institute.

Recent Publications:

Jiang H, et al. *Mol Cell Biol* 1999;19:8136–45.

Kashanchi F, et al. *J Virol* 2000;74:652–60.

Pise-Masison CA, et al. *Mol Cell Biol* 2000;20:3377–86.

Zhou M, et al. *Mol Cell Biol* 2000;20(14):5077–86.

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Biography: *Dr. Callahan obtained his Ph.D. in molecular genetics at Syracuse University. At the NIH, he studied protein factors required for in vitro translation of mRNA with Philip Leder and retrovirology with George Todaro. His recent studies have focused on identifying and characterizing mutations which contribute to human breast cancer and mammary tumorigenesis in a mouse model. He is chief of the Oncogenetics Section in the Basic Research Laboratory–Bethesda.*

Basic Research Laboratory–Bethesda
Somatic Mutations Associated with Breast Cancer

Keywords:

insertional mutagenesis
INT6
mammary tumorigenesis
mouse mammary tumor virus
NOTCH4/INT 3
transgenic mice
translation

Research: The accumulation of somatic mutations has been strongly implicated as a contributing factor in the malignant progression of human breast cancer. One goal of our research efforts has been to identify and characterize the putative target tumor suppressor genes within the chromosomal regions affected by loss of heterozygosity (LOH). We are currently taking the positional cloning approach to identify these target genes on chromosome 17q in primary breast tumors. In other related studies, we have developed a unique mouse mammary tumor model system for mammary tumorigenesis in which we have identified two novel genes (*Int3* and *Int6*) that are frequently activated by MMTV-induced insertional mutagenesis in mammary tumors. Currently we are undertaking the molecular characterization of these genes and determining how they contribute, in an in vivo setting, to malignant transformation of mammary epithelium.

Identification of Human Genes Associated with Neoplasia
In previous studies we were able to define three regions of chromosome 17q which are independently affected by LOH in primary human breast tumors. We surveyed 130 sporadic breast cancers at 17 polymorphic loci to define the smallest region on 17q12–21 affected by LOH. This corresponded to the approximately 120 to 150 kb interval between D17S846 and D17S746. Currently we are determining the nucleotide sequence of two overlapping P1 phage clones which span this region, and have found two candidate target genes. Whether these genes are mutated in breast tumors is being determined.

Mammary Tumorigenesis in Inbred and Feral Mice
Previous studies of our mouse model system led to the identification of the *Notch*-related *Int3* gene, which is involved in cell fate decisions during development, as a target for MMTV insertional mutagenesis in mammary tumors. One consequence of these integration events is the expression of a truncated Int3 RNA species initiated in the MMTV LTR, which encodes the transmembrane and intracellular domains of the normal gene product. We have completed the nucleotide sequence of the normal 6.4 Kb *INT3* RNA. It encodes a 200 KD protein which shares 60 percent homology with the mouse homolog of *NOTCH1*, and have therefore renamed this gene "*NOTCH4/INT3*." *NOTCH4/INT3* has several novel characteristics which distinguish it from other members of this gene family. The extracellular domain of *NOTCH4/INT3* contains 29 instead of 36 EGF-like repeats found in NOTCH1. Four novel EGF-like repeats have been created as a consequence of small deletions which have occurred within the gene during evolution. From the nucleotide sequence of host-viral junction fragments of 9 independent mammary tumors, it was determined that all of the integration events in *NOTCH4/INT3* have occurred within a 174 bp region 3′ of the LIN12 repeat sequences in the extracellular domain and 5′ of the transmembrane encoding sequences. This strongly suggests that loss of the LIN12 repeat sequences is required for viral-induced activation of *NOTCH4/INT3*.

Recently we found a second novel common insertion site for MMTV, designated *Int-6*. The nucleotide sequence of *Int-6* is identical to that of a component of translation initiation factor 3 (eIF3). The *Int-6* gene is ubiquitously expressed as a 1.5 kb RNA species in adult tissues. In each tumor tested, MMTV integration into *Int-6* results in the expression of a truncated *Int-6*/long terminal repeat (LTR) chimeric RNA species. Since the nonrearranged *Int-6* allele in these tumors contains no mutations, we favor the conclusion that truncation of the *Int-6* gene product either biologically activates its function or represents a dominant-negative mutation. The *Int-6* gene has been highly conserved through evolution. The deduced amino acid sequence of the mouse and human *Int-6* proteins are identical, whereas the *Drosophila* protein shows 60 percent identity. Currently we are determining whether the human *Int-6* gene is mutated in primary human breast cancer as well as in other human neoplasia.

Our collaborators are Lothar Hennighausen and Glenn Merlino, NIH; and Ray White, Eccles Institute of Human Genetics and Howard Hughes Medical Institute, University of Utah.

Recent Publications:

Imatani A, et al. *Oncogene* 2000;19:223–31.

Krebs LT, et al. *Genes Dev* 2000;14:1343–52.

Marchetti A, et al. *Int J Oncol* 2001;18:175–9.

Callahan R, et al. *J Mam Gland Biol Neoplasia* 2001;6:23–36.

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Biography: *Dr. Cho-Chung obtained her M.D. from Seoul Women's Medical School and her Ph.D. from McArdle Laboratory for Cancer Research, University of Wisconsin, where she studied feedback control mechanisms in Morris hepatomas with Van R. Potter and Henry C. Pitot. As a research associate, she studied yeast genetics with H. Edwin Umbarger at Purdue University. At the NIH, she studies the cellular regulatory mechanisms of cell growth and differentiation, particularly those involving the cAMP signaling pathway.*

Basic Research Laboratory–Bethesda
Molecular Mechanisms of cAMP-Mediated Growth Control, Differentiation, and Gene Regulation

Keywords:
antisense
cAMP-dependent protein kinase
cancer chemotherapy
CRE
differentiation
transcription factor-decoy
tumor marker

Research: Our research has been to define and elucidate the principles by which cAMP regulates cell growth/differentiation, a process generally disrupted in tumor cells. The identification and analysis of the key intracellular regulatory molecules that mediate cAMP-growth regulatory function will potentially provide molecular targets for therapeutic intervention and markers of preventive and prognostic/diagnostic value for cancer. Our study (Project 1) is to answer one of the most puzzling questions in the cAMP-regulation of cell growth—namely, the roles of cAMP-dependent protein kinase (PKA) isozymes; why do the ratios of type 1 and type 2 PKAs that contain an identical catalytic (C) subunit but distinct regulatory (R) subunits, RI and RII, respectively, change dramatically during cell development, differentiation, and transformation? This research topic stems from our early investigations of site-selective cAMP analogs. We initially discovered that site-selective cAMP analogs, such as 8–Cl-cAMP that preferentially bind to the RII subunit of PKA, induce rapid RII upregulation and RI downregulation and exert potent growth inhibitory effects on a wide variety of human cancer cell lines (IC_{50}: 0.1 to 20 µM). 8–Cl-cAMP inhibits growth selectively in transformed rather than nontransformed cells. Importantly, 8–Cl-cAMP induces growth inhibition by decreasing the RI:RII ratios in tumor cells. RI and RII, therefore, may provide targets for therapeutic intervention. Another study (Project 2) is to understand how cAMP signals result in the activation of cAMP response element (CRE)-directed transcription to stimulate/inhibit cell proliferation. cAMP regulates a striking number of physiologic processes, including intermediary metabolism, cellular proliferation, and neuronal signaling by

altering basic patterns of gene expression. However, the mechanism of the CRE-directed transcription in cell proliferation is largely unexplored. Studies of the CRE-transcription in tumor cells compared to normal cells should contribute to the knowledge base for pharmaceutical design.

Increased expression of the RIα subunit of PKA type 1 (PKA–I) has been shown during carcinogenesis, in human cancer cell lines, and in primary tumors. Taking advantage of the specificity of Watson-Crick base pairing, antisense oligonucleotides are being tested for use in the therapy of human diseases. We conducted in-depth studies of RIα antisense. This study led us to make the first discovery that the RIIβ protein increases its half life (RIIβ $t_{1/2}$, control cells: 2 hr; antisense treated cells: 11 hr) to compensate for the loss of RIα in the cell. When the concentration of free C subunit increases due to the loss of RIα (by the antisense), RIIβ rapidly responds to this perturbation via protein stabilization in a holoenzyme complex (PKA–IIβ). This results in an increase in the level of RIIβ protein in cancer cells that otherwise overexpress RIα. These results indicate that switching PKA–I with PKA–II is an important biochemical adaptation mechanism of the cell that determines tumor cell growth inhibition. By this unique mechanism of action, RIα antisense inhibits cancer cell growth leading to apoptosis/differentiation, the terminal endpoint of cancer cell survival. Our results that RIα antisense produces potent growth inhibition in a variety of cancers of epithelial cell origin as well as leukemia cells support its therapeutic application toward a broad spectrum of cancer. In addition, RIα antisense also overcomes multidrug resistance (MDR) as it is an effective inhibitor of MDR colon carcinomas in nude mice and it sensitizes MDR cancer cells to chemotherapeutic agents. Thus, RIα antisense targeting protein kinase A type 1 provides a gene-specific therapeutic approach to the treatment of cancer. GEM 231 (RIα antisense) is currently undergoing phase I–II clinical trials.

Alteration of gene transcription by inhibition of specific transcriptional regulatory proteins has important therapeutic potential. Nucleic acid molecules with high affinity for a target transcription factor can be introduced into cells as decoy cis-elements to bind the factors and alter gene expression. We used transcription factor decoy oligonucleotide to elucidate the CRE-gene transcription in cell growth. The CRE transcription factor complex is a pleiotropic activator that participates in the induction of a wide variety of cellular and viral genes. Because the CRE cis-element, TGACGTCA, is palindromic, a synthetic single-stranded oligonucleotide composed of the CRE sequence self-hybridizes to form a duplex/hairpin. We found that a 24mer CRE-palindrome-oligonucleotide (trioctamer of TGACGTCA) that self-hybridizes to form a duplex/hairpin can penetrate into cells, compete with CRE enhancers for binding transcription factors, and specifically interfere with both the CRE– and AP–1 directed transcription in vivo. Surprisingly, the CRE-decoy oligonucleotide restrained tumor cell proliferation without affecting the growth of noncancerous cells. The dual blockage of two important signal transduction pathways, CRE–PKA and AP–1–PKC, could be causally related, at least in part, to the cancer cell-specific growth inhibitory effect of the CRE decoy oligonucleotide. This finding led us to investigate a novel CRE-directed transcription pathway that is uniquely required for tumor cell proliferation. We anticipate

that outcome of this study should lead to a better understanding of a new, dynamic aspect of the CRE-directed transcription in growth control. This decoy oligonucleotide approach offers great promise as a tool for defining cellular regulatory processes and treating diseased conditions.

Recently, we have shown that the free catalytic (C) subunit of PKA is excreted in conditioned medium of various cancer cells and in serum of cancer patients. The ECPKA expression was upregulated 10-fold as compared with normal serum. We found that overexpression of RIα in an expression vector, which upregulates intracellular PKA–I, markedly upregulates ECPKA expression. Conversely, overexpression of RIIβ, which downregulates PKA–I in the cell and reverts the transformed phenotype, downregulates ECPKA. Importantly, ECPKA upregulation is reduced in cancer cells maintaining hormone dependency (a normal cell property), such as hormone-dependent breast cancer as compared to the hormone-independent breast cancer. Thus, this phenomenon of ECPKA expression can provide an innovative approach to cancer diagnosis, prognosis, and hormone-dependence detection of breast cancer, and a method for treatment of cancer.

A collaborator on our research is Sudhir Agrawal, Hybridon, Inc.

Recent Publications:

Nesterova M, et al. *Antisense Nucleic Acid Drug Dev* 2000;10:423–33.

Cho YS, et al. *Proc Natl Acad Sci USA* 2000;97:835–40.

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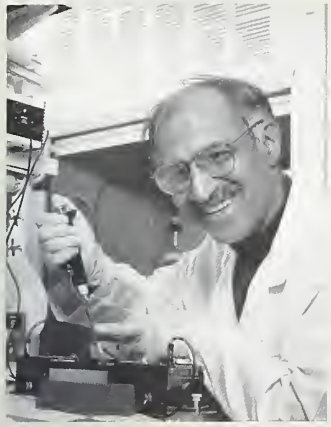
Nesterova M, et al. *Clin Cancer Res* 2000;6:3434–41.



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Biography: *Dr. Dhar obtained his Ph.D. from Calcutta University, India, and did his postdoctoral work at Yale University with Dr. Sherman Weissman. His major research interest has been the study of gene structure and regulation of DNA and RNA tumor viruses. More recently, he has been using fission yeast as a model system to study the mechanism of nuclear cytoplasmic trafficking.*

Basic Research Laboratory–Bethesda
Nucleocytoplasmic Trafficking in Fission Yeast

Keywords:
mRNA export
nuclear location signal
nuclear pores
Rae1p
spMex

Research: The research focus of the laboratory is to understand the mechanism of nucleocytoplasmic trafficking through the nuclear pore. We have used fission yeast *Schizosaccharomyces pombe* as a model genetic organism and have concentrated on the export of mRNA from the nucleus. We see this process as a three-fold problem: (1) identifying the components involved in mRNA export; (2) defining genetic and physical interactions among components; and (3) elucidating the biochemical function of these proteins.

The prevailing model of mRNA export is one in which mature mRNA binds to a set of proteins of which one or more carry a nuclear export signal (NES). This signal binds to a receptor which then directs the transport of the mRNP through interaction with the transport machinery within the pore. We have used as a starting point for this study the temperature sensitive mutants of *rae1* gene that rapidly accumulate poly(A)RNA in the nucleus upon shift to restrictive temperature. The rae1 protein (Rae1p) is a conserved WD repeat protein and associates with the nuclear pore. We have identified several genes that genetically interact with rae1: two genes encoding nucleosporins, *npp106* and *nup184*, and a gene, *mex 67*, that encodes a nuclear pore-associated protein. Moreover, *mex67* genetically interacts with *npp106* and *nup184*. Each of these genes in turn affects the export of mRNA. Moreover, it appears that Rae1p and Mex67p likely function in parallel mRNA export reactions. In contrast to *S. cerevisiae*, Mex67p is not essential in *S. pombe* whereas Rae1p is essential. It has been suggested that Mex67p functions in nuclear export of mRNA by directly associating with it and directing its export out of the nucleus. We have identified a novel domain within Mex67p that functions in mRNA export without direct interaction with mRNA. This domain can shuttle between the nucleus and the cytoplasm using novel import-export pathways. We are currently in the process of identifying its interacting partners and receptors that direct its nuclear import and export. In addition, our current work focuses on identifying components of the Rae1p-dependent mRNA export machinery and linking them with components of the Mex67p-dependent mRNA export pathways.

Recent Publications:

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Bharathi A, et al. *Gene* 1997;198:251–8.

Yoon JH, et al. *Mol Cell Biol* 1997;17:7047–60.

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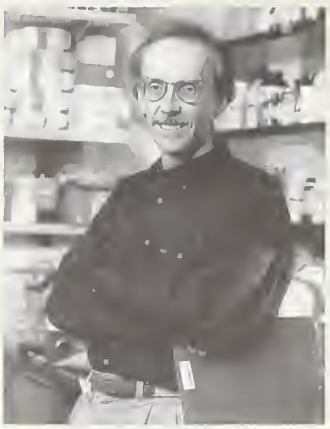

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Biography: *Dr. Fornace received his M.D. in 1972 from Jefferson Medical College in Philadelphia, PA. He was both a clinical fellow at Harvard Medical School and a research fellow at Harvard School of Public Health from 1975 to 1979, and has been at the NCI since 1979.*

Basic Research Laboratory–Bethesda
The Molecular Biology of Cellular Injury

Keywords:
DNA damage
DNA repair
gadd
p53

Research: A major focus of this research group is the study of the cellular responses to genotoxic stress in mammalian cells. This has included the cloning and characterization of a variety of DNA-damage-inducible (DDI) genes, including the gadd genes, and elucidation of the regulatory mechanisms controlling their expression. Cell cycle checkpoint activation and growth inhibition are universal responses to genotoxic stress. We have found five gadd (**g**rowth-**a**rrest and **D**NA-**d**amage-inducible) genes to be coordinately induced by cellular exposure to many DNA-damaging agents and certain other stresses that trigger growth arrest. Evidence for such responses has been found in all mammalian cells examined to date and indicate that this is a well-conserved stress response(s). In the case of gadd45, this was the first cellular gene found to be regulated by the key tumor suppressor p53 via a pathway that is activated by ionizing radiation (and many other stresses). This pathway also involves ATM, the gene defective in ataxia telangiectasia. Responses to ionizing radiation have been characterized in a variety of human tumor lines including the regulation of key cell death genes like BAX, BCL2, BCL–X, KILLER/DR5, TRID/TRAIL–R3. More recently, the laboratory has shown that a functional and physical interaction between p53 and WT1 plays a central role in the regulation of such genes after certain stresses, such as UV radiation. Interestingly, the same promoter control element, which binds to WT1, has been found to be suppressed by c-Myc, a growth-stimulatory signaling protein. Moreover, deletion of c-Myc has been found to markedly upregulate the gadd45 growth-arrest gene. Both c-myc and Brca1 are implicated in breast cancer, and have been shown to contribute to the regulation of the gadd45 gene. We have recently applied a functional genomics approach to the study of DDI genes. Using cDNA microarray hybridization, a very complex pattern of responses has been found in

various human cells which is dependent on p53 status, apoptotic potential, and a variety of other control factors. Characterization of such responses in tumor cells will be used to elucidate the status of signal transduction pathways and may have predictive value in treatment planning.

A second major focus is the characterization of the products encoded by particular stress genes with emphasis on p53-regulated genes. This project involves both a genetic and biochemical approach. Targeted disruption of the gadd45 gene has been carried out in mice and characterization of these mice is currently under way. Studies are being expanded to other engineered mice including strains with disruption of p53, cip1/waf1, and other selected genes. Defects in important parameters, such as genomic stability, growth control, resistance to carcinogenesis, and DNA repair, have been found in gadd45–/– mice. From analysis of these various knock-out strains, cip1/waf1, gadd45, and other effector genes contribute to the phenotype of p53–/– mice. Targeted disruption of related DDI genes is currently under way. Using a biochemical approach, this laboratory has already demonstrated interactions between Gadd45 with PCNA, p21Cip1/Waf1, Cdc2, and core histone proteins, and evidence for roles in DNA repair and cell cycle control. The goal of these studies is to contribute to the understanding of the function of these and related DDI genes and their potential as targets for therapy in the future.

Recent Publications:

Hollander MC, et al. *Nat Genet* 1999;23:176–84.

Amundson SA, et al. *Oncogene* 1999;18:3666–72.

Bulavin D, et al. *EMBO J* 1999;18:6845–54.

Smith ML, et al. *Mol Cell Biol* 2000;20:3705–14.

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Biography: *Dr. Franchini obtained her M.D. at the University of Modena in 1977 and became a board-certified hematologist in 1981. She is an elected member of the American Society for Clinical Investigation and is on the editorial advisory board of* AIDS Research and Human Retroviruses, Virology *and* AIDS Abstracts.

Basic Research Laboratory–Bethesda
Human Retroviral Diseases: Pathogenesis and Prevention

Keywords:
AIDS
animal models
HIV
HTLV–1
macaques
retroviruses
SIV
vaccines

Research: Human T cell leukemia/lymphotropic virus type 1 (HTLV–1) causes adult T cell leukemia/lymphoma (ATLL) and a progressive myelopathy designated "tropical spastic paraparesis/HTLV–1-associated myelopathy." HIV–1 is the causative agent of AIDS. Both of these human retroviruses induce disease affecting CD4+ T cells. The major aims of the laboratory at present are: (1) to investigate the T cells' physiological pathways affected by HTLV–1 transformation in vitro (HTLV–1-transformed T cell lines) and in vivo (in leukemic T cells of ATLL patients); (2) to develop strategies for preventive vaccines and gene therapy delivery systems based on human retroviral vectors; (3) to study the natural history of HTLV/STLV types I and II; and (4) to identify other related human retroviruses.

Efforts to understand the mechanisms of HTLV–1 oncogenesis include studies on cellular proteins involved in G1 and S-phases of the cell cycle. We have demonstrated that HTLV–1 infection interferes with the physiological regulations of the p53 and $p21^{waf1}$ proteins, and the effect of the HTLV–1 transactivation gene (*tax*) on Rb and $p16^{ink}$ is under investigation. We have found that in HTLV–1-infected cultured T cells, constitutive activation of the IL–2 signaling pathway (JAK–STAT) occurs. Such events correlate with the ligand (IL–2) independence observed in HTLV–1-transfected T cells. A viral protein ($p12^{I}$) which binds and downregulates the IL–2Rβ and γ_c-chains might be involved in these events, and its function is being currently investigated. Understanding the effects of viral genes on these events will allow a more accurate staging of the disease and the design of novel therapeutic approaches. ATLL is a viral-induced leukemia which could be prevented by an HTLV–1 vaccine.

We are investigating poxviruses (NY–VAC and ALVAC) as a vaccine delivery system to generate antiretroviral vaccines. Canarypox (ALVAC) is naturally restricted in its replication in mammalian cells. We have obtained encouraging results using ALVAC HIV–2 or SIV recombinant vaccines in primates. Some macaques were protected from infection by HIV–2, and AIDS progression was delayed in macaques vaccinated and infected with the SIV_{251} virulent strain. In addition, ALVAC-based HTLV–1 vaccine has

protected rabbits against an HTLV–1 cell-associated challenge. Our interest is to learn to modulate the host immunoresponse to vaccines by the addition of interleukins (IL–2, IL–12, for example). We are also investigating the usefulness of attenuated poxviruses expressing retroviral structural genes as a tool to provide viral protein *in trans* in a gene delivery system based on HIV–2 vectors.

During studies on HTLV–1/–2 natural history, we discovered new HTLV–1 clades, demonstrated that HTLV–1 is likely to be derived from horizontal transmission from African monkeys to humans, and identified a new oncornavirus related to HTLV–2 in Pigmy chimpanzees. These primate oncornaviruses might be a valuable source of reagents to identify related novel human retroviruses.

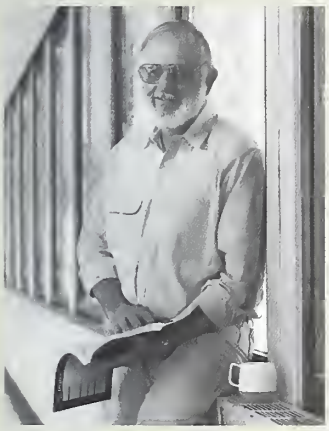
Recent Publications:

Takemoto S, et al. *Blood* 2000;15:3939–44.

Nicot C, et al. *Blood* 2000;96:275–81.

Nicot C, et al. *Blood* 2000; in press.

Dekaban GA, et al. *Virology* 2000; in press.



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Biography: *Dr. Hatfield received his Ph.D. in the Genetics Foundation at the University of Texas at Austin, and did his postdoctoral work on protein purification in Dr. J.B. Wyngaarden's laboratory at Duke University Medical School; on genetic coding and protein synthesis in Dr. M. Nirenberg's laboratory at the National Heart, Lung, and Blood Institute; and on bacterial genetics in Dr. J. Monod's laboratory at the Institut Pasteur, Paris, France. He then came to the NCI where he has continued a lifelong interest in genetic coding and protein synthesis.*

Basic Research Laboratory–Bethesda
Role of Selenium in Cancer and Human Health

Keywords:
selenium
selenocysteine

Research: The major aims of the section of the Molecular Biology of Selenium are to understand the means by which selenium is incorporated into protein as the amino acid selenocysteine and the role of specific selenoproteins in human health. Selenium has a role in preventing heart disease and has been shown to have a protective effect against prostate and other cancer forms and has been implicated in delaying the aging process. Undoubtedly, a detailed understanding of how selenium makes its way into protein and the role of specific selenoproteins in cellular metabolism will elucidate the mechanisms of selenium benefits on human health.

We are also examining the role of tRNA in ribosomal frameshifting in HIV and other vertebrate retroviruses. One of the tRNAs involved in frameshifting is asparagine (Asn) tRNA which normally contains the highly modified queuine (Q) base in the wobble position of its anticodon. As Q base is known to restrict wobble, it seems quite likely that the absence of Q base may give greater flexibility of movement of the Asn anticodon and may therefore enhance ribosomal frameshifting. Interestingly, Q base comes exclusively from the diet of mammals and thus diet can possibly play a role in the expression of some vertebrate retroviruses.

Our studies show that: (1) Sec, which is coded by UGA, is the 21st amino acid in the universal genetic code, and this expansion of the code marks the first addition to the universal genetic code since it was deciphered in the mid 1960s; (2) the Sec tRNA population in higher vertebrates consists of two isoacceptors that are 90 nucleotides in length; (3) Sec tRNAs are highly undermodified compared to other tRNAs and differ from each other by only a 2'0-methylribose in the wobble position; (4) the intracellular levels are influenced by selenium; (5) Sec tRNA is transcribed without a leader sequence; (6) the A and B boxes which are located inside the gene and are required in expression of all eukaryotic tRNA genes are not essential for basal level transcription of the Sec tRNA gene; (7) TBP utilizes a Pol II TAF for Sec tRNA gene transcription even though the gene is transcribed by Pol III, and thus this gene is expressed more like Pol II than Pol III genes; (8) the Ser but not the Sec form of the tRNA binds to eEF1, and thus suppresses UGA codons (this data also provides strong evidence that Sec-tRNA has its own EF); (9) biosynthesis of Sec tRNAs has been reconstituted in *Xenopus* oocytes; (10) replenishment of selenium-deficient rats with selenium shows dramatic changes in the distribution of the Sec tRNA isoacceptors and these changes occur in a tissue-specific manner; (11) mouse embryonic stem cells heterozygous for the Sec tRNA gene (i.e., the Sec tRNA gene has been selectively removed from one chromosome) show that the Sec tRNA population is about 60 percent of that observed for wild-type cells, but the biosynthesis of the selenoprotein, glutathione peroxidase, is not affected; thus, Sec tRNAs do not appear to be limiting in the biosynthesis of selenoproteins; (12) the *Drosophila* Sec tRNA population consists of one major isoacceptor that exists in three different conformational states, and the Sec tRNA gene maps on chromosome 2 at regions 47E or F; (13) a novel 15 kDa selenoprotein exists in high levels in prostate and thyroid tissues and the gene exists as a polymorphism in the human genome; (14) the seryl form of Sec tRNA serves as a suppressor tRNA in vivo; (15) a single UGA codon can serve eight functions in mammalian cells; (16) translational reading gaps occur in mammalian cells; and (17) Asn and Phe tRNAs lacking a hypermodified base that normally occurs within the anticodon loop of these tRNAs serve to enhance frameshifting in vertebrate retroviruses when AAC or UUU (Asn and Phe codons, respectively) are present at the frameshift site.

In the last year, we have (1) shown that transgenic mice carrying copies of a selenocysteine tRNA gene with a mutation at position 37 (removing the highly modified isopentenyladenosine moiety) results in selective inhibition of selenoprotein biosynthesis and inhibits selenocysteine tRNA maturation; these mice can be used as a model system to understand the role of specific

selenoproteins in health; (2) developed a chemically defined medium for growing *Drosophila* and found that the addition of selenium to the medium normalizes the life span of this organism; this is the first demonstration that selenium has a direct role in the life span of any organism; (3) participated with collaborators to identify the specific elongation factor and selenocysteine insertion binding protein demonstrating how selenocysteine is cotranslationally inserted into a protein as the 21st naturally occurring amino acid; (4) further characterized the 15kDa selenoprotein previously identified in this laboratory to provide evidence that it is linked to cancer prevention and that it exists intracellularly in a complex with UDP-glucose: glycoprotein glucosyltransferase, a protein that is involved in the quality control of protein folding, thus providing evidence that a selenoprotein may also function in protein folding; (5) found that the methylation of the ribosyl moiety at position 34 of the selenocysteine tRNA is governed by both primary and tertiary structure of the tRNA, whereas the biosynthesis of all other modified bases in this nucleic acid is far less dependent on primary and secondary structure; and (6) shown that yeast asparagine tRNA without Q base promotes eukaryotic frameshifting more efficiently than mammalian asparagine tRNA with or without Q base; and that phenylalanine tRNA without Y base is a shifty tRNA and promotes ribosomal frameshifting of mammalian retroviruses.

Our collaborators are Marla Berry, Harvard Medical School; Paul Copeland and Donna Driscoll, Cleveland Clinic Foundation; Alan Diamond, University of Illinois at Chicago; Vadim Gladyshev, University of Nebraska; and Byeong Lee, Seoul National University, Korea.

Recent Publications:

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Martin-Romero F, et al. *J Biol Chem* 2001; in press.

Carlson B, et al. *Virology* 2001;279:130–5.

Kumaraswamy E, et al. *J Biol Chem* 2000;275:35540–7.

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Biography: *Dr. Kraemer received his M.D. from Tufts Medical School and is board certified in dermatology and internal medicine. He has a longstanding interest in human cancer-prone genetic diseases and DNA repair. His studies focus on molecular, cellular, and clinical features of diseases including xeroderma pigmentosum and familial melanoma. He is a member of the American Society for Clinical Investigation and has received awards from the Society for Investigative Dermatology and the U.S. Public Health Service.*

Basic Research Laboratory–Bethesda
DNA Repair in Human Cancer-Prone Genetic Diseases

Keywords:
aging
cancer genetics
cancer prevention
chemoprevention
DNA repair
genetic disease
genetic polymorphism
melanoma
mutagenesis
mutation
retinoids
skin cancer
squamous cell carcinoma
UV mutagenesis
xeroderma pigmentosum

Research: Human cancer-prone genetic diseases are being studied to identify groups of people with an increased susceptibility to environmental agents. Present emphasis is on the skin cancer-prone disease, xeroderma pigmentosum (XP). We are attempting to: (1) understand the molecular basis of the cellular hypersensitivity of these diseases; (2) correlate cellular hypersensitivity with clinical abnormalities; (3) determine the degree of genetic heterogeneity within such groups, and (4) explore methods of cancer prevention. We developed plasmid assays to measure DNA repair and mutagenesis at the molecular level in human cells and to assign cells to complementation groups. These studies provide strong evidence that DNA repair genes play a major part in UV induction of skin cancer in XP. Cells from a patient with the rare xeroderma pigmentosum/Cockayne syndrome complex with severe clinical symptoms of Cockayne syndrome had the cellular characteristics of the XP–G DNA repair defect. An unusual XP–C patient had neurological abnormalities and a metabolic defect (hypoglycinemia) associated with a splice mutation. We also found an age-dependent increase in plasmid UV mutability in cultured blood cells from normal donors. This finding suggests that the ability to process UV-damaged DNA decreases with increasing donor age in the normal population. This may play a role in the greatly increased frequency of UV-induced skin cancer with age. Oral retinoids are effective in prevention of skin cancers in patients with XP although there are substantial side effects.

Collaborators on our research include J. DiGiovanna and R. Tarone, NIH; and L. Grossman, Johns Hopkins University.

Recent Publications:

Emmert S, et al. *Proc Natl Acad Sci USA* 1997;97:2151–6.

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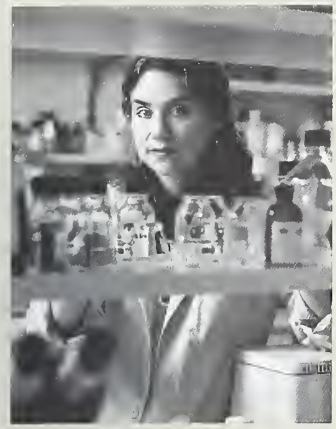
Tobi SE, et al. *Carcinogenesis* 1999;20:1293–1301.



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Biography: *Dr. Linnekin received a Ph.D. in physiology from the Uniformed Services University of the Health Sciences in 1989. She came to the NCI where, in the Laboratory of Molecular Immunoregulation, she carried out postdoctoral work that addressed signal transduction mechanisms of erythropoietin and granulocyte-macrophage colony stimulating factor. Her recent work is directed towards understanding the mechanism of action of stem cell factor.*

Basic Research Laboratory–Bethesda
Signal Transduction Pathways of Normal and Oncogenic Forms of c-Kit in Hematopoietic Cells

Keywords:
animal model
cytokines
growth factors
signal transduction

Research: Stem cell factor (SCF) is a growth factor critical for survival, growth, and differentiation of hematopoietic progenitor cells, as well as for the development of mast cells. The receptor for SCF is c-Kit, a receptor tyrosine kinase. Activating mutations in c-Kit are associated with mastocytosis, leukemia, and gastrointestinal stromal cell tumors. Because of the importance of c-Kit in normal hematopoiesis, as well as its role in human disease, the goal of this project is to understand the mechanism of action of both normal and oncogenic forms of this receptor in two important target populations, hematopoietic progenitor cells and mast cells.

One specific aim of our work is to define the biochemical mechanism of action of wild-type c-Kit. While SCF-mediated activation of the Ras-Raf-Map kinase cascade and PI3 kinase has been well studied, less is known about Src family members and the JAK/STAT pathway in relation to SCF signaling mechanisms. The important role of both of these pathways in the development and function of hematopoietic cells led us to examine if one or both plays a role in SCF-mediated responses.

In summary, we have shown that SCF induces association of Lyn with phosphorylated tyrosines 568 and 570 in the c-Kit juxtamembrane region and that Lyn activity increases at multiple points during SCF-induced cell cycle progression. Studies with antisense oligonucleotides specific for Lyn, as well as a pharmacological inhibitor of Lyn activity, suggested that Lyn plays a role in SCF-induced proliferation. To more definitively establish the role of Lyn in c-Kit stimulus-response coupling mechanisms, we have examined the capacity of both hematopoietic progenitor cells and mast cells from Lyn-deficient mice to respond to SCF. These studies demonstrate that Lyn is required for optimal SCF-mediated responses of both lineages. Studies to define downstream signaling pathways that couple Lyn to SCF-induced survival, growth, and functional responses are in progress.

The critical role of the JAK/STAT pathway in hematopoiesis has recently been appreciated. As described above, little is known about the role of this pathway in SCF-mediated responses. To determine if the JAK/STAT pathway plays a role in c-Kit stimulus-response coupling mechanisms, we first examined the capacity of SCF to activate JAK2 and Stat1. Our studies demonstrated that JAK2 and Stat1 associate with c-Kit, and that both these signaling components are rapidly, but transiently, activated by SCF. To determine the role of JAK2 in SCF-mediated responses, we are comparing the capacity of hematopoietic progenitor cells from the fetal liver of JAK2-deficient and wild-type mice to survive, proliferate, and differentiate in response to SCF. These preliminary results suggest an important role for JAK2 in SCF-mediated responses of primary progenitor cells. Using cell lines derived from wild-type and JAK2-deficient fetal liver, we plan to define signaling pathways downstream of JAK2 that couple SCF to survival and growth.

Mutation of aspartic acid 816 of human c-Kit (V816 c-Kit), or 814 of its murine counterpart (V814 or Y814), results in a constitutively active form of c-Kit that is oncogenic. This mutation is found in human patients with mastocytosis, leukemia, and germ cell tumors. A second specific aim of our work is to delineate signal transduction pathways mediating cellular transformation by this oncogenic c-Kit mutant. We have shown that V816 c-Kit constitutively associates with activated PI3 kinase. Further, studies with a PI3 kinase inhibitor and a mutant incapable of associating with $p85^{PI3K}$ (V816/F721 c-Kit) have demonstrated that constitutive recruitment of activated PI3 kinase is essential for tumorigenicity of this mutant in a myeloid progenitor cell line. Presently we are examining downstream effectors of PI3 kinase that mediate transformation of progenitor cells by V816 c-Kit. Also, because c-Kit signaling is lineage-specific, work is in progress to determine signaling pathways constitutively activated by the V816 c-Kit mutant in mast cells. These findings could have important implications in designing treatment strategies of diseases associated with this mutation, such as mastocytosis and leukemia.

Collaborators on this project include Leonie Ashman, Hanson Center for Cancer Research, Adelaide, Australia; Clifford Lowell, University of California, San Francisco; Dean Metcalfe, NIH; Klaus Pfeffer, Technical University of Munich, Germany; and Lars Rönnstrand, Ludwig Institute for Cancer Research, Uppsala, Sweden.

Recent Publications:

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Linnekin D. *Int J Biochem Cell Biol* 1999;31:1053–74.

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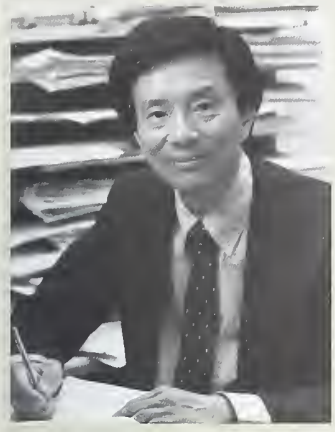

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Biography: *Dr. Miki received his Ph.D. in molecular genetics from Institute for Virus Research, Kyoto University, Japan, in 1979. He was an assistant professor in the Department of Biochemistry, Yamaguchi University School of Medicine, before he joined the Laboratory of Cellular and Molecular Biology as a guest researcher in 1985. He became a visiting scientist in 1987, a principal investigator in 1994, and a senior investigator and chief, Molecular Tumor Biology Section of the Basic Research Laboratory in 2000.*

Basic Research Laboratory–Bethesda
Molecular Mechanisms of Tumor Formation

Keywords:
cell division
cytokinesis
exchange factor
Rho GTPases
transformation

Research: The objective of our entire project is to understand the mechanisms of tumor formation at the molecular level. An approach we chose for this purpose was the isolation of molecules whose activation induces malignant transformation. A tool to achieve the aim was an efficient expression cloning system. We first performed a genetic screen for genes that can induce foci of morphologically transformed cells when they are expressed from a cDNA library introduced in host cells. The signaling molecules isolated by this approach can be mainly divided into two groups; isoforms of fibroblast growth factor receptor 2 (FGFR2) and guanine nucleotide exchange factors for the Rho family GTPases. Initial characterization of these groups of genes created two projects as described below.

Activation Mechanisms of FGF and KGF Receptors

The isolation of the keratinocyte growth factor receptor (KGFR) led us to a finding that this receptor is encoded by an alternative transcript of the gene for FGFR2. This finding established that two growth factor receptors with different ligand-binding specificity are encoded by alternative transcripts of a single gene. Following this initial finding, a constitutively activated FGFR2 containing an altered C terminus was isolated from rat osteosarcoma cells. Acquisition of a new sequence, designated FRAG1, played a major role on the activation, and this activation can override the negative regulatory effect of the normal ligand-binding domain. Alteration of the C terminus by either deletion or point mutation also activated the receptor. Interestingly, the receptor activity was also modulated by alterations of the C terminal domain by alternative splicing. Finally, we found that glycosaminoglycan-modified forms of FGFR2 exhibit sustained activation of MAP kinase and are required for stimulation of DNA synthesis. This modification event is also regulated by alternative splicing events.

Biological Functions of Oncogenes Encoding Exchange Factors for the Rho Family GTPases

A group of oncogenes isolated by our expression cloning strategy shared structural motifs of the guanine nucleotide exchange factors for the Rho family GTPases. While these oncogenes, designated *ECT2*, *OST*, *TIM*, *NET1*, and *NTS*, share common motifs (Dbl-homology and pleckstrin homology domains), they also display distinct features. Ost comprises multiple isoforms, catalyzes guanine nucleotide exchange on RhoA and Cdc42, and associates with Rac1 in its GTP-bound form. Ost regulates the JNK/SAPK MAP kinase pathway and induces actin reorganization in fibroblasts. *NET1* is one of the genes induced with oxidative stress. NTS, an exchange factor for Rac1, is a major nucleolar antigen expressed in proliferating but not in resting cells. ECT2 contains a homology domain which is shared by a number of molecules involving cell cycle checkpoint control and repair. ECT2 is phosphorylated specifically in G2/M phases and phosphorylation is required for its guanine nucleotide exchange activity. ECT2 exhibits nuclear localization in interphase, spreads throughout the cytoplasm in prometaphase, and is condensed in the midbody during cytokinesis. Expression of an ECT2 derivative, containing the amino-terminal domain required for the midbody localization but lacking the carboxyl-terminal catalytic domain, strongly inhibits cytokinesis. Therefore, ECT2 appears to be a critical regulator of cytokinesis. Currently we are isolating proteins which can associate with the regulatory domain of ECT2 to clarify the signaling pathways towards cytokinesis. We are also studying the role of *Xenopus* ECT2 in mitosis using a *Xenopus* in vitro cell cycle control system. We are increasing our endeavor in this project to clarify the role of Rho GEFs in cell division control.

Our collaborators include Mary Dasso, Shioko Kimura, Kyung Lee, and Jeffrey Rubin, NIH; and Shuh Narumiya, Kyoto University, Japan.

Recent Publications:

Sakaguchi K, et al. *Mol Cell Biol* 1999;19:6754–64.

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Kimura K, et al. *J Biol Chem* 2000;275:17233–6.

Sakata H, et al. *Hepatology* 2000;32:193–9.

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Biography: *Dr. Robert-Guroff received her Ph.D. from Georgetown University and was a postdoctoral fellow of the Leukemia Society of America at the NCI and of the Friedrich Miescher-Institut, Basel, Switzerland. She was an ASM Foundation Lecturer, is on the editorial board of* AIDS Research and Human Retroviruses, *and is a member of the National Action Plan on Breast Cancer's Etiology Working Group. Her research interests include AIDS vaccine development, retroviral-cell interactions, and the role of viruses in breast cancer.*

Basic Research Laboratory–Bethesda
Humoral, Cellular, and Mucosal Immune Responses to Human and Nonhuman Primate Retroviruses

Keywords:
adenovirus vectors
AIDS
animal models
antibody
antigen
breast cancer
cell-mediated immunity
chemokines
coreceptors
HIV
immune response
retrovirology
SIV
vaccines
virus-cell interactions

Research: Our laboratory is interested in retroviral pathogenesis and strategies for the diagnosis, treatment, and prevention of retroviral-induced human diseases. HIV and AIDS are the main focus of research efforts, but a new project on breast cancer has been initiated.

AIDS Vaccine Development

We are utilizing adenovirus recombinants for delivery of AIDS vaccines. Our studies in chimpanzees have shown that live, replication competent Ad-HIV recombinants together with a viral envelope booster immunization elicit humoral, cellular, and mucosal immune responses, including antibodies capable of neutralizing both lab-adapted and primary viral isolates, antibodies in secretory fluids, and viral-specific cytotoxic T lymphocytes. Long-lasting protection against syncytial-inducing HIV isolates was demonstrated. Moreover, protection was achieved against a primary, nonsyncytium-inducing HIV isolate, representative of the type which is readily transmitted between people. Notably, these advances were achieved using a vaccine based only on the viral envelope. We expect greater vaccine efficacy if multicomponent vaccines are employed. These findings have provided the basis for moving ahead into phase I human trials, in which the safety and immunogenicity of Ad-HIV recombinants administered orally or intranasally will be compared. Further studies will compare the immunogenicity of replication competent and incompetent Ad-HIV recombinants, in order that design of next generation vaccine materials can be based in the most appropriate vector. The ability of the Ad-HIV priming/subunit boosting approach in protecting against a mucosally administered pathogenic isolate will also be evaluated.

To further exploit this system, an adenovirus host range mutant-SIV recombinant system has been utilized. The full spectrum of immune responses is again seen in rhesus macaques immunized with a host range mutant recombinant carrying the inserted SIV envelope gene, in combination with envelope protein boosting. Moreover, decreased viral burden during the acute phase of pathogenic SIV infection was observed in

immunized rhesus macaques. Long-term followup of the animals revealed that some developed slow disease progression. The immunologic basis for modulation of the disease course is under investigation. This model is being used to explore multicomponent vaccines, development of mucosal immune responses in the intestine and comparison with what is observed in the periphery, protection from mucosal challenge, and design of novel vaccine approaches. Forms of innate immunity are also under study. We have observed that vaccination can induce a CD8+ T cell HIV and SIV suppressive activity, and that this activity correlates with decreased viremia and slower disease progression in SIV-infected macaques. Optimization of immunization regimens may lead to greater induction of this suppressive activity.

The protein booster immunization is an important part of our vaccine strategy, and collaborative studies are under way to improve this component of the candidate vaccine. A conformationally constrained peptide polymer representative of the CD4-binding domain of the viral envelope mimics the native conformational structure of the viral envelope in this important conserved region and its immunogenicity and effect on protective efficacy are under investigation. Conserved regions in the viral envelope transmembrane protein, gp41, are also of great interest. The gp41 protein contains the fusion peptide, and efforts to elicit humoral and cellular immune responses to elements of the fusion-active domain are being pursued.

Breast Cancer Etiology

A possible association of the human endogenous retrovirus type K (HERV–K) in breast cancer is under study. Preliminary results have suggested that expression of HERV–K may be linked with aggressive breast cancers, such as inflammatory breast cancer. Some ethnic groups have a high incidence of inflammatory breast cancer. We are carrying out serologic studies to determine if HERV–K expression is associated with this type of breast cancer. Such an association could have potential diagnostic utility.

Our collaborators include Martin Cranage, Centre for Applied Microbiology and Research; John Eldridge, Wyeth-Lederle Vaccines and Pediatrics; Barbara Ensoli, Istituto Superiore di Sanita; Mary Klotman, Mt. Sinai Medical Center; Paul Levine, George Washington University; Christopher Miller, University of California–Davis; Frank Robey, NIH; James Tartaglia, Virogenetics Corporation; Carol Weiss, Center for Biologics Evaluation and Research; and Carl Wild, BBI–Biotech Research Laboratories, Inc.

Recent Publications:

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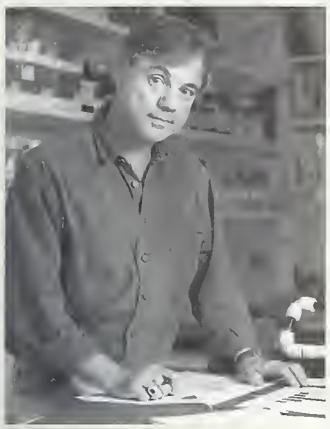

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Biography: *Dr. Salomon obtained his Ph.D. degree from the State University of New York at Albany. He was a postdoctoral fellow at the Roche Institute of Molecular Biology in Nutley, NJ, and spent 6 years as a staff fellow in the Laboratory of Developmental Biology in the National Institute of Dental Research. Currently, as chief of the Tumor Growth Factor Section in the Laboratory of Tumor Immunology and Biology, he has been studying the interaction of growth factors and oncogenes in the etiology of breast and colon cancer.*

Basic Research Laboratory–Bethesda
Role of EGF–Related Peptides in the Pathogenesis of Breast and Colon Cancer

Keywords:
breast cancer
cripto
growth factors
signal transduction

Research: The Tumor Growth Factor Section is engaged in studying the biology of novel epidermal growth factor (EGF)-related peptides such as amphiregulin (AR), heregulin (HRG), and cripto-1 (CR–1) and their relationship to the pathogenesis of breast and colorectal cancer. This laboratory has found that several of these growth factors such as TGFα, AR, and CR–1 are differentially overexpressed in a majority of primary human breast and/or colorectal tumors and in premalignant lesions in these tissues relative to normal, noninvolved mammary or colonic epithelium. We have found that the expression of TGFα, AR, and HRG can be upregulated by specific oncogenes such as an activated c-Ha-ras gene or by an over-expressed c-erb B–2 gene and can function as autocrine growth factors for these oncogene-transformed mammary and colonic epithelial cells. We have also found that expression of TGFα and AR can be enhanced by estrogens and can be decreased by antiestrogens such as tamoxifen in malignant human mammary epithelial cells.

We have recently demonstrated that these peptides can impair to varying degrees the expression of milk proteins such as β-casein and whey acidic protein in response to lactogenic hormones such as prolactin in mouse mammary epithelial cells. This may be relevant in vivo since the expression of these peptides such as HRG and CR–1 are enhanced during pregnancy and lactation in the mouse mammary gland. We have found that TGFα and CR–1 can function as dominant transforming genes in mouse and human mammary epithelial cells in vitro. In addition, the in vivo administration of recombinant AR or CR–1 or transduction of the CR–1 or AR genes into mammary epithelial cells can stimulate ductal branching in the virgin mouse mammary gland and can lead to the development of mammary hyperplasias. We have found that CR–1 does not bind to the EGF receptor nor does it directly activate the c-erb B–2, c-erb B–3, or c-erb B–4 type 1 tyrosine kinases. CR–1 can rapidly enhance the tyrosine phosphorylation of p46 Shc and can activate the MAPK isoform, p42erk2. CR–1 is expressed in approximately 80 percent of breast and colorectal carcinomas, in 60 percent

of pancreatic carcinomas, and in 50 percent of gastric carcinomas, while only 10 to 15 percent of adjacent and noninvolved epithelium in these tissues express CR–1. In addition, CR–1 is expressed in 50 to 70 percent of colon adenomas and gastric intestinal metaplasias where the frequency of expression correlates with the degree of dysplasia in these premalignant lesions. CR–1 is also expressed in a majority of mammary adenocarcinomas that arise in transgenic mice that overexpress the TGFα, c-neu, int-3, polyoma middle t or SV40 large T transgenes. The differential overexpression of CR–1 in several different types of carcinomas suggests that CR–1 might be a significant tumor marker and may contribute to the pathogenesis of this disease in these tissues.

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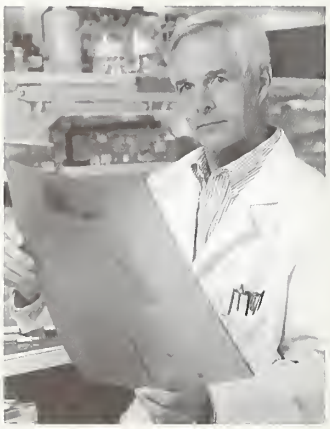

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Biography: *Dr. Gilbert H. Smith, a research biologist in the laboratory's Oncogenetics Section, develops and investigates biological models designed to elucidate the cellular, molecular, and genetic basis for breast cancer.*

Basic Research Laboratory–Bethesda
Biology of Mammary Gland Development and Tumorigenesis

Keywords:
aging
animal models
breast
breast cancer
breast cancer model
cancer cell growth regulation
cancer genetics
cancer susceptibility
carcinogen resistance
carcinoma
cell proliferation
cell proliferation control

Continued on page 327

Research: In conjunction with our studies on mammary tumorigenesis in feral and inbred mice, our efforts have focused upon understanding the cellular basis of malignant progression in the mammary gland. Taking the point of view that mammary carcinomas arise as clonal populations of transformed tissue-specific stem cells and their differentiating progeny, we have initiated a long-term project aimed at elucidating the cellular, molecular, and genetic events underlying mammary epithelial cell growth, regeneration, and functional development.

Previous studies have demonstrated that mammary gland development and function were severely impaired in transgenic mice expressing a gain-in-function mutation of the Notch4/*Int3* gene from the MMTV viral promoter. Both mammary ductal growth and secretory lobule development were curtailed in these mice. To confirm and extend these findings, mutated

Keywords (continued):
cell signaling
development
gene
gene discovery
genetically engineered mouse models
malignant conversion
mammary
mammary gland
mammary tumorigenesis
mouse mammary tumor virus
mutations in sporadic breast tumors
stem cells
susceptibility/resistance genes
TGFß
TGFß1
transformation
transgenic mice
transgenics
transplantation

Notch4/*Int3* was expressed from the whey acidic protein (WAP) promoter whose activity, unlike the MMTV LTR, is restricted to the secretory mammary epithelial population. In transgenic mice carrying the *WAP/Int3* construct, mammary ductal growth was unaffected in virgin females, but growth and differentiation of secretory lobules during gestation was profoundly inhibited. Coincidental with the block in lobular secretory differentiation, mammary dysplasia and tumorigenesis occurred in all females. The *WAP/Int3* mammary tumors were highly malignant and most tumor-bearing females, irrespective of breeding history, developed metastatic lung lesions. These results suggest that WAP promoter-targeted *Int3* function is associated with mammary secretory cell differentiation and maintenance in this transgenic model. Consistent with this conclusion, transplants of *WAP/Int3* gland into nontransgenic mammary fat pads produced complete mammary ductal outgrowths in virgin FVB/N mice, but failed to develop secretory lobules when the females were impregnated.

The study of mammary gland development and differentiation in several transgenic mouse models utilizing the mammary fat pad transplantation technique as an experimental approach strongly suggested that two separate and distinct mammary epithelial progenitor cells were responsible for the generation of the ductal and lobular components of the mature functional mammary gland. An in vivo transplantation system was used to evaluate the developmental capacities of specific mouse mammary epithelial cell populations. Specifically, mouse mammary epithelial cells with distinctly limited developmental potentials were identified using this procedure. Two distinct epithelial cell progenitors have been identified by experiments designed to determine whether basal lobular and ductal phenotypes could develop independently under conditions imposed by a limiting dilution. The prediction that these separate epithelial progenitors must exist was based upon the results from transplantation experiments carried out in epithelium-divested mammary fat pads of syngeneic mice with mammary epithelium from two different transgenic mouse models (WAP-Int3; WAP–TGFβ1). The results demonstrated the following points: (1) lobular (i.e., secretory) progenitor cells are present as distinct entities among the mammary epithelial cells found in immature virgin female mice; (2) similarly, ductal epithelial progenitors are present within the same population; (3) lobular progenitors are present in greater numbers, although both cell populations are extremely small; (4) some inocula produced outgrowths with simultaneous development of both lobular and ductal phenotypes. This indicates either cooperative interaction between the two epithelial progenitors or represents evidence for a multipotent epithelial stem cell capable of producing both ductular- and lobular-committed daughters. We have recently shown that the progeny of a single multipotent mammary epithelial cell may comprise a regenerated fully functional mammary gland. In addition to producing all the types of epithelium necessary to form the gland, these individual stem cells are capable of self-renewal and give rise to roughly 1100 additional multipotent epithelial stem cells during the repopulation of a single mammary fat pad. These individual multipotent mammary epithelial cells are present in the gland throughout life with undiminished proliferative capacities and represent a cellular subpopulation at great risk for malignant transformation.

Collaborating on this research are Lothar Hennighausen, Chamelli Jhappan, and Glenn Merlino, NIH.

Recent Publications:

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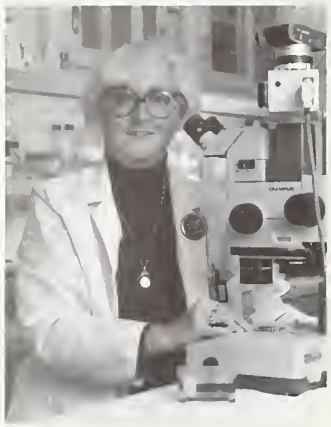

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Biography: *Dr. Vonderhaar received her Ph.D. from the University of Wisconsin–Madison. After postdoctoral training in mammary gland biology with Yale Topper at the NIH, she joined the NCI where she studies prolactin action in breast cancer. She was the first to purify a prolactin receptor from any source and the first to characterize a monoclonal antibody directed against the human prolactin receptor. She was also the first to demonstrate that human breast cancer cells synthesize and secrete significant amounts of biologically active prolactin.*

Basic Research Laboratory–Bethesda
Prolactin Action in Mammary Gland Development and Tumorigenesis

Keywords:
animal model
breast
breast cancer
mammary gland
mammary tumorigenesis
progesterone
prolactin
receptor

Research: The emphasis of our research is on understanding the mechanisms of prolactin (PRL) action in concert with estrogen (E), progesterone (P), epidermal growth factor (EGF), and insulin-like growth factors (IGF–I and IGF–II) in mammary gland development, differentiation, and tumorigenesis. Both in vivo and in vitro approaches are used to confirm physiological relevance.

Development of the Normal Gland

Lobulo-alveolar development of the mammary gland requires the priming action of both E and P. During priming, induction of DNA synthesis, ductal branching, the homeobox containing MSX–2 gene, and EGF receptors are primarily due to P; E increases TGFα and P receptors in the gland. During pubescence, P receptors are located on a limited number of ductal epithelial cells. In situ hybridization and immunohistochemistry suggested that they are located in clusters around putative branch points. P, in concert with PRL, increases DNA synthesis in the branching ducts. PRL, in concert with insulin, glucocorticoids, and EGF or TGFα, promotes full lobulo-alveolar development in vitro. Subsequently, the gland undergoes reductive remodeling (involution) after removal of the PRL stimulus. Both in vivo and in vitro, onset of nonrandom DNA degradation occurs within 12 hrs,

reaching a peak at day 3. These changes are paralleled by an increase in the expression of TGFβ1 and TGFβ3 but not of TGFβ2.

PRL as Mitogen in Breast Cancer

Growth of both ER– and ER+ human breast cancer cells is inhibited 70 percent to 85 percent by anti-PRL antibodies; 53 percent inhibition is achieved by treating the cells with antisense RNA for PRL. Conditioned media from breast cancer cells contains biologically active PRL. A significant regulator of PRL synthesis by the mammary gland is PRL itself. By RT–PCR analysis, 92 percent of human breast cancer cell lines and 83 percent of human breast carcinomas contain PRL mRNA. PRLR mRNA is expressed in 90 percent of the cell lines and 100 percent of the surgical samples, demonstrating the widespread expression of both PRL and PRLR in normal and malignant human breast cells. The data suggest an autocrine role of PRL in human breast cancer. Comparisons between matched pairs of cancerous and adjacent, noninvolved tissue from the same breast surgical specimens show that, on average, both PRL and PRLR mRNA expression are significantly higher in the cancerous tissue.

PRL Receptors and Signal Transduction

NIH 3T3 cells or human breast cancer cells MDA–MB–435 transfected with either the long or the short forms of the PRL receptor respond to PRL with differentiation or mitogenesis. Only the long form activates a β-casein-CAT construct. In 3T3 transfectant cell lines, PRL rapidly and transiently activates MAP kinase. Maximal stimulation occurs at 5 min. In PRL-responsive human breast cancer cell lines, *raf*-1, MEK, and MAP kinases are activated within 5 min of PRL exposure. The tyrosine kinase, JAK2, is associated with the receptor within 1 min of exposure to PRL. This is followed by rapid phosphorylation of SHC and its association with Grb2, and Sos. This signaling pathway continues through *ras, raf*, MEK, and MAP kinase, and may act in concert with the JAK2/*stat* pathway.

Long-Term Effects of Neonatal Exposure to the Synthetic Estrogen Diethylstilbestrol (DES) or the Antiestrogen Tamoxifen (TAM) on Mammary Gland Development and Tumorigenesis

The mammary glands of adult C3H/HeN (MMTV+) mice, exposed to a single injection of 12.5, 25, or 50 micrograms of TAM or 25 micrograms of DES within the first 36 hr of birth, contain only sparse ducts, devoid of alveolar buds. Preneoplastic hyperplastic alveolar nodules appear 2 to 3 months earlier in neonatally exposed mice compared to controls. Serum PRL levels in the treated animals are constitutively elevated. In BALB/c (MMTV–) mice, glands from neonatally DES exposed mice develop a sparse pattern of dilated ducts by the age of 12 weeks. These ducts frequently are engorged with milk. Glands from mice exposed to the higher levels of TAM occasionally develop dilated ducts. These data suggest that neonatal exposure to relatively low doses of DES or TAM has long-acting deleterious effects on mammary gland development and may possibly contribute to tumorigenesis.

Our collaborators are Mikiko Asai and Mario Ikeda, Yokohama City University School of Medicine; Craig Atwood, Case Western University; Charles Brooks, Ohio State University; Rina Das, Walter Reed Army Institute of Research; Helen Davey, Ruakura Research Centre, New Zealand; Sandra Haslam, Michigan State University; John Lydon and Jeffrey Rosen, Baylor College of Medicine; Lina Matera, University of Turin, Italy; Hichem Mertani, IMCB, Singapore; Chris Ormandy, Garvan Institute, Australia; Karen Plaut, University of Vermont; Jose Russo, Fox Chase Cancer Center; Sami Shousa, Imperial College of Medicine, London; and Jim Xiao, Boston University.

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Hovey RC, et al. *Mol Endocrinol* 2001;15:819–31.

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The CCR's Basic Research Laboratory (BRL) plans and conducts research on the cellular, molecular, genetic, biochemical, and immunological mechanisms affecting the progression, diagnosis, and treatment of cancer. Many of the researchers in the BRL actively collaborate with scientists in other research programs within both the NCI and the NIH.

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Basic Research Laboratory–Frederick

Regulation of Cell Growth and Differentiation: Roles of Osteopontin and the p300/CBP Related Protein p270

Keywords:
cell growth regulation
cell signaling
differentiation
gene regulation

Research: The general focus of our research is on the process of differentiation, especially the coordination of the numerous cellular and molecular events that must occur as proliferating cells transition to terminal differentiation. Two key events in this transition are cell cycle exit and tissue-specific gene expression. The lack of coordination between cell proliferation and terminal differentiation underlies the molecular mechanisms of cancer. An attractive model in which to study these processes is osteoblast differentiation. These cells differentiate in a controlled multistep program with distinct proliferative and postproliferative stages. The murine preosteoblast cell line MC3T3–E1, when given ascorbic acid and allowed to become confluent, expresses a series of tissue-specific genes in a highly ordered manner over a period of weeks leading to the terminally differentiated state.

The retinoblastoma (pRb) and p300 protein families regulate various aspects of both tissue-specific gene expression and cell cycle control in most cell types. These proteins are bound and inactivated by the adenovirus 12S.E1A protein. To examine the role of these two protein families, a model was developed. We have generated MC3T3–E1 cell lines stably expressing either the wild-type 12S.E1A gene or mutants that selectively target either p300/CBP or pRb/p130/p107 and compromise their functions. Studies using the adenovirus E1A protein, stably transfected into the preosteoblast cell line, revealed that inactivation of these two families of proteins resulted in the suppression of two osteoblast marker genes, alkaline phosphatase and osteopontin. Alkaline phosphatase is dependent on both the pRb and p300 families for normal upregulation in response to ascorbic acid. The unique dependence of ALP expression on both the pRb and p300 families implies a specific mechanistic link between ALP expression, cell cycle regulation, and terminal differentiation, which we are continuing to investigate

Osteopontin, however, does not require the pRb and p300 family but instead is dependent on the expression of alkaline phosphatase and the subsequent inorganic phosphate produced by the enzyme in the extracellular

environment. The discovery that inorganic phosphate in the medium results in the upregulation of osteopontin defines a novel and potentially important regulatory mechanism for gene regulation. Osteopontin is expressed in most tissues and therefore this novel mechanism has the potential to be globally important. Osteopontin protein levels are upregulated during cell injury and associated with the pathology of many diseased tissues including kidney stones, atherosclerosis, and transformed cells. Studies involving reduced levels of osteopontin have shown that it is required for wound healing and as a key cytokine required for efficient type 1 immune responses. It will be interesting to determine whether osteopontin is unique in its response to inorganic phosphate or whether there are other genes (promoters) that exhibit this sort of regulation. It will also be interesting to identify key components of the pathway required for inorganic phosphate regulation, including critical signal transducers and any phosphate-responsive elements in the osteopontin promoter.

A related project in the lab revolves around the role of p270 in differentiation. p270 is an integral member of human SWI/SNF complexes, first identified through its shared antigenic specificity with p300 and CBP. SWI/SNF complexes were discovered in yeast cells where they are involved in the regulation of multiple inducible genes including those required for the mating type switch and sucrose fermentation pathways. More recent isolation of *Drosophila* and mammalian homologs of many of the yeast complex members suggest that SWI/SNF complexes play fundamental roles in the regulation of gene expression during cell growth and development in all organisms. Although there appear to be multiple SWI/SNF-related complexes of variable composition in eukaryotic cells, all are able to alter chromatin structure through ATP-dependent mechanisms. These changes may subsequently permit the recruitment of other general and gene-specific activator molecules while facilitating the transcriptional process. A recently defined motif, LXXLL (L is leucine and X is any amino acid), has been demonstrated to be necessary and sufficient for interaction with liganded nuclear hormone receptors. The list of proteins containing this functional motif is growing, but to date it appears it is the hallmark of the protein family of transcriptional "coactivators." The most studied proteins of this group are p300 and CBP (CREB-binding protein). The function of these proteins is to assist in the formation of a complex between upstream transcriptional activators/enhancers and the basal transcriptional machinery, including TBP. p270 has a group of four LXXLL motifs located towards the C terminus of the protein. The potential interaction between various nuclear hormone receptors and p270, a protein associated with a complex that contains chromatin modifying activity, is very exciting. It suggests a role for p270 as an integrator of hormone signaling and transcription initiation. Although it is becoming clear that proteins with multiple LXXLL motifs are important in nuclear hormone receptor mediated transcriptional activation, the specificity relating to the many combinations of nuclear hormone receptors and coactivators is far from clear.

Our collaborators are Elizabeth Moran, Temple University, and Brad Zerler, Collagenex Pharmaceuticals.

Recent Publications:

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Parreno M, et al. *J Virol* 2000;74:3166–76.

Beck GR Jr, et al. *Cell Growth Differ* 2001;12:61–83.

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Photo not available

Biography: *Dr. Benveniste obtained his Ph.D. in molecular biology at the University of Wisconsin–Madison, where his research with Julian Davies involved novel mechanisms of resistance to aminoglycoside antibiotics in gram-negative bacteria. He obtained his M.D. from the University of Miami (Ph.D. to M.D. program). He has been at the NIH since 1972, where his research has focused on the isolation of novel retroviruses, the discovery that retroviruses have been transferred between species during the course of evolution, and the development of vaccines and antivirals against AIDS.*

Basic Research Laboratory–Frederick
AIDS Pathogenesis, Antiviral and Vaccine Development

Keywords:
AIDS
antiviral
immune response
retroviruses
vaccines
viral envelope genes

Research: Approximately 35 million persons are currently infected with HIV worldwide; two thirds of these infected individuals live in sub-Saharan Africa. A new person becomes infected on average once every six seconds worldwide. A vaccine to prevent new infections, as well as effective treatments for those already infected, is thus urgently needed.

Our studies have focused on the development of vaccines and the testing of antivirals. Using our pathogenic molecular clones (SIV/Mne) obtained from a pigtailed macaque naturally infected with AIDS, we have also examined the virologic and molecular changes in SIV that are associated with progression to AIDS. The time interval between infection and death, expressed as a percentage of total life span, is very similar between macaques infected with these SIV clones and humans infected with HIV–1. These SIV/Mne viruses have therefore been used by several investigators as challenge stocks for a variety of AIDS vaccine protocols.

AIDS Vaccines

Our studies have focused on various strategies for AIDS vaccines.

- **Vaccinia recombinants.** Envelope-based vaccines, when used in a recombinant vaccinia virus priming and subunit protein-boosting regimen, protect from intravenous infection by the cloned homologous virus. However, only partial protection was observed from an intravenous heterologous virus challenge. In order to identify the regions of the virus needed for increased protection against a heterologous challenge, we designed a study in which various regions of the AIDS virus were systematically added, as recombinant vaccinia constructs, to the envelope-based vaccine regimen.

These studies indicate that the inclusion of the transmembrane protein in the envelope-based vaccine is essential in order to obtain protection. In addition, the presence of both envelope and core antigens were found to now protect against a heterologous virus challenge, indicating that responses to core antigens contributed to the broadening of protective immunity. Our results argue for the inclusion of multiple antigenic targets in the design of recombinant vaccines against AIDS, and should be directly applicable to designing vaccines to protect humans against HIV isolates that differ in their sequence.

- **Role of cell-mediated immunity (CMI) in AIDS vaccines.** A possible role for CMI in protection against HIV infection is suggested by the finding that a significant number of potentially HIV-exposed individuals from different risk groups, who show no evidence of infection, exhibit a strong HIV-specific CMI. We have tested this hypothesis in the SIV model, and shown that exposure to a subinfectious virus dose resulted in lymphocyte proliferation to SIV antigens and protection from an infectious SIV challenge delivered 16 months later.

- **DNA AIDS vaccines.** Studies are under way to test the effectiveness of DNA immunizations in protecting against a subsequent infectious virus challenge. The immunogen consisted of DNA containing the SIV genome that had been rendered noninfectious by removal of a region of the nucleocapsid gag protein that is responsible for the packaging of viral RNA as well as a postcell entry replication step. The results of a preliminary study suggest that these DNA vaccines may be effective and safe routes of immunization.

- **Antiviral studies.** The efficacy of pre- and postexposure treatment with the acyclic nucleoside reverse transcriptase inhibitor (R)–9–(2-phosphonyl-methoxypropyl) adenine (PMPA) was tested as a model for HIV. Previously we showed that PMPA treatment for 28 days prevented infection even when therapy was initiated 24 hrs after intravenous virus inoculation. We have now determined the maximum interval between infection and effective initiation of PMPA treatment, as well as the optimum duration of treatment. These results clearly show that both the times between virus exposure and initiation of PMPA treatment and the duration of treatment are crucial factors for the prevention of acute infection.

These results may be applicable in designing experiments that combine this antiviral in order to reduce viral loads together with therapeutic vaccination protocols. This antiviral approach may also be applicable in reducing the transmission of HIV from mother to infant. A significant number of those transmissions are believed to occur at the time of delivery, when placental membranes rupture and there is a potential transfer of blood from mother to infant. Since PMPA can prevent infection when administered up to 24 hrs after infection, treatment of newborns with this compound may prevent infection in those infants that currently fail the AZT protocol.

Collaborators include Larry Arthur, Robert Gorelick, Jeffrey Lifson, and Louis Henderson, SAIC–Frederick; Shiu-Lok Hu, William Morton, and Che-Chung Tsai, University of Washington; and Gene Shearer, NIH.

Recent Publications:

Gorelick RJ, et al. *J Med Primatol* 2000;29:209–19.

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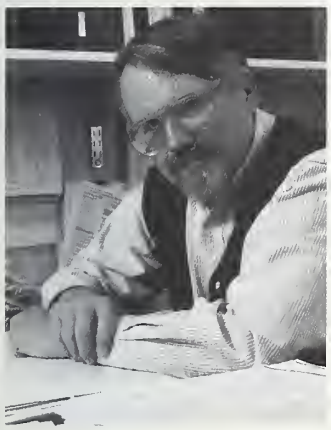

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Biography: *Dr. Blair received his B.S. degree in chemistry from the California Institute of Technology and his M.S. and Ph.D. degrees from the University of California–San Diego. He received postdoctoral training in retrovirology with Dr. Peter Vogt at the University of California Medical School, Los Angeles, and held a staff and faculty position in the Cancer Research Laboratory, University of Western Ontario, London, Ontario, prior to coming to the NIH. He is currently principal investigator and chief of the Oncogene Mechanisms Section, Basic Research Laboratory, CCR.*

Basic Research Laboratory–Frederick
Oncogenes in Signal Transduction and Transcriptional Regulation

Keywords:
bone morphogenetic proteins
cell transformation
oncogenes
tumor suppression

Research: Changes in the function of signaling pathways that control cell growth and differentiation lie at the heart of many, if not all, neoplastic events. The analysis of specific pathways involved in these processes and the molecular components of these pathways in both human cells and animal model systems will lead both to an increased understanding of the processes that underlie human cancer and to potential targets for interdiction. Our group has utilized tissue culture model systems involving transformation by the MOS and ETS oncogenes to analyze how altered or inappropriately expressed gene products act in vivo and in vitro to alter cell growth and differentiation. Our studies of MOS transformation led to the identification of DRM/Gremlin, a gene whose properties and expression pattern suggest it might play a novel role in cell transformation.

The MAP kinase cascade plays a critical role in cell signaling pathways involved in cell growth and proliferation in response to extracellular signals. MAP kinase kinase (MKK–1/MKK–2) is activated in many transformed cells and is an early target of MOS, a soluble serine/threonine kinase originally identified as the oncogene present in the Moloney strain of murine sarcoma virus. We isolated a nontransformed mutant of MOS-transformed cells in which the MAP kinase cascade is resistant to activation by the v-*mos* and v-*raf* serine/threonine kinases. However, the cascade can be activated in these cells by serum and v-*ras*, and revertant cells could be transformed by *ras* or by constitutively activated mutants of MKK–1 or MKK–2. Analysis of the MOS-resistant cell line led to the identification of a novel gene, DRM, which is

highly conserved between rodents and man and which is downregulated in transformed rat fibroblast cells. We cloned DRM from rat, mouse and human cDNA and genomic libraries, and demonstrated that it was highly conserved and possessed structural features suggesting it was related to DAN, a previously identified BMP antagonist with tumor suppressor functions, and Ceberus, a *Xenopus* protein with a role in development. DRM is a secreted protein expressed in a tissue-specific fashion and is found at highest levels in terminally differentiated, nondividing cells in the lung, colon, and brain. Our analysis showed that DRM was rarely expressed in transformed cells in culture and that its reexpression in some of these tumor-derived cell lines affected cell growth and other transformation-associated phenotypes.

DRM was also identified in *Xenopus*, where Gremlin, the *Xenopus* homolog of DRM, functions early in development as a BMP antagonist. Studies have indicated DRM/Gremlin plays a role in limb bud formation and lung and kidney development and function in mammals. These results suggest DRM/Gremlin could play multiple roles in differentiation and the control of cell proliferation. We are currently analyzing DRM/gremlin and other components of the BMP signaling pathway in human and murine tumors using immunohistochemical and molecular techniques, as well as measuring the effects of DRM/Gremlin overexpression in transgenic mice. We are attempting to characterize the function of DRM in normal adult cells and tissues, its potential role as a tumor suppressor in oncogenesis, and how these relate to its functions in mouse development.

Our collaborators on this project include G. Calothy and M. Marx, Institute Curie, Orsay France; Alan Perantoni, NIH; and T.G. Wood, University of Texas Medical School, Galveston.

Recent Publications:

Zhang Q, et al. *Cytogenet Cell Genet* 2000;89:242–51.

Topol LZ, et al. *J Biol Chem* 2000;275:8785–93.

Athanasiou M, et al. *Leukemia* 2000;14:439–45.

Blair D, et al. *Oncogene* 2000;19:64872–81.

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Biography: *Dr. Colburn is chief of the Gene Regulation Section, Basic Research Laboratory (BRL), as well as adjunct professor of genetics at George Washington University and of pathology at the University of Maryland Medical School. She obtained a Ph.D. in biochemistry from the McArdle Laboratory at the University of Wisconsin, where she studied molecular carcinogenesis with Dr. Roswell Boutwell and the late Dr. Charles Heidelberger. Subsequently, she held faculty appointments at the Universities of Delaware and Michigan, and research appointments at Michigan and the NCI.*

Basic Research Laboratory–Frederick
Gene Regulation in Carcinogenesis

Keywords:
AP–1
cancer prevention
gene regulation
molecular targets
NFκB
Pdcd4
transcription factors
tumor promotion

Research: The overall objective of these projects is to understand gene regulation events that occur during rate-limiting phases of carcinogenesis and to target these events for cancer prevention. Genes being examined include (1) those that control or are controlled by transcription factors AP–1 or NFκB, and (2) those that are differentially expressed during neoplastic progression. The inquiry utilizes the mouse epidermal JB6 model of variants that are sensitive (P+) or resistant (P–) to tumor promoter-induced transformation as well as human keratinocyte and transgenic mouse progression models.

The Role of AP–1 and NFκB in the Cause and Prevention of Carcinogenesis

Our 1989 observation that JB6 P+ but not P– cells responded to tumor promoters by activating AP–1 (Jun/Fos)-dependent transcription suggested that AP–1 activation might (1) explain the differential sensitivity, and (2) be required for progression to tumor phenotype. A test of the "requirement" hypothesis revealed that inhibition of AP–1 activation by AP–1 transrepressing glucocorticoids and retinoids as well as by dominant-negative jun (TAM 67) was accompanied by a block in tumor promoter-induced neoplastic transformation. These observations have been extended to demonstrate blocked invasion in mouse papilloma keratinocytes and blocked progression in human papilloma virus-immortalized human keratinocytes expressing dominant-negative jun driven by a keratin 14 promoter (K14–TAM 67). Transgenic mice expressing the same transgene show protection against skin tumor promotion. This block in tumor promotion occurs specifically, without effects on growth or differentiation or even on induced hyperplasia. Thus the K14–TAM transgenics provide a valuable tool for discovering new molecular targets for cancer prevention. Clonal P– JB6 cells owe their AP–1 nonresponsiveness and transformation resistance to a deficiency of extracellular regulated kinases (ERKs). ERK-dependent events required for AP–1 activation are being elucidated. Other current inquiries are concerned with identifying (using microarray analysis) transformation-relevant target genes that are AP–1 or NFκB dependent and inhibited by

dominant-negative jun, and with determining the role of AP–1 transactivation in multistage carcinogenesis in other transgenic mouse models. The molecular interactions that regulate retinoid transrepression of AP–1 are being probed, as are those that regulate crosstalk between NFκB and AP–1.

Characterization of Genes Differentially Expressed During Tumor Promotion and Progression: Pdcd4, Plekstrin, and Others

Differential display analysis has yielded two recently cloned genes preferentially expressed in transformation-resistant (P–) JB6 cells. One of them, Pdcd4, is a novel protein that functions as an inhibitor of transformation, shown by a gain of transformation response when antisense Pdcd4 is expressed in P– cells and by a loss of response when sense Pdcd4 is expressed in P+ cells. Pdcd4 expression inhibits AP–1 activation but not activation of other transcription factors such as NFκB or SRE nor does it inhibit ornithine decarboxylase activation, all events required for transformation. Although Pdcd4 inhibits AP–1 activation, it does not interact with AP–1 proteins. A yeast two-hybrid analysis has revealed a novel binding partner of Pdcd4 that is a translation initiation factor; the functional significance of this interaction is being investigated. Other current inquiries are directed to generating a K14–Pdcd4 transgenic mouse and a Pdcd4 conditionally null mouse. These mice will be used to test the hypothesis that Pdcd4 functions as a suppressor of tumorigenesis in vivo. Analysis of human progression models is ongoing; at least one model has been identified in which progression to advanced cancers appears to involve downregulation of Pdcd4. Other differentially expressed proteins of continuing interest to us include Plekstrin, induced by tumor promoters to express at high levels in P– cells, and HMG–I/Y, induced preferentially in P+ cells.

Recent Publications:

Young M, et al. *Proc Natl Acad Sci USA* 1999;96:9827–32.

Cmarik J, et al. *Proc Natl Acad Sci USA* 1999;96:14037–42.

Yang H, et al. *Oncogene* 2001;20:669–76.

Hsu T, et al. *Cancer Res* 2001;61:4160–8.

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Biography: *Dr. Derse obtained his Ph.D. from the State University of New York at Buffalo where he worked on inhibitors of herpesvirus and cellular DNA polymerases. Dr. Derse came to the NCI in 1984 where he did postdoctoral work on the molecular biology and gene regulation of complex retroviruses. Dr. Derse is an adjunct associate professor in the graduate program in genetics at George Washington University in Washington, DC, and is on the editorial board of* Virology.

Basic Research Laboratory–Frederick
Molecular Mechanisms of Retroviral Pathogenesis

Keywords:
gene regulation
gene splicing
retroviruses
RNA splicing
T cell transformation
transcription
viral pathogenesis
virus-cell interactions

Research: Our current research efforts are directed towards understanding the molecular genetics of human T cell leukemia virus (HTLV–1) and human immunodeficiency virus (HIV–1); the former is associated with adult T cell leukemia and degenerative neurologic diseases, and the latter is the etiologic agent of AIDS. The premise that virus-encoded regulatory factors and elements largely determine pathological consequences of infection, such as tissue tropism, disease spectrum, latency, and cytopathology, has motivated our efforts to identify and characterize the complex regulatory circuits that modulate virus gene expression and alter host cell metabolism. Analyses of HIV–1 and HTLV–1 genetics combined with comparative analyses of distant relatives of the human viruses have provided novel insights into the regulatory strategies and pathogenic mechanisms of these viruses. HTLV–1 and closely related simian and bovine leukemia viruses form a unique subfamily of retroviruses that do not encode typical oncogenes. Rather, they encode essential transcriptional (tax) and posttranscriptional (rex) regulatory proteins that have profound effects on cell proliferation and gene expression. Infectious molecular clones of HTLV–1 and methods for examining their replication were recently developed in this laboratory. We are applying these new tools to examine the genetic and biochemical determinants of virus infectivity, replication, and T cell transformation. Virus effects on cellular responses to growth factors and cytokines are a key component of oncogenic transformation; current and future efforts focus on understanding how HTLV–1 infection subverts signal transduction pathways in T lymphocytes. HIV–1 codes for proteins that activate viral mRNA synthesis (tat) and processing (rev) via interactions with cis-acting RNA elements. We have examined mechanisms of action and structure/function relationships of both tat and rev by mutagenesis and by comparative virology. The latter approach takes advantage of the similar mechanisms of action but distinct structural differences among homologous proteins encoded by distantly related viruses. These proteins were thus shown to contain a virus-specific, RNA-binding domain and an effector domain which interacts with cellular cofactors. We have recently characterized interactions between Rev and cellular splicing factors, termed "SR-proteins," and have correlated these interactions with

alternative splicing of viral RNAs in transfected cells. As an extension of this work, we have begun to determine how SR-proteins interact with other Rev and Rex proteins and to address how these interactions influence cell and virus gene expression.

Collaborators on this research include Tom Hope, The Salk Institute, and David Waters, SAIC–Frederick.

Recent Publications:

Gontarek R, et al. *Mol Cell Biol* 1996;16:2325–31.

Harris ME, et al. *Mol Cell Biol* 1998;18:3889–99.

Hill SA, et al. *Virology* 1999;263:273–81.

Derse D, et al. *Molecular Pathogenesis of HTLV–1: Current Perspective.* Arlington, VA: ABI Professional Publications, 1999, pp. 123–30.

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Biography: *Dr. Felber received her Ph.D. in molecular biology from the University of Bern, Switzerland. After carrying out postdoctoral studies in the Laboratory of Biochemistry, NCI, she joined the Molecular Mechanisms of Carcinogenesis Laboratory, ABL–Basic Research Program, in 1985. In 1990, Dr. Felber established the Human Retrovirus Pathogenesis Group. In 1998, Dr. Felber received her tenure appointment and, in 1999, she joined the Center for Cancer Research, NCI. Her work focuses on the posttranscriptional mechanisms of gene regulation in human retroviruses and the study of pathogenesis of simian immunodeficiency virus.*

Basic Research Laboratory–Frederick

Posttranscriptional Control: A General and Important Regulatory Mechanism of HIV–1 and Other Retroviruses

Keywords:

AIDS
animal models
binding protein
gene expression
HIV–1
molecular interactions
protein transport
regulatory elements
retroviruses
transport
vaccine

Research:

Posttranscriptional Regulation of mRNA Expression and Its Role in Retroviral Pathogenesis

Our section has two major research goals: (1) to study the mechanisms of posttranscriptional mRNA regulation, and (2) to study the pathogenicity and immunogenicity of attenuated Rev-independent HIV and simian immunodeficiency virus (SIV) strains.

A critical step for mRNA expression is the transport from the nucleus to the cytoplasm. The analyses of retroviral systems and, especially, human retroviruses such as HIV and human T cell leukemia virus were responsible for the rapid progress in this research field. All lentiviruses depend on the posttranscriptional regulation mediated by the viral Rev protein binding to the Rev-responsive element (RRE) to express their structural proteins.

Continuing our studies on the regulation of lentiviral expression, we showed that Rev participates in a common export pathway with cellular proteins sharing a similar leucine-rich export signal. We further showed that the nucleosporins Nup98 and Nup214 play a direct role in the export of Rev from the nucleolus to the cytoplasm.

In contrast to HIV–1, type D retrovirus expression is mediated via a cellular factor, which interacts with the viral cis-acting transport element (CTE) RNA element. We characterized the CTE RNA structure and we found that the cellular protein TAP binds and stimulates the nucleocytoplasmic export of CTE RNA. TAP is the first cellular protein known to be directly involved in mRNA export. We demonstrated that TAP is a dynamic protein able to shuttle between the nucleus and the cytoplasm, supporting TAP's role as the export factor of the CTE-containing mRNAs.

We also demonstrated that the RRE- and the CTE-regulated mRNAs utilize distinct nucleocytoplasmic export pathways. This is the first demonstration that specific export pathways are responsible for mRNA export. These studies are important for understanding the basic cellular pathways that are also involved in viral regulation and carcinogenesis.

Attenuated SIV strains have been shown to be effective in protecting against virus challenge, but the strains studied so far have also been shown to have residual pathogenicity, preventing their use as live attenuated vaccines. We studied HIV and SIV variants that have the Rev/RRE regulatory system replaced by CTE in order to evaluate their pathogenicity and ability to induce a protective immune response. We found that Rev-independent clones of HIV–1 and SIV are infectious but show reduced replicative capacity. Furthermore, we demonstrated that the SIV variant virus can persistently infect rhesus macaques, albeit at low levels, without causing disease. Therefore, our data demonstrate that the Rev/RRE regulatory mechanism is required for high levels of virus propagation in vivo, and that this posttranscriptional regulatory control plays an important role in the pathogenicity of SIV. It appears that replacement of the Rev/RRE regulatory system is a promising approach to attenuate SIV and HIV. These systems are also useful in the identification of determinants for protective immunity against HIV/SIV.

Our collaborators include Elisa Izaurralde, European Molecular Biology Laboratory, Heidelberg; Marta Marthas, California Regional Primate Center, University of California–Davis; Nancy Miller, NIH; and Ruth Ruprecht, Dana-Farber Cancer Institute.

Recent Publications:

Bear J, et al. *Mol Cell Biol* 1999;19:6306–17.

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Tan W, et al. *RNA* 2000;6:1762–72.

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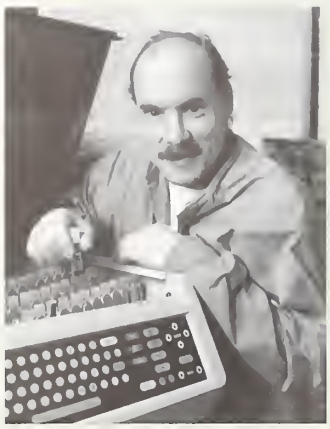

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Biography: *Dr. Ferris is a scientist employed by SAIC to perform basic research at the NCI–Frederick. He received his Ph.D. in biology in 1986 from Virginia Polytechnic Institute and State University where he studied developmental biology with Charles A. Rutherford. Dr. Ferris performed postdoctoral research in lymphokine-mediated signal transduction in William L. Farrar's laboratory at the NCI–Frederick and since 1989, he has studied the molecular mechanisms regulating cell cycle progression.*

Basic Research Laboratory–Frederick
Characterization of Polo-Related Kinases

Keywords:
cancer
cell cycle
polo-like kinase

Research: The processes of cell growth and division are stringently regulated to ensure fidelity of DNA replication and correct segregation of genetic information. So essential are these processes to cellular homeostasis that many of the gene products regulating passage through the cell cycle are highly conserved both in amino acid sequence and function between organisms as evolutionarily divergent as yeast and man. Within the last few years, a variety of related enzymes known as cyclin-dependent protein kinases (CdKs) have been identified that are activated and inactivated at specific times during cell cycle progression. The CdKs must form complexes with a cyclin family member to be activated and are subject to both positive and negative phosphorylation by other protein kinases, and dephosphorylation by protein phosphatases. Together, the family of CdKs constitute critical components of the engine that propels the cell through the cycle. Many proteins that are phosphorylated in a cell cycle-specific fashion have been identified as putative targets of CdK regulation including nuclear lamins, nucleolin, and other matrix proteins, as well as cytoskeletal proteins. In addition to such architectural proteins, the tumor suppressor gene products p53 and RB are both subject to regulatory phosphorylation by CdK family members. Thus, it is clear that reversible phosphorylation reactions play a major role in regulating cell cycle progression, and the list of enzymes known to be involved is rapidly expanding.

During the last few years we have been working to characterize members of the polo family of protein kinases. Three mammalian polo-related kinases have been cloned, polo-like kinase (Plk), serum-inducible kinase (Snk) and fibroblast growth factor-inducible kinase (Fnk). Aside from similarities in their N terminal kinase domains, these enzymes share a highly conserved sequence of 30 amino acids known as the polo box. Related kinases have also been cloned from *Drosophila*, yeasts, nematodes, and amphibians. Recently we have been involved in purifying Plk from cell extracts with the goal of identifying other proteins that interact with Plk. We found that Plk is a member of a large complex and is associated with alpha, beta, and gamma

tubulins independent of microtubule polymerization state. We demonstrated that Plk phosphorylates the associated tubulins in vitro and that the association between Plk and tubulins is dependent on the kinase domain of Plk, but that the kinase activity is irrelevant to the association. In light of the clear involvement of Plk in regulating the centrosomes and the mitotic spindle, these results help provide a molecular explanation for the role of Plk in cell cycle progression.

We have cloned a *C. elegans* homolog of the *Drosophila melanogaster polo* gene (designated *plk-1* for *C. elegans* polo-like kinase-1) and determined the subcellular localization of this protein during the meiotic and mitotic cell cycles in *C. elegans* embryos. Disruption of PLK–1 expression by RNA-mediated interference (RNAi) resulted in embryos which failed to develop properly and arrested before the first cell division. These embryos, in addition to being unable to divide, displayed defects in the completion of meiosis as characterized by the inability to properly separate maternal chromosomes and extrude polar bodies. Further, the oocytes of *plk-1* RNAi animals failed to undergo nuclear envelope breakdown (NEBD) before ovulation. The anomalous presence of an intact nucleus was also observed in newly fertilized *plk-1* RNAi embryos as well as in embryos that were depleted of the cyclin-dependent kinase NCC–1 (*C. elegans* homolog of Cdc2). These results indicate that PLK–1 is required for several aspects of early *C. elegans* development including cytokinesis and meiotic progression. In addition, PLK–1 and NCC–1 are required for the regulation of nuclear envelope breakdown in *C. elegans* oocytes.

Recent Publications:

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Feng Y, et al. *Biochem J* 1999;339:435–42.

Chase D, et al. *Genesis* 2000;26:26–41.

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Basic Research Laboratory–Frederick
Oncogene Working Group

Keywords:
ATPase
cell cycle
NFκB
oncogenes
posttranslational regulation
proteasome
protein folding
protein kinase
protein-protein interaction
proteolysis
receptor signaling
signal transduction
transcription factor
ubiquitin

Research: The ubiquitin-proteasome (Ub-Pr) mediated degradation pathway has been shown to regulate many important cellular processes. The pathway consists of two sequential steps: the target protein is first conjugated with multiple ubiquitin molecules, then transferred to the 26S proteasome, unfolded and degraded. The multisubunit proteasome has ATPase activity which presumably provides the energy needed for the chaperoning and unfolding of the substrate before its degradation. We previously identified Valosin-containing protein (VCP), a highly conserved ATPase, physically and functionally associated with both the ubiquitinated Ub-Pr substrates and the highly purified proteasome. We hypothesize that VCP is an ATPase chaperone that carries the ubiquitinated substrates to the proteasome. VCP and its orthologs, Cdc48p and p97, belong to a highly conserved AAA (ATPases associated with a variety of cellular activities) family. Despite the high sequence and structural similarities among VCP/Cdc48p/p97, these proteins are paradoxically involved in many seemingly unrelated functions—e.g., cell cycle control, T and B cell activation, homotypic membrane fusion, vesical-mediated transport, and the Ub-Pr degradation. The apparent paradox suggests that VCP may function in a common mechanism that underlies these activities. Since Ub-Pr pathways have been shown to regulate many substrates that are also involved in these same activities, our hypothesis of VCP being a chaperone in the Ub-Pr pathways would provide the underlying mechanism explaining the paradox for VCP. We demonstrate that VCP preferentially binds the ubiquitinated substrates, and this binding is mediated through a direct interaction between N terminal 200 residues of VCP and the polyubiquitin chains. Preliminary data also reveal that VCP prevents aggregate formation, an indicative characteristic of molecular chaperones. Our current and future research focuses on demonstrating the required chaperone role of VCP in Ub-Pr-mediated degradation, and to elucidate the structure/function relationships in VCP.

Our collaborators are Henry Bose and Radmila Hrdlickova, University of Texas; Carlos Gorbea, University of Utah; Robert Kirken, M.D. Anderson Medical Center; Sandra Ruscetti, NIH; and Yu-Chung Yang, Indiana University.

Recent Publications:

Dai RM, et al. *J Biol Chem* 1998;273:3562–73.

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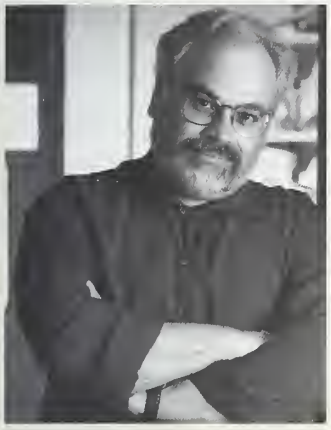

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Biography: *Dr. Pavlakis received his M.D. from the University of Athens, Greece, and his Ph.D. from Syracuse University. He has been associated with the NCI since 1980 and is currently chief of the Human Retrovirus Section. Dr. Pavlakis has focused his research on the biology and pathogenesis of human retroviruses, especially HIV–1. His interests include the molecular biology of HIV–1; the pathogenic mechanisms leading to AIDS; and the development of models of human disease, new technologies to study gene function, and improved methods for gene transfer and gene therapy.*

Basic Research Laboratory–Frederick
Analysis of HIV–1 Expression, Protein Function, and Pathogenic Mechanisms of AIDS

Keywords:
AIDS
fluorescence
gene regulation
HIV–1
immune dysfunction
MRna export
retroviruses
vaccines
virus-cell interaction

Research: The Human Retrovirus Section studies the molecular biology and pathogenic mechanisms of HIV–1. We are interested in new vaccine approaches against AIDS and in identifying and validating new targets for antiviral therapies. Highlights of recent accomplishments are described below.

HIV RNA Expression Regulation

Expression of HIV–1 RNA is totally dependent on the presence of the viral Rev protein. In the absence of Rev, instability elements (INS) scattered throughout the HIV–1 mRNA trap these messages within the nucleus, thus interfering with their expression. We have demonstrated that these INS elements are targets for binding the poly(A)-binding protein (PABP1). Binding of PABP1 and other factors in the nucleus interfere with mRNA transport and lead to more splicing or degradation. We eliminated the INS sequences from the HIV–1 messages by using multiple point mutations that maintain the amino acid composition of the corresponding protein. This technique resulted in high HIV–1 mRNA expression levels even in the absence of Rev, and led to the development of simple expression vectors that produce large amounts of the Gag, Pol, and Env proteins in many cell types and in animals. Increased expression of these proteins in mice and primates produced a strong immune response. Both cellular and humoral immunity components were activated in these animals after DNA injection, indicating the potential of this approach in AIDS vaccine applications. Currently, several vaccination protocols are under way that use these vectors.

HIV–Cytokine Interactions

We are studying the complex interactions of HIV–1 with cytokines such as interleukin 4 (IL–4) and tumor necrosis factors (TNFs). We have found dichotomous effects on the propagation of HIV strains with different receptor specificity due to the regulation of both virus and receptor gene expression by these cytokines. These studies have led to the proposal that both IL–4 and TNF are important regulators of HIV in vivo and that they participate in the long-term selection in the body leading to altered receptor utilization. Because receptor-blocking drugs are now under development, receptor switching by HIV needs to be better studied and understood.

Protein Localization and Trafficking

We have developed and applied new technologies in our study of the localization and trafficking of proteins in live cells. In particular, we generated several mutants of the *Aequorea victoria* green fluorescent protein (GFP) with enhanced fluorescence. Crystallization and analysis of these mutants led to the identification of the molecular basis for spectral variation in GFP and facilitated the design of additional beneficial mutants. We have used these mutants extensively in tagging proteins, cells, virions or viruses, and in gene expression studies. Among our findings was the identification of a new trafficking pattern for primarily cytoplasmic proteins such as PABP1 and the product of the von Hippel-Lindau tumor suppressor gene (pVHL).

We are also studying the function of several HIV–1 proteins. We have elucidated the parameters of rapid and continuous trafficking between the nucleus and cytoplasm for the Rev protein. We have shown that the mechanism of transdominance of a Rev mutant over the normal protein involves the binding of Rev to transdominant Rev in the nucleolus and the subsequent inhibition of export. We have demonstrated that the HIV–1 Vpr protein is a transcriptional coactivator of nuclear receptors. This finding, in turn, explains several actions of Vpr on the expression of cellular genes. We also showed that the HIV–1 Nef protein colocalizes with the AP2 adaptor protein complex, which is involved in protein sorting from the plasma membrane.

In addition to the above techniques, we have developed improved cationic liposome formulations for in vivo gene delivery. This method opens up new approaches for gene delivery and has applications in gene therapy and gene vaccine methods.

Our collaborators include George Chrousos, Tomoshige Kino, Richard Klausner, Stephen Lee, and Alexander Wlodawer, NIH; Markus Neumann and Ralf Schneider, GSF–National Research Center for Environment and Health, Germany; and Xiao-Fang Yu, Johns Hopkins University.

Recent Publications:

Lee S, et al. *Mol Cell Biol* 1999;19:1486–97.

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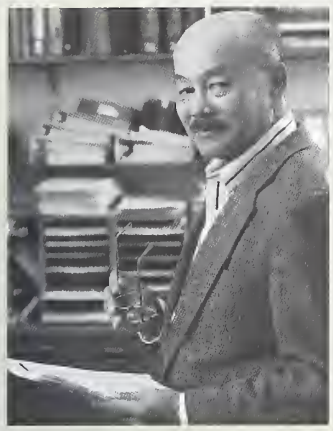
Rosati M, et al. *J Immunol* 2001;167: in press.



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Biography: *Dr. Phang received his M.D. from Loma Linda University School of Medicine and his clinical training in internal medicine from Stanford Medical Center. He was a clinical associate with the NCI's Metabolism Branch. After additional training in biochemistry and molecular biology with the Laboratory of Chemical Biology, National Institute of Diabetes and Digestive and Kidney Diseases, Dr. Phang was appointed a senior investigator in the Metabolism Branch, NCI, and later became chief of the Endocrinology Section. From 1989 to 1998, he served as chief of the Laboratory of Nutritional and Molecular Regulation, and in 1998, he formed the Metabolism and Cancer Susceptibility Section in the Basic Research Laboratory.*

Basic Research Laboratory–Frederick
Metabolic Mechanisms for Modulating the Cancer Susceptible Phenotype

Keywords:
colon cancer
nitric oxide
proline
prostate-specific antigen
reactive oxygen species

Research: An underlying theme is the epigenetic modulation of proliferative, apoptotic, and stress-related signaling. Recent advances in three areas have focused our efforts: (1) the identification of cancer susceptibility genes and their products—for example, *Apc*, which is responsible for familial adenomatous polyposis (FAP) and which is mutated in 85 percent of sporadic human colorectal cancers; (2) the participation of metabolic enzymes in carcinogenesis. A number of metabolic enzymes are induced accompanying p53-mediated apoptosis; and (3) the implication of reactive oxygen species (ROS) in mitogenic and cytokine-mediated signaling and/or programmed cell death.

The *Apc* Genotype in Colorectal Carcinogenesis and Modulation of the Phenotype by Nitric Oxide

Using conditionally immortalized murine colonic epithelial cells contrasting in *Apc* genotype, we showed that NOS II and PGHS–2 are overexpressed with inflammatory stimuli, and the levels are higher in cells with mutant *Apc*. The role of NOS II was emphasized by showing that inhibitors of its catalytic activity blocked the response to inflammatory stimuli suggesting a signaling role for nitric oxide (NO). NO donors increased the formation of β-catenin: Tcf/LEF:DNA complexes. The mechanism of this effect is linked to the degradation of E-cadherin and release of β-catenin bound to plasma membranes. In cells with a mutated *Apc*, β-catenin accumulates to a higher level in response to NO because the degradation of β-catenin is defective in these cells. These studies show that NO modulates the defective signaling found with the mutant *Apc* genotype and mechanistically links colorectal carcinogenesis to inflammatory stimuli. These studies also implicate the effect of NO on metalloproteinases and on proteins mediating cell-cell and cell-matrix interactions.

Proline and Pyrroline-5-Carboxylate in Cell Regulation

Pyrroline-5-carboxylate (P5C) is the immediate precursor and product of proline. Catalytic cycling of these two molecules can mediate redox transfers between mitochondria and cytosol and can regulate several pathways: (1) augments the production of ribose and PP-rib-P for the synthesis of ribonucleotides; (2) regulates gene expression, especially of hypoxia induced genes; and (3) modulates programmed cell death in cells undergoing p53-mediated apoptosis. The mechanisms underlying these effects are being studied. In cells undergoing apoptosis, proline oxidase is induced and added proline stimulates the formation of reactive oxygen species (ROS). Importantly, in p53 negative cells transfected to overexpress proline oxidase, the addition of proline is sufficient to induce apoptosis. A developmental role for this metabolic pathway can be inferred from findings in patients with deficiencies of these enzymes. We have pursued this possibility in studies of *Xenopus* embryos. Ornithine aminotransferase (OAT), the enzyme that converts ornithine to P5C, may be important in neurodevelopment. From studies of in situ hybridization, the expression of OAT is seen predominantly in neural tissue in early embryonic development. Microinjection of OAT mRNA alters the tadpole morphology characterized by a short tail. The metabolic cascade responsible for this abnormality is being investigated. These studies show that proline and pyrroline-5-carboxylate may be important in metabolic signaling in development, proliferation, and apoptosis.

Prostate-Specific Antigen and Prostate Cancer Cells

Although prostate-specific antigen (PSA) has been an important clinical marker for prostate cancer, its biologic function has been recognized only recently. We showed that PSA causes prostate cancer cells to increase their production of reactive oxygen species (ROS) as monitored with dichlorofluorescin and laser-activated cell fluorometry. Testosterone can stimulate ROS formation, but the effect is mediated through its stimulation of PSA production. Since PSA is a serine protease, its biologic effect may be due to its proteolytic activity. However, we found that stimulation of ROS formation by PSA can be dissociated from its proteolytic activity. These findings suggest that PSA may interact with cellular proteins to activate signaling cascades.

Recent Publications:

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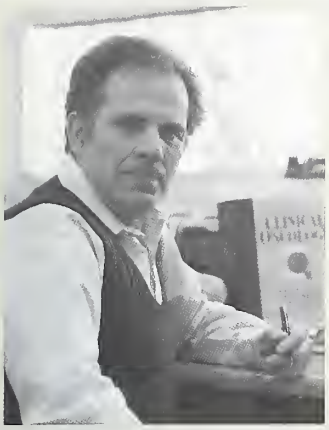
Mei JM, et al. *Cancer Res* 2000;60:3379–83.

Donald SP, et al. *Cancer Res* 2001;61:1810–5.

Sun XY, et al. *Carcinogenesis* 2001; in press.



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Biography: *Dr. Ruscetti obtained his Ph.D. from the University of Pittsburgh working on prokaryotic RNA metabolism under Lew Jacobson and subsequently carried out postdoctoral work on hematopoiesis under Dane Boggs at Pitt Medical School. From 1975 to 1983, he studied human retroviruses and T cell biology in the Laboratory of Tumor Cell Biology, NCI. He has continued his studies on cytokine regulation of hematopoiesis and human retrovirology as a senior investigator. He is currently head of the Leukocyte Biology Section, Basic Research Laboratory, Center for Cancer Research, NCI–Frederick.*

Basic Research Laboratory–Frederick

Balance Between Positive and Negative Regulation in Hematopoiesis: Disruption by Human Retroviruses

Keywords:
AIDS
antiproliferative agents
antiviral
apoptosis
Bcl-2
bone marrow
cancer cell growth
cell cycle inhibitors
differentiation
Fas
HIV
HTLV–1
pathogenesis
stem cells
T lymphocytes
TGFβ1
virus-cell interactions

Research: Cell cycle control of cell growth and differentiation is tightly regulated by a series of complex interactions. It is the main tenet of our studies that unrestricted growth control and inappropriate transcriptional activation, due to defects in negative regulatory control, play a major role in oncogenesis and retroviral pathogenesis.

Balance Between Positive and Negative Regulators in Hematopoiesis
We and others have shown that to initiate and maintain the growth and differentiation of primitive progenitor cells, multiple cytokine stimulation (synergy) is required. More recently, we showed that such cooperation also occurs between negative regulators of cell growth, and that the ability of primitive progenitors to proliferate depends on the balance of positive and negative signals the cell receives. Transforming growth factor-β (TGFβ) directly and reversibly inhibits hematopoietic stem cells with marrow repopulating ability (LT–HSC). Also, short-term incubation with TGFβ does not damage the self-renewal potential of these stem cells. TGFβ has inhibitory effects on the cell surface expression of many cytokine receptors that directly correlates with its effect on cell growth. For example, stem cell factor receptor (c-kit) expression is downregulated by TGFβ, in part by affecting c-kit mRNA stability. These results indicated that c-kit expression could be negatively regulated on LT–HSC. Indeed, we were able to characterize a novel LT–HSC lacking c-kit expression and to show that in bone marrow cell development, this cell matures into a c-kit+ LT–HSC. Also, TGFβ prevents S-phase cell cycle progression through an intracellular mechanism involving regulation of transcription factors and cell cycle regulatory proteins. In vivo results demonstrated that TGFβ can protect mice from both the lethal hematopoietic toxicity of 5–FU as well as the nonhematopoiesis toxicity of DXR. These findings show that a negative regulator of hematopoiesis can be successfully used systemically to mediate chemoprotection in vivo. Previous results from many labs also indicated that TGFβ treatment of donor cells before bone marrow transplantation (BMT) could have a beneficial effect by blocking the immune reactivity. We were able to show suppression of graft-versus-host

disease (GVHD) after allogeneic BMT through a TGFβ mediated mechanism. Treatment of donor CD4+ T cells with TGFβ and IL–10 made the donor T cells hyporesponsive and less able to promote GVHD. In many instances, growth inhibition following terminal differentiation or anticancer drug treatment results in apoptosis (programmed cell death). We have previously shown vitamin E succinate (VES) to induce apoptosis in human B lymphoma cells. We recently demonstrated that VES-mediated growth arrest of the estrogen receptor negative (antiestrogen therapy resistant) breast cancer cell lines, MDA–MB–231 and SKBR–3, results in apoptotic cell death. In human breast tumors, estrogen receptor negative status is associated with low expression of bcl-2 and a higher frequency of p53 mutations. Both MDA–MB–231 and SKBR–3 cells have been previously shown to contain mutant p53 and to express undetectable bcl-2 protein. Recent data indicates that the apoptotic and growth inhibitory effects on VES are not directed through TGFβ but are mediated through the fas pathway, but only in cells that have a dysfunctional Bcl-2 pathway.

Negative Regulation of Viral and Cellular Genes During Retroviral Infection

We previously demonstrated HIV–1 latency in monocytes in vivo. The presence of latently infected cells constitutes a possible source for reactivation of virus production during the normal immune response even in the face of potent antiviral therapy. We have identified immune activation of latently infected cells to be a mechanism by which latent HIV–1 can be reactivated to produce infectious virus. Studies on the development and function of CD4+ Th1 and Th2 cells during the progression to AIDS may increase the understanding of AIDS pathogenesis. The preferential replication of HIV in either Th1 or Th2 cells could alter the delicate balance of the immune response. Th1 (IFN-γ positive, IL–4 and IL–5 negative) and Th2 (IFN-γ negative, IL–4 and IL–5 positive) clones, developed from several normal healthy donors, pedigreed by RT–PCR and ELISA, have similar cell surface expression of CD4 and several chemokine receptor cofactors necessary for viral entry. After activation by specific antigens and infection with HIV–1, both Th1 and Th2 clones showed similar levels of viral entry and reverse transcription. Methylation of the viral long terminal repeat is a second mechanism by which HIV–1 and HTLV–1 proviral expression is silenced. We have recently demonstrated that these viruses upregulate expression of DNA methyltransferase (Metase) in infected cell lines and primary cells. Further, we have identified the IFN-γ gene as a target of this increased DNA Metase activity. Thus, increasing DNA Metase expression may be another mechanism by which HIV–1 evades the immune response. Although largely unknown, the cellular events triggered by HTLV–1 infection in the lymphoproliferative process are of major importance to the establishment of leukemia. We have recently characterized an infectious clone of HTLV–1 as well as a susceptible expressing cell line to study the molecular interactions of this infectious clone. We have shown the clone to be highly transforming for T cells.

Our collaborators are Steve Bartelmez, University of Washington; Steve Baylin, Johns Hopkins Medical School; Bruce Blazer, University of Minnesota; and Bernard Poiesz, Upstate Medical Center, Syracuse, NY.

Recent Publications:

Mikovits J, et al. *J Virol* 1998;72:5231–8.

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Zeller JC, et al. *J Immunol* 1999;163:3684–91.

Ortiz M, et al. *Immunity* 1999;10:173–82.

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Basic Research Laboratory–Frederick
Molecular Basis for the Pathogenesis of Murine Retroviruses

Research: The focus of our research is devoted to understanding the molecular basis for the pathogenesis of rodent retroviruses. We have been studying retroviruses that cause leukemia or neurological disease in rodents to obtain basic information on how molecular changes in normal cells can result in pathological consequences. We hope to use the information gained from these studies to design rational strategies to counteract the molecular events that are responsible for the diseases induced and apply this to similar diseases in man.

The major focus of our research is on the erythroleukemia induced by the Friend spleen focus-forming virus (SFFV), which serves as an important model for understanding how deregulation of hematopoietic pathways can lead to leukemia. Although normal erythroid cells require erythropoietin (Epo) for proliferation and differentiation, those expressing the SFFV envelope protein proliferate and differentiate in the absence of Epo, resulting in acute erythroid hyperplasia and polycythemia. In addition, SFFV integration at the *sfpi-1* locus results in expression of nonphysiological levels of the myeloid transcription factor PU.1 in erythroid cells, causing a block in differentiation and the outgrowth of transformed cells. To understand how expression of the SFFV envelope protein in erythroid cells alters their growth and differentiation and how expression of PU.1 leads to their transformation, we have been studying signal transduction pathways known to be activated by Epo to determine if any of these are deregulated after SFFV infection. In nontransformed erythroid cells infected with SFFV, we have shown that most Epo-induced signal transducers, including Stat proteins, components of the Raf-1/MAP kinase pathway, PI 3-kinase, protein kinase C and Akt kinase, are

constitutively activated through activation by the virus of a redundant signal transduction pathway involving IRS-related adaptor proteins. Our recent studies indicate that a truncated form of the receptor tyrosine kinase Stk, which was recently implicated in susceptibility to SFFV-induced erythroleukemia, is activated after covalent interaction with the SFFV envelope protein, suggesting that this kinase may be responsible for activating signal transducing molecules in SFFV-infected cells. Although Stat proteins are activated when SFFV infects erythroid cells, we observed that transformation of these cells was associated with a block in the activation of Stats 1 and 3 DNA-binding activity. This block is specific for Stat proteins and is at the level of DNA-binding, not tyrosine phosphorylation or nuclear transport. There is a direct correlation in SFFV-infected erythroid cells between expression of PU.1 and inhibition of Stat DNA-binding, suggesting that PU.1 may be involved in the block. Consistent with this idea, we recently demonstrated that PU.1 blocks the transactivation of the oncostatin M promoter by Stats 1 and 3. Thus, PU.1 may block differentiation of SFFV-infected erythroid cells by interfering with the interaction of certain Stat proteins with the promoters of genes required for erythroid cell differentiation. To date, our studies on SFFV-induced erythroleukemia have identified a number of molecular events associated with deregulation of cell growth and transformation that we can potentially target for therapeutic intervention.

As a second retroviral model system, we have been studying PVC–211 murine leukemia virus (MuLV), a variant of the erythroleukemia-inducing Friend MuLV that causes a rapid neurodegenerative disease in rodents. PVC–211 MuLV provides an important model for understanding how retroviruses can undergo genetic changes that alter their interaction with cells in the host to cause novel biological effects. Using chimeric constructs between PVC–211 MuLV and its nonneuropathogenic parent Friend MuLV, we previously demonstrated that PVC–211 MuLV had undergone subtle changes in its envelope gene that allow it to efficiently infect brain capillary endothelial cells (BCEC), which are generally resistant to infection by MuLVs. This expanded host range allows PVC–211 MuLV to be expressed at high levels in the neonatal rodent brain and this results in a rapid neurodegenerative disease. Since viral infection of BCEC is crucial to the development of this neurological disease, we have concentrated our efforts on mapping changes in the envelope gene responsible for BCEC tropism and understanding the interaction of PVC–211 MuLV with its BCEC receptor. We demonstrated that the BCEC tropism of PVC–211 MuLV is the result of two amino acid changes in the region of the viral envelope glycoprotein that binds to CAT1, a cationic amino acid transporter that is the cellular receptor for ecotropic MuLVs. Recent studies indicate that one of these changes creates a unique heparin-binding site that may allow PVC–211 MuLV to bind to glycosaminoglycans on the surface of BCEC to facilitate receptor binding. Although BCEC tropism is essential for the neuropathogenicity of PVC–211 MuLV, it is not sufficient. We are, therefore, carrying out studies to determine how expression of PVC–211 MuLV in BCEC leads to degeneration of neurons, a mechanism which must be indirect since the virus cannot be detected in neurons or significantly in any other cells in the central nervous system. Finally, our studies of PVC–211 MuLV demonstrate that the BCEC tropism of this virus can be further extended to capillary endothelial cells (CEC) from other organs, and we have initiated studies in an attempt to exploit this

unique property of PVC–211 MuLV for use in gene transduction targeted to the proliferating endothelium associated with developing tumors.

Collaborators on this research are Paul Hoffman, Department of Veterans Affairs Medical Center, Baltimore, MD, and Michiaki Masuda, University of Tokyo, Japan.

Recent Publications:

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Nishigaki K, et al. *J Virol* 2000;74:3037–45.

Muszynski K, et al. *J Virol* 2000;74:8444–51.

Nishigaki K, et al. *J Virol* 2001; in press.

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Basic Research Laboratory–Frederick
Structural and Functional Analysis of Molecular Recognition in Antibody-Antigen Protein-Protein Complexes

Keywords:
antibody
antigen
association kinetics
biochemistry
docking
epitopes
immunology
molecular dynamics
molecular interactions
molecular models
molecular structure
monoclonal antibodies
protein chemistry
protein folding
protein-protein interactions
protein stability
protein structure
thermodynamics

Research: Macromolecular interactions are central to cellular regulation and biological function. Our research focuses on defining the structural features of a protein receptor that determine specific binding properties for a protein ligand, using a combination of computational, structural, and experimental approaches. An understanding of the structural and functional mechanisms underlying molecular recognition will facilitate rational design of proteins of predefined specificity and kinetic properties. Complexes of three mAbs with the protein antigen hen egg white lysozyme (HEL) have been defined by x-ray crystallography. They differ in the nature of their interactions with HEL, and therefore are useful for investigating different aspects of molecular recognition. The HyHEL5/HEL complex is predominated by salt links. Several other mAbs also recognize the same epitope, but with lower affinities. Studies with chain and domain swaps among these Abs as well as site-directed mutants of HyHEL5 are yielding important insights into the role of electrostatic forces and water in recognition. In contrast, no specific residues predominate the interaction of HyHEL10 with HEL. Three other mAbs, HyHEL26, HyHEL8, and HyHEL63, recognize epitopes that are highly coincident with that of HyHEL10, and share over 90 percent sequence

homology with HyHEL10, but they differ significantly from HyHEL10 in their specificity properties. High-resolution x-ray crystallography structures of Fab63 uncomplexed and in complex with HEL confirm that it recognizes the same binding site as that recognized by HyHEL10. A high degree of cross-reactivity with mutant antigens correlates with conformational flexibility and unspecific chemical complementarity, while high specificity correlates with a relatively rigid binding site and specific chemical complementarity. Binding of these complexes can be described by a two-phase kinetic model consistent with a binding sequence of encounter followed by docking which may include conformational changes. Estimates of encounter rate constants k_{+1} and k_{-1} are in good agreement with rate constants determined by atomic force microscopy. We have recently demonstrated for the first time that cross-reactivity correlates with free-energy changes associated with the docking phase, consistent with our hypothesis that cross-reactivity reflects conformational flexibility of the combining site.

This model has allowed additional insight into the dynamics of antibody-antigen association: (1) the biological half life of the encounter complex, defined by a new parameter T_{50}, is unique for each antibody-antigen complex; the encounter complexes of unmutated Fabs with HEL have T_{50} of 5 to 10 mins, while the decreased affinities with mutant antigens often reflect significantly slower docking with longer T_{50} of an hr or longer; (2) T_{50} is likely a more biologically relevant measure of activity than net affinity (K_A); (3) the same antigenic mutation affects different steps in the associations of each antibody complex; (4) faster net off rates accompanying decreased antibody affinity for mutant antigens may reflect significantly slower docking with longer T_{50} but not necessarily faster rate constants k_{-1} and k_{-2}; (5) free energy barriers to conformational rearrangement are highest in noncross-reactive antibodies and lowest in cross-reactive antibodies, especially in complexes with mutant antigens; (6) our results predict that H8 would be the most and H26 the least entropically driven, a result that is confirmed with calorimetric measurements; (7) temperature differentially affects the encounter and docking steps, altering T_{50} and the distribution of free energy change between these two steps; and (8) the encounter step is entropically driven, and docking is entropically driven with a large entropic penalty. These results suggest that an understanding of the forces that control protein flexibility are important to the design of antibodies and other receptors with predefined dynamic properties as well as specificity. These antibody-antigen protein-protein complexes are important models which will provide significant insight into the general process of molecular recognition.

We have collaborated in this research with David Davies and Eduardo Padlan, NIH; Peter Hinterdorfer, University of Linz; Roy Mariuzza, Center for Advanced Research in Biotechnology; and Richard Willson, University of Houston.

Recent Publications:

Raab A, et al. *Nat Biotechnol* 1999;17:902–5.

Li Y, et al. *Biochemistry* 2000;39:6296–309.

Lipschultz CA, et al. *Methods* 2000;20:310–8.

Li Y, et al. *Biochemistry* 2001;40:2011–22.

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Biography: *Dr. Grace Yeh obtained her Ph.D. in biochemistry from the University of Maryland–College Park and carried out postdoctoral work in the Metabolism Branch of the NCI. She then joined the faculty of the Uniformed Services University of the Health Sciences as associate professor in the Department of Pharmacology and held a joint appointment in the Medicine Branch of the NCI. Dr. Yeh joined the NCI as principal investigator in 1990 and became chief of the Cellular Defense and Carcinogenesis Section in 1997.*

Basic Research Laboratory–Frederick
Cellular Defense Mechanisms Against Carcinogens

Research: The goal of the Cellular Defense and Carcinogenesis Section is to elucidate the biochemical and molecular mechanisms involved in the chemopreventive effect of nutritive and nonnutritive factors in human diets. Numerous epidemiological studies have demonstrated that the consumption of fruits and vegetables has a protective effect against several forms of cancer. Many natural phytochemicals found in fruits, vegetables, spices, and tea have been shown in recent years to be protective against cancer in various animal models. Compounds of particular interest include: the flavonoids, a group of more than 4000 different polyphenolic derivatives of benzo-γ-pyrone that are widely distributed in the plant kingdom and thus are components of all human diets; curcumin, a polyphenolic compound present in the plant *Curcuma longa*, found in the spice turmeric; rosemary extract, a complex mixture of flavonoids, diterpenes, and rosmarinic acid derived from the plant *Rosmarinus officinalis*; retinoids, analogs of vitamin A; and synthetic chemopreventive compounds, including 4-hydroxyphenyl-retinamide, oltipraz, a synthetic dithiolthione, and compound 8354 (fluasterone), a fluorinated derivative of the steroid dihydroepiandrosterone (DHEA).

We have established several in vivo and in vitro model systems that allow us to investigate the effect of these phytochemicals on different mechanisms involved in the cellular defense against carcinogens.

- **The cellular efflux of aryl hydrocarbons by P-glycoprotein and the modulation of P-glycoprotein activity by dietary compounds.** We have developed a series of multidrug-resistant clones of MCF–7 cells that express P-gp. Using these cells, we have demonstrated that P-gp can mediate the efflux of the aryl hydrocarbons dimethylbenzanthracene (DMBA) and benzo[a]pyrene (BP), and thus may be an important defense mechanism against carcinogens. We have also demonstrated that some dietary compounds, particularly the flavonols quercetin, kaempferol, and galangin, increase P-gp activity toward these carcinogens.
- **Mechanisms of resistance towards aryl hydrocarbons.** We have developed MCF–7 clones by continuous exposure to BPs, which exhibit resistance to aryl hydrocarbons. We are characterizing some of the molecular

and biochemical changes in these cells that result in resistance, with particular attention to carcinogen activation, detoxification, and DNA repair.

- **The effect of phytochemicals on the carcinogen activation pathway mediated by the aryl hydrocarbon receptor.** Many carcinogens, including the aryl hydrocarbons, require activation from procarcinogen forms to be genotoxic. This activation is carried out by cytochrome P1A. The expression of cytochrome P4501A1 (CYP1A1), the primary activating enzyme in MCF–7 cells, is regulated by the aryl hydrocarbon receptor (AhR), a cytosolic transcription factor that mediates the transcription of both activating and detoxifying enzymes. We have investigated the effect of several flavonoids, curcumin, dibenzoylmethane, and resveratrol on carcinogen activation, CYP1A1 activity, and AhR function.

- **Modulation of cytochrome P450 aromatase activity and expression by natural and synthetic chemopreventive compounds.** Cytochrome P450 aromatase catalyzes the conversion of C19 androgens to C18 estradiol and is overexpressed in mammary tumor cells. Inhibition of this enzyme may reduce the local production of estradiol, which serves as a growth factor in mammary tumor cells.

Recent Publications:

Ciolino HP, et al. *J Biol Chem* 1999;274:35186–90.

Ciolino HP, et al. *Mol Pharmacol* 1999;56:760–7.

MacDonald CJ, et al. *Cancer Res* 2001;61:3919–24.

Yeh GC, et al. *J Biol Chem* 2001; in press.

Laboratory of Biochemistry

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The Laboratory of Biochemistry has a long tradition of commitment to basic research. The common goal of 11 small independent groups with diverse expertise in biochemistry, molecular and cell biology, developmental biology, genetics, microbiology, immunology, and structural biology is to solve basic mechanisms of cellular regulation. The success of this multidisciplinary approach is achieved by a sustained policy to recruit and support outstanding young independent investigators who continuously bring to the laboratory new ideas and new techniques. Sriram Subramaniam has joined the laboratory to head a newly created Biophysics Section, whose focus will be the elucidation of the structure of membrane proteins.

The presence within one laboratory of many talented investigators with overlapping interests and complementary expertise and the advice of two senior members of the laboratory, Maxine Singer and Edward Kuff, provide a stimulating environment well suited for the training of postdoctoral fellows. The laboratory is also eager to host outstanding postdoctoral fellows interested in developing their own research programs with the support of NCI scholarships.

The laboratory shares with the Laboratory of Molecular Cell Biology state-of-the-art facilities including protein and DNA sequencers, a FACS, a spectropolarimeter, a spectrofluorometer, an atomic absorption spectrometer, an analytical ultracentrifuge, a mass spectrometer, two silicon graphics workstations, and a *Drosophila* genetics facility equipped with a confocal microscope.

Laboratory of Biochemistry Staff

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May Liu	Support Staff
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Lisa Wheeler	Technician

Laboratory of Biochemistry Staff (continued)

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Control of DNA Replication

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DNA Recombination in Yeast

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Lea Jessop	Postdoctoral Fellow
Ruajie Sha	Postdoctoral Fellow
Robert Shroff	Postdoctoral Fellow
Tzu Chen Wu	Technician

Protein Folding

Yawen Bai	Tenure Track Investigator
Wuhong Pei	Postdoctoral Fellow
Jiro Takei	Special Volunteer
Ngoc-Diep Vu	Staff Scientist

Transcriptional Regulation of Development

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Xiaolin Bi	Postdoctoral Fellow
Zoya Demidenko	Postdoctoral Fellow
Tamara Jones	Technician
Xiang Yang	Postdoctoral Fellow

Cell Cycle Regulation

Barbara Thomas	Tenure Track Investigator
Jing Chen	Postdoctoral Fellow
Xiang Gao	Postdoctoral Fellow
Miho Tanaka-Matakatsu	Special Volunteer

Regulation of Apoptosis

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Anna DiPietrantonio	Postdoctoral Fellow
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Biography: *Dr. Klee came to the NIH in 1959 after receiving her M.D. from the Faculty of Medicine, Marseille, France. In 1974 she joined the Laboratory of Biochemistry, NCI, and became chief of the laboratory in 1989. Her research interests are focused on the role of protein-protein interactions in the regulation of enzyme activity.*

Laboratory of Biochemistry
Stimulus Response Coupling Mediated by Ca^{2+} and Calmodulin

Keywords:
calcineurin
calcium
calmodulin
immunosuppression
iron
oxidation
protein phosphatases
superoxide dismutase

Research: Stimulus response coupling mediated by changes in intracellular Ca^{2+} involves the participation of a family of structurally related subclass of Ca^{2+}-binding proteins, which act as sensors and modulators of Ca^{2+} transients. These proteins undergo conformational changes upon binding of Ca^{2+} that enable them to interact with and activate target enzymes. Calmodulin is a unique member of this subclass of proteins because of its ability to activate a large number of target proteins. The activation of the calmodulin-stimulated protein phosphatase, calcineurin, by calmodulin is used as a model system to elucidate the mechanism of action of calmodulin. The important and diverse roles of calcineurin in T cell activation, muscle hypertrophy, and long-term memory emphasize the need for a better understanding of the complex regulation of this enzyme by two structurally similar but functionally distinct Ca^{2+} regulatory proteins, calmodulin and calcineurin B. Calcineurin reconstituted from its two subunits expressed in *E. coli* is used to crystallize the calmodulin/calcineurin complex to identify the structure of the activated form of the enzyme. Reconstitution of calcineurin with calcineurin B mutants deficient in Ca^{2+} binding at any one of the four Ca^{2+} sites is being used to elucidate the role of Ca^{2+} binding to calcineurin B in the Ca^{2+} and calmodulin-dependent activation of calcineurin.

Recent experiments have revealed an additional level of complexity in the activation of calcineurin by calmodulin. In crude tissue extracts, calcineurin is subject to a time- and Ca^{2+}/calmodulin-dependent reversible inactivation facilitated by small, heat stable inactivators. A factor that prevents the inactivation of calcineurin in vitro and in vivo was identified as superoxide dismutase. The inactivation is the result of oxidative damage to the Fe-Zn active center of calcineurin. The involvement of the redox state of iron in the regulation of calcineurin activity provides a mechanism to desensitize the enzyme and to couple Ca^{2+}-dependent protein dephosphorylation to the redox state of the cell. The protection of calcineurin against inactivation by superoxide dismutase adds a new aspect to the physiological roles of superoxide dismutase—namely, the many cellular processes under calcineurin control such as the regulation of the cell cycle, T cell activation,

and neuronal function. Lack of this protective effect could be associated with diseases caused by superoxide dismutase mutations as, for example, familial amyotrophic lateral sclerosis.

Collaborators on this research include Adriaan Bax, NIH; Ching Kung, University of Wisconsin–Madison; Jill Trewhella, Los Alamos National Laboratory; and Jay Zweier, Johns Hopkins University.

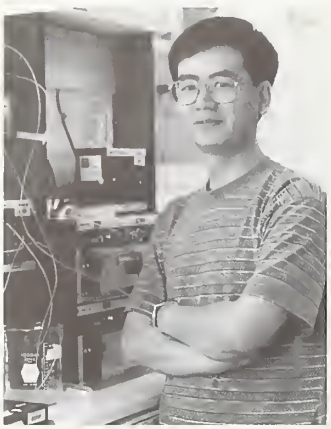
Recent Publications:

Yang SA, et al. *Biochemistry* 2000;39:16147–54.

Aramburu J, et al. *Curr Top Cell Regul* 2000;36:237–95.

Wang X, et al. *Nature* 1996;383:434–7.

Kuboniwa H, et al. *Nat Struct Biol* 1995;2:768–76.



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Biography: *Dr. Yawen Bai received his Ph.D. in biophysics from the University of Pennsylvania's medical school in 1994. He completed postdoctoral training with Dr. Peter E. Wright at the Scripps Research Institute, La Jolla, and was offered a tenure track fellowship in the Laboratory of Biochemistry in September 1997.*

Laboratory of Biochemistry
Protein Folding and Protein Design

Keywords:
protein design
protein folding

Research: To understand proteins' function and to design new proteins with new functions, it is essential to know the physical principles that control the structure, folding, stability, and dynamics of protein molecules. Our research interest is to investigate these principles and use them to solve practical problems in basic biomedical research. Currently, we are studying the mechanism of protein folding and learning to design proteins by phage display.

Protein Folding Mechanism

To learn how proteins fold, their folding processes need to be characterized, which includes intermediates and transition states. This is a difficult task because established methods for structural determination of native proteins are not applicable. So far, amide hydrogen exchange (HX) coupled with NMR has been one of the best techniques to study the structure of protein folding intermediates. This techniques allows hydrogen bond formation to be detected. Based on this technique, a native-state HX method was developed to detect folding intermediates under native equilibrium conditions a few years ago, which allows the structure and stability of the folding intermediates to be studied under equilibrium conditions. Since

then, the relationship between the native-state HX results and the kinetic folding pathways of proteins has been an interesting topic in the folding field. In the last 4 years, we have investigated the kinetic folding pathways of several proteins including cytochrome c, barnase, and a stable variant of apocyt b562 designed by a phage-display method (see below). We found that the kinetic folding behavior of these proteins has a simple relationship with their native-state HX results—that is, that the intermediates detected by the native-state HX method populate after the major kinetic barrier—the "barrier-early" hypothesis. These results are inconsistent with the folding paradigm established by the earlier studies on the folding pathway of barnase, which suggests an intermediate populates early in the folding process. In addition, we also proposed a kinetic criterion to test whether fold intermediates are on- or off-pathway. We demonstrated that the folding intermediate of cyt c and lysozyme are on-pathway intermediates. More surprisingly, we found that there are no definable nucleation sites at the rate-limiting transition state of barnase under native conditions, suggesting that barnase may fold by multiple pathways—a "new view" of protein folding. Our future work will also include investigations on the possible role of folding intermediates in protein function.

Protein Design by Phage Display

Until now, ~10,000 protein structures have been solved. However, we still do not understand the basic principles that control the uniqueness and stability of these structures. In the last several years, we have tried to test whether phage display coupled with proteolysis can be used to design proteins that fold uniquely. The design procedure involves the following steps: (1) rational design of target fold; (2) generation of multiple mutations in the core of the target protein; (3) displaying the mutants on the surface of phage; and (4) selection for stably folded proteins by challenging the protein library with protease. We have successfully applied this procedure to convert a partially unfolded four-helix bundle protein to a stably folded four-helix bundle protein. Further work will be to extend this method as an engineering tool for breeding protein molecules.

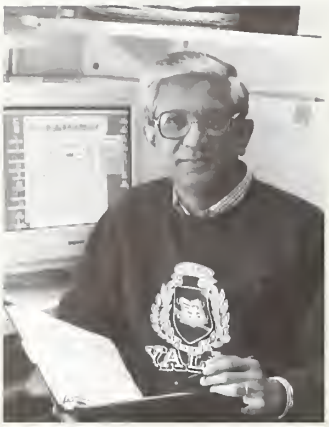
Recent Publications:

Bai Y. *Protein Sci* 2001;10:1056–66.

Takei J, et al. *Proc Natl Acad Sci USA* 2000;97:10796–801.

Bai Y. *Proc Natl Acad Sci USA* 1999;96:477–80.

Chu RA, et al. *Biochemistry* 1999;39:14119–24.



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Biography: *Dr. Chattoraj received his Ph.D. in biophysics in 1970 from the University of Calcutta, India, where he studied chromatin structure. As a postdoctoral associate, he studied nucleoprotein interactions involved in phage DNA replication and recombination at the University of Wisconsin with Dr. Ross Inman, and at the University of Oregon with Dr. Frank Stahl. His current research interest is on replication and segregation of chromosomes in the bacterial cell cycle. He has been associated with the NCI since 1980.*

Laboratory of Biochemistry
Control of DNA Replication

Keywords:
cell cycle
DNA binding proteins
DNA replication
E. coli
transcriptional control

Research: In all living organisms, DNA replication is a highly controlled process. Using primarily *E. coli* plasmids, we study features common to all replicons that ensure replication during each cell cycle but prevent a second round of replication in the same cell cycle. Our interest also includes the mechanisms that coordinate DNA replication in a multichromosome bacterium (*V. cholerae*) and that couple chromosome replication to chromosome segregation.

Activation of Replication by DNA Bending

Separating the strands of the origin of replication is a crucial step in the initiation of DNA replication. Since DNA replication is normally controlled at the stage of initiation, our premise is that steps leading to origin opening are important for controlling replication. Origin opening in plasmid P1 requires the host initiator DnaA. The protein has a set of binding sites at each end of the origin but either one of the sets suffices for origin function. However, one of the sets requires nearby binding of a DNA bending protein, IHF. In a variety of DNA transactions, IHF allows interactions between proteins bound to distant sites. We are pursuing a model where IHF is activating replication by bringing in distal DNA into close proximity to the initiation complex. The nonspecific binding of the distal DNA to initiators could suffice to stabilize the complex. A similar mechanism has been suggested for the activation of transcription by DNA bending in some cases.

Replication-Induced Transcription

We have discovered that transcription of the plasmid encoded initiator gene, *repA*, is activated by replication. The *repA* promoter maps within the RepA-binding sites in the origin, and RepA binding to them represses the promoter efficiently (autorepression). The passage of the replication fork apparently cleans the promoter of bound RepA and provides a window of opportunity for maximal *repA* expression. In contrast, autorepression was not efficiently released upon RepA titration by extra binding sites. Thus

replication seems to be a requirement to induce transcription. A similar situation seems to exist for induction of the autorepressed *dnaA* gene of *B. subtilis*.

Regulation of Replication Frequency by Origin-Pairing
It has been proposed that replication of plasmids like P1 is controlled by RepA-mediated pairing of origins that causes steric hindrance to origin activity. To address the role of pairing, we have compared copy numbers of plasmid monomer and plasmid dimer from otherwise isogenic cells. Our premise is that pairing would occur more readily when the two origins are in cis, as in a dimer, because of higher local concentration of one site in the vicinity of another than when the sites are in trans as in monomers. The dimer copy number was four-fold lower as compared to that of the monomer in support of the pairing model. This is the first physiological evidence that origin pairing can control replication. The assay developed here can be applied to any protein, such as a transcription factor, with DNA looping activity.

Coordination of DNA Replication and Chromosome Segregation
Genetic information in most bacteria is present in a single chromosome while it is distributed into two chromosomes in *V. cholerae*. In order to determine whether the replication of the two chromosomes are independently or coordinately regulated, we are trying to identify regulators that can be used to selectively switch off replication of individual chromosomes. A checkpoint control will be evident if the replication of one chromosome depends on the other. The replication switch-off experiments will also be used to understand if there is coordination between DNA replication and chromosome segregation. The two processes are generally coupled in bacteria, making *V. cholerae* ideally suited to study mitosis in a bacterium.

Recent Publications:

Park K, et al. *J Biol Chem* 1998;273:24906–11.

Chattoraj DK. *Mol Microbiol* 2000;37:467–76.

Mukhopadhyay S, et al. *Proc Natl Acad Sci USA* 2000;97:7142–7.

Park K, et al. *J Mol Biol* 2001;310:69–81.

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Biography: *Dr. Florence Davidson received her Ph.D. from the University of California–San Diego. She received postdoctoral training in the laboratory of Dr. H. Gobind Khorana at the Massachusetts Institute of Technology (MIT), studying folding and function relationships in rhodopsin. She completed her postdoctoral studies in the laboratory of Dr. Hermann Steller at MIT, studying the molecular genetics of apoptosis caused by rhodopsin-folding mutants in* Drosophila. *Dr. Davidson joined the Laboratory of Biochemistry in March 1998.*

Laboratory of Biochemistry
Neuronal Cell Death Regulation in *Drosophila*

Keywords:
aging
apoptosis
cell biology
developmental biology
disease association
Drosophila
genetics
membrane proteins
neuronal survival
neurons
programmed cell death
protein folding
rhodopsin
vision

Research: Apoptosis is a genetically programmed form of cell death required for the development of normal neural tissue. Yet, it is essential to suppress apoptosis in nonregenerating adult neurons. In these cells, inadequate suppression causes irreversible loss of neural function, as the cells are lost. We are studying neuronal survival and the regulation of apoptosis. Our primary experimental model is the activation of apoptosis in adult photoreceptor cells (PRCs) by presumptive folding mutants of the transmembrane protein rhodopsin. Our interest in this system stems from three distinct attributes. First, the terminally differentiated state of PRCs eliminates contributions of cell cycle, growth, and differentiation from the analysis of apoptotic signaling. Second, the easy accessibility of PRCs for assays of in vivo function allows analysis of the relationship between manipulation of the death program and physiological outcome. Third, the opsin mutants under study cause age-related blindness in both humans and flies. We are thus using *Drosophila melanogaster* as a genetic model organism to explore the molecular mechanism of cell death and its suppression in PRCs.

A primary focus of the lab is to determine the genes required for apoptosis and those that suppress cell death in PRCs. To this end, we are screening for dominant and recessive modifiers of the retinal degeneration phenotype brought about by dominant opsin mutations. In addition, mediators of cell death in cycling cells are being tested for their role in PRC apoptosis. These include regulators of apoptosis in *Drosophila* and mammalian neurons, neuronal precursors, and other cell types. In addition to determining the basic mechanism, we are thus determining the relationship of cell death activation and suppression in PRCs to that in other cell types. This sometimes reveals surprising relationships between gene function in developing and mature animals. For example, we have found that a *Drosophila* homolog to the tumor necrosis factor receptor-associated factor (TRAF) family of proteins plays an unexpected and essential role in both neurogenesis and myogenesis. Its action in PRC cell death suppression is under investigation.

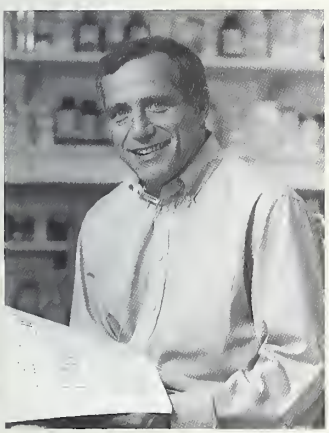
A second focus for the lab is to analyze the effect of artificial manipulation of apoptosis on physiological function, for both the cell and the whole animal. We previously found that apoptosis of PRCs induced by opsin and other retinal degeneration mutants could be blocked by eye-specific expression of the baculoviral cell survival factor p35. Surprisingly, by blocking the apoptotic program with p35, the flies were able to retain sight through most or all of their lifetimes, as assayed by electrophysiological and behavioral techniques. This was the first demonstration of preservation of physiological function by inhibition of the core cell death pathway. On the one hand, this result provides hope for the development of pharmaceutical strategies to treat degenerative diseases. On the other hand, it was particularly surprising because of previous data suggesting that opsin misfolding mutants inhibit the processing of wild-type opsin. We are pursuing this avenue of research, using cell biology and functional assays to learn more about the control of membrane protein folding, editing, and trafficking in polarized neurons. Initial studies are focused on the fate of mutant opsins when neurons are rescued from apoptosis.

Recent Publications:

Davidson FF, et al. *Proc Natl Acad Sci USA* 1994;91:4029–33.

Mollaaghababa R, et al. *Proc Natl Acad Sci USA* 1996;93:11482–6.

Davidson FF, et al. *Nature* 1998;391:587–91.



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Laboratory of Biochemistry
Genetics of Cancer Risk-Related Behaviors and Complex Traits

Keywords:
AIDS
behavior
cigarette smoking
genetics
HIV

Research: Many aspects of human personality and behavior are genetically influenced. Our laboratory has been studying the role of genes in cancer risk-related behaviors such as cigarette smoking, the major preventable cause of cancer in the United States, and other complex human traits.

Behavioral genetic studies have shown that cigarette smoking is 53 percent heritable and that there are different genes for starting and continuing to smoke. Some of these genes may directly affect nicotine sensitivity whereas others probably act indirectly through personality traits. We are seeking to

identify such genes through DNA linkage and allelic association studies. Toward this end, we are collecting behavioral data, personality test scores, and DNA samples from a series of nuclear families in which at least one of the siblings is a heavy cigarette smoker. We are also screening a series of candidate genes, such as the neuronal nicotine receptor loci, for functional DNA sequence polymorphisms. Lastly we are developing statistical methods to identify significant gene-environment-phenotype associations and interactions within our dataset.

To date, two potentially relevant associations have been discovered. First, a coding sequence polymorphism in the dopamine D4 receptor gene has been linked to the temperamental trait of novelty seeking, which is related to sensation-seeking behaviors including the initiation of cigarette smoking. Second, an upstream regulatory region polymorphism in the serotonin transporter gene has been associated with anxiety-related traits that contribute to the persistence of addictive behaviors. Our long-term aim in understanding the interaction between genes, environment, and cancer risk factors is to develop better methods for behavioral intervention.

The laboratory also studies the role of genes in personality and sexual orientation and recently has initiated a program on HIV/AIDS. The aim is to use molecular biology to develop novel therapeutic agents. The availability of potent antiretroviral regimens has focused attention on the need for strategies that target HIV latency or regenerate the immune system. Current projects include immunotoxins, dominant-negative mutants, and nucleic acid mimetics.

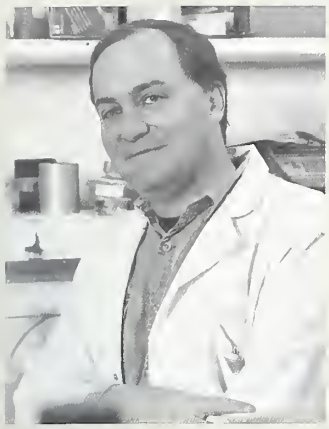
Recent Publications:

Lueders KK, et al. *Mamm Genome* 1999;10:900–5.

Hu S, et al. *Mol Psychiatry* 2000;5:181–8.

Greenberg BD, et al. *Am J Med Genet* 2000;96:202–16.

Myakishev MV, et al. *Genome Res* 2001;11:163–9.



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Biography: *Dr. Lichten is a research microbiologist in the Microbial Genetics and Biochemistry Section of this laboratory. He received his Ph.D. in 1982 from the Massachusetts Institute of Technology and received his research training with Dr. Maurice S. Fox and Dr. James E. Haber. He joined this laboratory as a senior staff fellow in 1987, became a senior investigator in 1995, and became a member of the Senior Biomedical Research Service in 2000.*

Laboratory of Biochemistry
Mechanism and Control of Meiotic Recombination

Keywords:
cell cycle control
chromatin structure
chromosome alterations
DNA repair
higher order chromatin structure
meiosis
microarray
recombination

Research: We study genetic recombination and chromosome structural changes that occur during meiosis using the yeast *S. cerevisiae* as a model system. Our goal is a description of the molecular steps of meiotic recombination from start to finish, as well as the changes in chromosome structure that occur as homologous chromosomes pair and recombine during meiosis.

Meiotic recombination in *S. cerevisiae* is initiated by double-strand DNA breaks (DSBs), formed by the type 2 topoisomerase paralog Spo11p. Recent identification of Spo11 homologs in *C. elegans*, *Drosophila*, and mammals makes it likely that DSBs initiate meiotic recombination in all eukaryotes. We are interested in factors that control where and when DSBs occur. We have shown that chromatin structure determines DSBs' sites, since DSBs occur at chromatin nuclease hypersensitive sites, and changes in chromatin structure cause parallel changes in DSB. However, chromatin structure is not the only factor that determines where breaks form. DSBs occur nonuniformly along yeast chromosomes, being concentrated in 50 to 100 kb regions and absent from intervening regions of similar size. We have shown that these "hot" and "cold" regions are imposed independently of primary sequence or chromatin accessibility and that the following features of chromosome structure determine where DSBs form:

- **Centromeres**—Removing a centromere from its normal location and inserting it elsewhere on the same chromosome causes a substantial increase in DSBs in a ~30 kb region adjacent to the old centromere location, and a marked decrease in DSBs near the new centromere location. The results indicate that yeast centromeres actively repress DSBs in their vicinity.
- **DNA replication**—Deleting active replication origins from an arm of a chromosome causes a substantial delay in the time of both replication and DSB formation in that chromosome arm in wild-type cells, and a severe loss of breaks in mutant strains (rad50S or sae2) that are unable to process or repair DSBs. Similar results are seen in the vicinity of telomeres. We conclude that replication potentiates DSB formation in a segment-autonomous manner,

and that mechanisms exist to prevent DSB formation in late-replicating regions in rad50S/sae2 cells.

During meiosis, DSB formation is preceded by chromatin structural changes that occur specifically at active DSB sites. These chromatin changes are thought to reflect the binding of multiprotein complexes that perform the biochemistry of DSB formation. Circumstances that delay replication in a region (see above) cause a corresponding delay in this chromatin transition, suggesting that DNA synthesis in a region is required to potentiate the binding of proteins that form DSBs. We are currently using combined chromatin immunoprecipitation and microarray approaches to identify the proteins that bind to active DSB sites, both on a regional and on a whole-genome level. A similar approach is being used to identify the chromatin structure changes that occur and recombination proteins that bind in the vicinity of breaks during the process of DSB repair.

The Holliday junction is thought to be a central intermediate in the recombination events that occur during DSB repair. This four-stranded DNA structure is lost during standard extraction protocols due to branch migration. To characterize recombination intermediates that contain Holliday junctions, we developed an extraction protocol that restrains branch migration, thus preserving meiotic recombination intermediates containing four-way junctions. We combined this protocol with a sensitive assay to detect heteroduplex DNA in the following experiments.

- **Structure of meiotic recombination intermediates**—A central intermediate in current recombination models contains two Holliday junctions flanking a region of heteroduplex DNA. We showed that double-junction molecules form during meiosis, and that these intermediates contain heteroduplex DNA.

- **Differential timing and regulation of different types of meiotic recombination**—Unitary models of meiotic recombination postulate that a central intermediate containing Holliday junctions is resolved to generate either noncrossover or crossover recombinants, both of which contain heteroduplex DNA. Contrary to this expectation, we find that, during meiosis, noncrossover heteroduplex products are formed at the same time as Holliday junction intermediates. Crossovers appear later, when these intermediates are resolved. Furthermore, noncrossover and crossover recombination are regulated differently. ndt80 mutants arrest in meiosis with unresolved Holliday junction intermediates and very few crossovers, while noncrossover heteroduplex products are formed at normal levels and with normal timing. These results suggest that crossovers are formed by resolution of Holliday junction intermediates, while most noncrossover recombinants arise by a different, earlier pathway.

We are examining the relationship between meiotic recombination and chromosome pairing by measuring recombination between dispersed homologous sequences (ectopic recombination). Ectopic recombination between sequences on heterologous chromosomes is less frequent than ectopic recombination between sequences on homologous chromosomes, and ectopic recombination between dispersed sequences on homologs decreases as a function of distance. These results imply that, on average, meiotic

recombination occurs when homologous chromosomes are paired in end-to-end alignment, and that homolog pairing either promotes allelic recombi-nation or restricts ectopic recombination. We tested these suggestions by examining ectopic recombination in two circumstances in which normal interhomolog relationships are disrupted. In the first, one member of a homolog pair was replaced by a homologous chromosome that had diverged sufficiently to prevent interhomolog recombination. In the second, ndj1 mutants were used to delay homolog pairing and synapsis. A substantial increase in the frequency of ectopic recombination between homologous inserts on heterologous chromosomes was observed in both cases. These findings suggest that, during normal yeast meiosis, progressive homolog colocalization, alignment, and synapsis restrict the ability of ectopically located homologous sequences to find each other and recombine. In the absence of such restrictions, the meiotic homology search may encompass the entire genome.

Collaborators on this project include Alastair Goldman, University of Sheffield; James Haber, Brandeis University; Thomas Petes, University of North Carolina; and Kunihiro Ohta, RIKEN, Japan.

Recent Publications:

Borde V, et al. *Science* 2001; in press.

Goldman ASH, et al. *Proc Natl Acad Sci USA* 2000;97:9537–42.

Allers T, et al. *Mol Cell* 2001;8:225–31.

Allers T, et al. *Cell* 2001;106:47–57.

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Biography: *Dr. Mortin earned his Ph.D. from Indiana University working with Thomas Kaufman, where he studied the function of structural components of RNA polymerase II and their interactions with transcription factors using* Drosophila *development as a model system.*

Laboratory of Biochemistry
Transcriptional Regulation of Development

Keywords:
apc
armadillo/ß-catenin
development
genetics
RNA polymerase II
transcription
wingless/WNT signaling

Research: We are interested in transcriptional regulation of development. We use the fruit fly, *Drosophila melanogaster*, as a model system, which allows us to combine classical and molecular genetic techniques with a biochemical approach to investigate regulatory mechanisms. Our research group has identified a novel regulatory step controlling the functioning of the homeodomain transcription factor, prospero. We have identified two nuclear export signals in the DNA binding domain. The subcellular localization of prospero protein is dependent upon cell type, with it residing in the cytoplasm of neuroblast stem cells but residing in the nucleus of their ganglion mother cell progeny, which are committed to drop out of the cell cycle and differentiate.

Current and Future Plans

We have used our initial mutant prospero allele to demonstrate that the C terminus of prospero regulates its own subcellular localization in vivo. A number of questions were raised by this initial study:

- What is the smallest region that can function as a nuclear export signal (NES)?
- How does the masking region function and how is it regulated?
- What accessory factors help regulate subcellular localization?

We are the first to notice NES regulation of the subcellular localization of prospero and are in an ideal position to follow up our initial observations. Regulation of the subcellular localization of transcription factors is a key control step in regulating the function of RNA polymerase II.

- We have generated fusion proteins between regions of the C terminus of prospero and green fluorescent protein (GFP). The fusion proteins were expressed in mammalian CV–1 cells, and resulted in a number of observations: (1) the DNA binding homeodomain of prospero contains two separable nuclear export signals of 29 (HDA) and 36 amino acids (HDB) in length; (2) the HDA export signal functions via the exportin pathway as it is inhibited by leptomycin B; and (3) the sequence of this NES does not match that of a canical exportin NES; however, there are three absolutely conserved

Leucine residues and a phenylalinine, which, when mutated, abrogate nuclear export.

- We have reconstituted NES-masking function by fusion of the entire C terminal 163 amino acids of prospero to the C terminus of GFP. Mutation of this fusion protein has allowed us to elucidate a number of properties of the mask: (1) the entire 163 amino acids of the C terminus of prospero are required for masking function, which is dominant over the two nuclear export signals. Deletion of approximately 30 amino acids from any portion of this region abrogates masking function; (2) we predict that the tertiary structure of the intact hemeo/prospero domain is required for masking function as either of two double amino acid substitutions abrogates mask function. These mutations were selected because they are predicted to disrupt helical regions either between the export signals and the prospero domain or within the prospero domain itself.

- The masking region might work by directly contacting the NES in an intramolecular interaction. Alternatively, the masking region might recruit an additional factor(s) to perform its function. To test the first hypothesis and to identify proteins required for the nuclear export of prospero, we are using a yeast two-hybrid expression system to isolate proteins that directly interact with the NESs and the NES-mask. We are using either the homeodomain alone or the homeo/prospero domain as bait to screen a *Drosophila* expression library in yeast. To date we have identified approximately 150 positive clones using the homeodomain as bait. Sequence analysis of the first seven clones demonstrates that they represent two genes. The first is a *Drosophila* gene with high-sequence homology to yeast and human genes but with no known function. We are currently characterizing the remaining clones.

Recent Publications:

Avedisov S, et al. *Mol Cell Biol* 2000;20:8220–9.

Yang X, et al. *Development* 2000;127:3695–702.

Dekel I, et al. *FEBS Lett* 2000;472:99–104.

Dernidenko Z, et al. *Development* 2001;128:1359–67.

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Biography: *Dr. Paterson received his Ph.D. in zoology in 1971 from the University of California–Berkeley with Dr. R. Strohman, studying muscle-specific gene expression during myogenesis in vitro. He continued this work at the Weizmann Institute of Science in Rehovot, Israel, with Dr. D. Yaffe, from 1971 to 1974, followed by an additional year with Dr. J. Bishop at the University of Edinburgh, Scotland, studying mRNA complexity changes during myogenesis. He has been in the Laboratory of Biochemistry since 1975.*

Laboratory of Biochemistry
Molecular Studies on Eukaryotic Gene Regulation

Keywords:
cell cycle
MyoD
myogenesis
terminal differentiation

Research: Our research focus is on the determination, proliferation, and differentiation of muscle cells during development in both vertebrates and *Drosophila*. This process of myogenesis depends upon the complex regulated expression, dimerization, and function of the MyoD family of basic helix-loop-helix proteins. In the vertebrates, the MyoD-related muscle gene regulatory proteins, or MRFs, were thought to directly autoregulate their expression levels through MRF-binding sites in the promoters of these genes. However, we have demonstrated that this pathway is indirect, at least for avian MyoD (CMD1), and does not involve any presently known muscle-specific transcription factors.

In *Drosophila* we have shown that the single MyoD gene, *nau*, is also not autoregulated directly and expression depends upon an earlier mesodermal transcription factor, DMEF2. The single DMEF2 gene encodes a MADS domain containing SRF homolog essential for muscle formation in *Drosophila*. DMEF2 is expressed just after the onset of *twist* expression, the key mesodermal determination gene in flies. Our studies indicate that, in addition to DMEF2, *twist* regulates *nau* to a lesser extent, suggesting *twist* is epistatic to *nau* and is a possible regulator of *nau* during embryogenesis at the earliest point of mesoderm formation.

The MyoD family of proteins are phosphoproteins. A single serine residue outside the HLH domain in the carboxyterminal portion of vertebrate MyoD regulates the formation of the homodimer in vitro and in vivo. This site and its function are conserved in *Drosophila* MyoD (*nau*). Phosphorylation of this site also regulates a transcriptional activation domain and may be responsive to signal transduction pathways that modulate MyoD function during myogenesis.

Recently, we have shown that gene function in *Drosophila* can be determined by the direct injection or transfection of the corresponding double-stranded mRNA either into the embryo or onto S2 cells, respectively. The dRNA directs the targeted degradation of the cognate mRNA in a process known as RNA interference (RNAi). Using RNAi, we demonstrated

the MyoD homolog *nautilus* was essential for embryonic muscle formation and the *daughterless*-dependent conversion of S2 cells into a myogenic phenotype. RNAi is being used to study gene function during *Drosophila* myogenesis. We are also currently studying the mechanism of RNAi in *Drosophila*.

We have demonstrated the role of nonconserved hydrophilic amino acid groups in the HLH domain of the MRFs and their importance in dimerization specificity among the MyoD/E-protein family members by using a newly developed competitive EMSA. Information from this work was used to establish the heterodimer-dependent myogenic conversion of nonmuscle cells from both vertebrates and *Drosophila* and predicted the ultimately successful rescue of a MyoD lethal mutation in *C. elegans* with *Drosophila* MyoD.

MyoD is not only a tissue-specific transcription factor but also appears to play a role in the decision to exit the cell cycle during differentiation. New experiments have established that MyoD can bind to and regulate cyclin-dependent kinase 4 (cdk4) by inhibiting the cdk4 kinase directly as well as the DNA-binding activity of MyoD. Nuclear localization of cdk4 is dependent upon cyclin-D_1. The fragment of MyoD containing the cdk4-binding site can itself inhibit DNA replication and cell proliferation. Thus, cyclin D_1 modulates the nuclear levels of cdk4 in response to mitogens to regulate MyoD function and terminal differentiation of the myoblast.

Recent Publications:

Misquitta L, et al. *Proc Natl Acad Sci USA* 1999;96:1451–6.

Zhang J, et al. *EMBO J* 1999;18:926–33.

Zhang J, et al. *EMBO J* 2000;18:6983–93.

Wei Q, et al. *Dev Biol* 2000;228:239–55.

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Biography: *Dr. Subramaniam obtained his Ph.D. in physical chemistry from Stanford University in 1987 and carried out postdoctoral work in the departments of chemistry and biology at the Massachusetts Institute of Technology. In 1992, he joined the faculty at the Johns Hopkins University School of Medicine as an assistant professor, and was promoted to associate professor in 1998. He joined the Laboratory of Biochemistry in 1998 as chief of the Biophysics Section and continues to maintain a visiting faculty appointment with the Johns Hopkins University School of Medicine.*

Laboratory of Biochemistry
Structural Analysis of Macromolecular Assemblies by High Resolution Electron Microscopy

Keywords:
electron crystallography
electron microscopy
high-resolution electron microscopy
macromolecular complexes
membrane proteins
structural biology
tomography

Research: Our research program is focused on the analysis of three-dimensional structures of macromolecular assemblies using high resolution electron microscopy. Members of our group work on different, yet complementary, aspects of structural analysis. These include electron crystallographic studies of two-dimensional protein crystals, "single particle" approaches to analyze the structures of protein complexes, and determination of the structures of large subcellular assemblies using electron tomography. A significant fraction of our research effort is devoted to developing and implementing novel methods for specimen preparation, high throughput data acquisition, and computational analysis.

Currently, our collaborators are Suresh Ambudkar, Bernie Brooks, Manu Hegde, Richard Leapman, and Jacqueline Milne, NIH; David Blair, University of Utah; Kevin Campbell, University of Iowa; Richard Henderson and Richard Perham, Cambridge; Shahid Khan, Syracuse University; Peter Maloney, Johns Hopkins University; and Paul Mooney, Gatan Inc.

Recent Publications:

Faruqi AR, et al. *Q Rev Biophys* 2000;33:1–27.

Subramaniam S, et al. *Nature* 2000;406:653–7.

Zhang P, et al. *J Struct Biol* 2001; in press.

Heymann J, et al. *EMBO J* 2001;20:4408–13.

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Biography: *Dr. Barbara Thomas obtained her Ph.D. from the Department of Genetics and Development at the Columbia University College of Physicians and Surgeons. She received postdoctoral training in the laboratory of Dr. S. Lawrence Zipursky at the University of California–Los Angeles and joined the NCI's Laboratory of Biochemistry in 1996.*

Laboratory of Biochemistry
Cell Cycle Regulation During *Drosophila* Eye Development

Keywords:
cell cycle
cyclin kinase inhibitor
developmental biology
eye development
G1

Research: The G1 phase of the cell cycle represents a critical stage at which cells can respond to extracellular cues either to commit to another round of cell division, to withdraw temporarily from the cell cycle, or to terminally differentiate. Studies from both yeast and mammalian systems suggest that progression through G1 is regulated in response to extra- and intracellular signals that act directly on the cell cycle machinery. We are interested in exploring the mechanisms involved in regulating G1 progression in vivo and the requirement for G1 in the developmental decision to proliferate or to differentiate. To this end, we have been studying a gene, *roughex (rux)*, that is required to arrest cells in G1 in the developing compound eye of *Drosophila*.

A striking feature of development in the *Drosophila* eye is the simultaneous synchronization of cell cycle progression in G1 and the onset of pattern formation mediated by intercellular signaling molecules. The adult eye of *Drosophila* develops from a tissue called the eye imaginal disc, which is formed during embryogenesis from a small group of cells that are determined to form eye tissue. These cells proliferate steadily during the first two stages of larval growth; differentiation initiates during the third and final stage of larval development within a physical constriction in the eye disc epithelium called the morphogenetic furrow (MF). The onset of differentiation in the MF is marked by a synchronization in cell cycle progress and arrest in the G1 phase of the cell cycle. Thus, anterior to the MF, cells are undifferentiated and cycle asynchronously, while posterior to the MF, cells begin to differentiate and undergo a single, synchronous cell division. We have previously shown that rux mutants fail to arrest in G1 in the MF, and instead all cells ectopically reenter S phase. The loss of G1 leads to subsequent defects in pattern formation and cell fate determination. This suggests that G1 must be actively established and maintained during development by a pathway that requires rux, and that cell fate determination is dependent on G1 arrest. The rux locus encodes a polypeptide of 335 amino acids with an N terminal cyclin-binding motif and a C terminal bipartite NLS. Previous genetic studies indicated that Rux is required to inhibit the kinase activity associated with the G2 cyclin, Cyclin A (CycA). Molecular experiments indicate that Rux functions by

binding to and inhibiting CycA-dependent kinase activity, and may function in part by dissociating the cyclin from its kinase partner. Further, in vivo studies show that CycA protein becomes mislocalized to nucleus and is degraded when Rux is overexpressed. We are currently exploring the idea that Rux may target CycA for destruction in G1 cells. Interesting, Rux protein itself is degraded in cells that normally reenter S phase for a final wave of cell division behind the MF. S phase in higher eukaryotes is marked by the expression and activation of a cyclin complex containing the G1 cyclin, CycE. We have shown that Rux also binds to CycE, and we propose that Rux is targeted for destruction by a CycE-dependent kinase activity in cells that reenter S phase. In support of this notion, the mislocalization and subsequent degradation of CycA resulting from Rux overexpression is reversed in cells that also overexpress CycE. This effect is dependent on four consensus sites for phosphorylation by cyclin-dependent kinases present in the Rux protein and a mutant derivative of Rux lacking these sites is stabilized in S phase cells. Interestingly, the CycA-binding-defective version of Rux is also stable in S phase cells, suggesting that this site may also mediate binding to CycE.

A large-scale screen to identify new dominant suppressors of *rux* has identified a number of novel loci as well as the *Drosophila* patched (ptc) gene. Ptc functions in the Hedgehog signaling cascade, suggesting that this approach will be useful to identify genes that are required for regulating cell cycle progression in response to developmental signals. The high degree of conservation of cell cycle components between species makes it likely that many of the pathways for regulating cell cycle progression during development will be conserved during evolution.

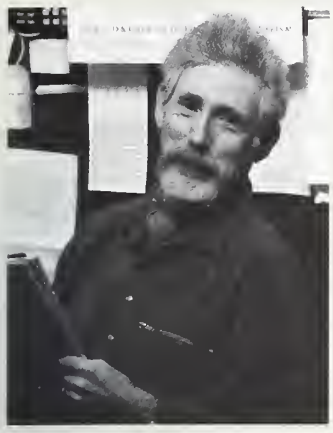
Recent Publications:

Avedisov SN, et al. *Mol Cell Biol* 2000;20:8220–9.

Thomas B, et al. *Trends Genet* 1999;15:184–90.

Thomas B, et al. *Genes Dev* 1997;11:1289–98.

Dong X, et al. *Genes Dev* 1997;11:94–105.



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Biography: *Dr. Yarmolinsky has held positions at Johns Hopkins University, from which he received his doctorate, the Centre National de la Recherche Scientifique Institute of Molecular Biology (Paris), the Frederick Cancer Research Facility, and the NIH.*

Laboratory of Biochemistry
Mechanisms of Plasmid Maintenance: Segregational Stability

Keywords:
gene silencing
partitioning
plasmid

Research: Our aim is to elucidate molecular mechanisms that control the genetic stability of entire genetic elements, ensuring their equitable partition between daughter cells. The studies have focused on mechanisms that stabilize two of the simplest of stably inherited elements, the P1 plasmid prophage in *E. coli* and the pTAR plasmid in *Agrobacterium tumefaciens*.

Silencing of Genes Flanking the P1 Plasmid Centromere

In dividing bacteria, low-copy-number plasmids, such as P1 and F, are stabilized by active partitioning, which generally requires two plasmid-encoded Par proteins and a DNA site (a centromere analog). We have shown that P1 ParB, like its F homolog, can silence genes flanking the centromere (*parS*) and several kilobases away. No comparable examples of long-range gene silencing in prokaryotes are known to us. We have been studying the mechanism of this silencing and its possible relevance to the elusive process of partitioning, the bacterial analog of mitosis.

ParB could be crosslinked in vitro with formaldehyde to both *parS* and the silenced DNA. Provision of a repressor able to bind DNA sites between *parS* and a reporter gene alleviated silencing of the reporter and diminished the associated ParB–DNA binding. We propose that ParB can polymerize along DNA flanking a centromeric nucleation site. A partitioning deficiency of silencing-defective mutant forms of ParB that retained the capacity to bind to *parS* suggests that the silenced structure is important for partitioning. This conclusion is reminiscent of evidence that the silencing of genes in the vicinity of the centromeres of chromosomes in fission yeast and in *Drosophila* appears to be essential to their functioning. Further analysis of P1 and F partition protein-mediated silencing may offer clues to the mechanism of partitioning and may assist in understanding the widespread phenomenon of gene silencing in higher organisms.

Mode of Action of the P1 Plasmid Addiction Cassette

Plasmid addiction operons serve to kill or inhibit the growth of plasmid-free (cured) segregants. Such an operon is paradoxically programmed to function *in absentia*; it is the *cessation* of transcription of the operon that

triggers an endotoxin to become active. The operational principle is illustrated by the addiction operon of P1. It encodes a small endotoxin protein (Doc) responsible for death on curing and a small, relatively unstable, antidote protein (Phd) that can prevent host death.

Potentially lethal fluctuations in the transcription of this operon are damped by an autoregulatory circuit in which both Phd and Doc participate. We have exploited the unaltered regulatory properties of a nontoxic mutant form of Doc to show that the protein dramatically enhances both the affinity of Phd for a pair of adjacent binding sites and the cooperativity of that binding. The regulatory features of the P1 addiction operon appear to be shared by other plasmid addiction operons that differ with respect to the nature of their lethal potential.

We have found that the target of Doc differs from that of other plasmid addiction toxins. Although the plasmid addiction cassette normally functions to kill cured cells, we find that Doc can be bacteriostatic. Intoxicated cells retain their normal morphology and motility. Studies of the effect of Doc on the incorporation of labeled precursors into macromolecules indicate that protein synthesis is specifically inhibited first.

Characterization of a Diminutive Par Cassette

The diminutive partition cassette of the *Agrobacterium* plasmid pTAR, claimed to encode only a single protein with similarities to ParA of P1, appears, upon resequencing of the DNA, to encode an additional small protein that we name ParB as well as a third small protein. ParA and ParB and an upstream centromeric site to which ParB binds are necessary and sufficient for efficient plasmid partitioning in pTAR's normal host. As in the case of P1 or F, transcription of the partition operon is under autogenous control, although the circuitry differs in detail.

Recent Publications:

Magnuson R, et al. *J Bacteriol* 1998;180:6342–51.

Rodionov O, et al. *Science* 1999;283:546–9.

Kalmin K, et al. *J Bacteriol* 2000;182:1889–94.

Yarmolinsky M. *Curr Opin Microbiol* 2000;3:138–43.

Laboratory of Biosystems and Cancer

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The mission of the Laboratory of Biosystems and Cancer (LBC) is to use fundamental knowledge of molecular and integrative biology to understand the molecular and environmental causes of cancer. Pursuit of both molecular and environmental causes of cancer is a synergistic approach to cancer prevention and treatment. Knowing the target genes for environmental agents allows the development of improved methods for the identification of environmental carcinogens in both laboratory studies and environmental studies. The LBC is actively involved in basic research on the identification of new cancer genes and understanding of mechanisms of action of specific genes in cell signaling, cell proliferation, and cell death. Based on advances in gene discovery, the LBC develops new models of carcinogenesis that are used to understand the cancer process in vivo and the influence of environmental agents on cancer.

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Biography: *Dr. Barrett received his B.S. in chemistry at William and Mary College, Williamsburg, Virginia in 1969 and his Ph.D. in biophysical chemistry at Johns Hopkins University, Baltimore, Maryland in 1974. Following a 3-year postdoctoral fellowship in the Division of Biophysics at Johns Hopkins University, Dr. Barrett began his career at the National Institute of Environmental Health Sciences (NIEHS), NIH, Research Triangle Park, North Carolina as leader of the Environmental Carcinogenesis Group, Laboratory of Pulmonary Pathobiology. In 1987 he became chief of the Laboratory of Molecular Carcinogenesis where he conducted research on critical target genes in carcinogenesis molecular mechanisms of environmentally induced cancers. Dr. Barrett's current research is centered on the relationship between aging and cancer, the genes involved in cellular senescence and apoptosis, and the function of KAI1, and other cancer metastasis suppressor genes. He is editor-in-chief of* Molecular Carcinogenesis, *associate editor of* Cancer Research, *and a member of editorial boards of 12 journals. He has been a chairperson, organizer, or keynote speaker at various conferences, workshops, and symposia and has authored or coauthored over 400 publications.*

Laboratory of Biosystems and Cancer
Cancer and Aging Section

Keywords:
aging
cellular senescence
dietary restriction
environmental carcinogenesis
invasion
metastasis

Research: Neoplastic development of cancers is a multistep process requiring multiple genetic changes. Significant advances have been made in the elucidation of the genes involved in genetic predisposition to cancer but less is known about the genes involved in the later stages of malignant progression. Identification of the target genes for different steps in the cancer process is important in understanding the environmental causes of cancer as well as the endogenous causes of cancer, which include spontaneous mutations, aging, and hormones. The role of aging in cancer is studied by cloning and characterizing genes involved in cellular aging. Unlike tumor cells, normal cells have a finite life span and enter a state of irreversible growth arrest, termed "cellular senescence," at the end of their life span. We have shown that cellular senescence is genetically controlled and that multiple senescence genes are altered in immortal cancer cells. Only a few senescence genes have been identified and efforts to clone new genes are actively being pursued. Studies of the regulation of these genes by environmental factors may elucidate the causes of aging and cancer.

Oxidative stress is a major form of endogenous and exogenous damage to cells. The role of oxidative stress in cell senescence and cell death (apoptosis) is under study. Interestingly, the same genes (e.g., p53 and Rb) are involved in cell senescence and cell death. A better understanding of the molecular mechanism of signal transduction leading to cell senescence or cell death through divergent pathways is required to understand cellular responses to environmental stresses, particularly oxidative damage.

As cells progress to cancer, their responses to apoptotic signals change. Cancer cells die at a higher rate than normal cells, suggesting that environmental modulators of apoptosis can influence the rate of tumor growth. One example of this is dietary restriction of animals, which reduces cancer progression by stimulating apoptosis of precancerous cells. We have shown that this is in part due to modulation by dietary restriction of circulating IGF–I levels, which blocks apoptosis of cancer cells. Further studies on the genetic controls of apoptosis may help elucidate the role of diet and other environmental factors in cancer.

Another area of active investigation is the mechanism of metastatic progression. The malignant phenotype of a cancer cell is under both positive and negative controls but little is know about the genes that control metastasis. We have recently cloned a novel metastasis suppressor gene, KAI1, which may be important in prostate, breast, lung, and possible other cancers. Further studies of this and related genes may yield important new insights into cancer diagnosis and treatment. In addition, molecular markers for the later stages of cancer progression may help define the environmental factors that influence malignant development.

Hormones are major factors in human cancers and the Cancer and Aging Section is actively involved in studying multiple aspects of hormonal carcinogenesis, including molecular alterations of hormonally associated cancers (breast, prostate, ovarian and endometrial), and the mechanisms of estrogen-induced chromosomal changes.

Recent Publications:

Yin Y, et al. *Nature* 1998;391:707–10.

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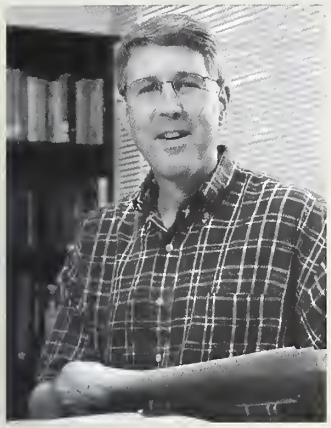

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Biography: *Dr. Hursting received his B.A.in biology from Earlham College and his M.P.H. (nutritional epidemiology) and Ph.D. (nutritional biochemistry) from the University of North Carolina at Chapel Hill. Following postdoctoral training in molecular biology and cancer prevention at the NCI, he developed a research program focused on mechanism-based cancer prevention strategies using transgenic mouse models at the University of Texas (M.D. Anderson Cancer Center). He joined the NCI in 1999 as deputy director of the Office of Preventive Oncology, Division of Cancer Prevention, and as an Adjunct Investigator in the Laboratory of Biosystems and Cancer in 2000.*

Laboratory of Biosystems and Cancer
Mechanism-Based Cancer Prevention Studies in Transgenic Mouse Models

Keywords:
caloric restriction
chemoprevention
IGF–I
nutrition
p53-deficient mice

Research: Future progress in cancer prevention research will be facilitated by the use of animal models with specific genetic susceptibilities for tumor development. The recent development of mouse strains with carcinogenesis-related genes overexpressed or inactivated provides investigators with new models for studying the carcinogenesis process and for testing preventive strategies that can offset specific and highly relevant genetic susceptibilities to cancer in humans. Our work has focused on using mice deficient in the p53-tumor suppressor gene (the most frequently altered gene in human cancer) to ask the question: Can we offset increased cancer risk due to a genetic lesion, such as loss of p53 tumor suppressor activity, by preventive (particularly nutritional) approaches?

We have reported that calorie restriction (CR; a potent inhibitor of many types of rodent tumors) significantly delays spontaneous tumor development in p53-null (–/–) mice by slowing lymphocyte cell cycle traverse and enhancing apoptosis in the lymphoma-susceptible thymocyte population. We are currently testing the hypothesis that these effects are mediated by reduced levels of insulin-like growth factor-1 and leptin, and are evaluating the molecular changes in response to CR. In addition, we have shown that several other dietary interventions (i.e., phytochemicals) and chemo-preventive agents (i.e., NSAIDs) known to suppress endogenous oxidant production and inflammation (including nitric oxide and prostaglandins) can significantly delay spontaneous tumorigenesis in these mice. These studies established that p53–/– mice provide a useful in vivo model of genetic susceptibility since tumorigenesis in these mice is spontaneous, rapid, relevant to human cancer, and responsive to experimental manipulation. Heterozygous p53-deficient (p53+/–) mice, with only one p53 allele inactivated, have some analogy to humans susceptible to heritable forms of cancer due to decreased p53 gene dosage, such as individuals with Li-Fraumeni Syndrome. While p53+/– mice have low rates of spontaneous

tumorigenesis for up to 12 months of age, we and others have reported that they display increased susceptibility to chemically-induced tumor development relative to wild-type mice. We are thus developing new models based on the induction of specific tumor types via low-dose or chronic carcinogen or hormone exposure for studying gene-environment (with emphasis on diet) interactions. We have established that p-cresidine- and 4-aminobiphenyl-induced bladder tumors, nitrosomethylurea-induced lymphomas, and azoxymethane-induced aberrant crypt foci and colon tumors, all appear significantly earlier in p53+/– mice than in similarly-treated p53+/+ mice. We have recently focused on the p-cresidine-bladder model, establishing that the cyclo-oxygenase (COX) pathway is a key prevention target, and that NSAIDs, selective Cox inhibitors, and anti-inflammatory nutrients are effective anticancer agents in the bladder. In addition, we are characterizing the hormonal and molecular changes underlying the preventive effects of CR in this model, again with a focus on the IGF–I pathway.

We have also been characterizing a rapid and spontaneous p53+/– mouse mammary tumor model developed by crossing p53+/– mice with MMTV–Wnt-1 transgenic mice. In these mice, CR, a one day/week fast, the synthetic retinoid fenretinide, the chemopreventive steroid fluasterone, and a high soy diet each delay spontaneous mammary tumor development. Mammary tumors from these mice are estrogen receptor-positive, over-express Cox-2 and β–catenin, and show reduced BRCA–1 expression, suggesting that this model, which involves alterations in two critical carcinogenesis pathways, may be highly relevant for the development of breast cancer prevention strategies.

Collaborators on these projects include Carl Barrett and Barbara Davis, NIH; John DiGiovanni and Susan Fischer, M.D. Anderson Cancer Center; Larry Donehower, Baylor College of Medicine; and Diana Haines, SAIC–Frederick.

Recent Publications:

Hursting SD, et al. *J Natl Cancer Inst* 1999;91:215–25.

Hursting SD, et al. *Mut Res* 1999;443:234–49.

Poetschke HL, et al. *Carcinogenesis* 2001;21:1959–64.

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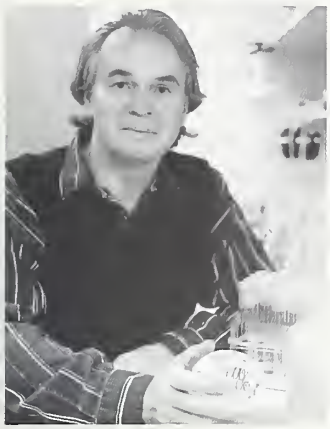

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Biography: *Dr. Larionov obtained his Ph.D. from the Institute of Experimental Medicine, the USSR Academy of Medical Sciences, Leningrad in 1977 for work on extrachromosomal elements in yeast. After postdoctoral fellowship at the Leningrad State University and Enhelhardt Institute of Molecular Biology (Moscow), he came to the Institute of Cytology, Russian Academy of Sciences, as a staff scientist. He received his D.Sc. from the Institute of Cytology for work on genetic control of chromosome transmission in 1983. One year later he became the chief of the Laboratory of Genetics in the same Institute. He moved to the Laboratory of Molecular Genetics in the National Institute of Environmental Health Sciences, NIH, as a visiting scientist in 1991. In 1997 he became the leader of the Gene Isolation Unit in NIEHS. He joined the Laboratory of Biosystems and Cancer in the NCI as a Senior Investigator in 2000. His recent studies have focused on studying the organization and function of centromeric regions of human chromosomes and on characterization of human disease genes.*

Laboratory of Biosystems and Cancer
The Organization and Function of Chromosomal Regions That Are Required for Stability of the Human Genome

Keywords:
centromere
functional genomics
genome targeting
human artificial chromosome

Research: Our work can be divided into two interrelated parts. We are studying the general organization of centromeric regions of human chromosomes. The major focus of the research being conducted in our laboratory is to determine minimal structural requirements for functional centromere and to generate a portable human artificial chromosome (HAC) for gene delivery. We are also working on isolation and characterization of human disease genes. Ongoing work is presently focused on two major areas of research.

The Organization of Human Centromere

The mammalian centromere is a specialized region of the chromosome, which controls proper chromosome segregation. Structural studies of mammalian centromeres have shown that they contain long stretches of tandemly repeated DNA sequences. These regions represent approximately 15 percent of human genome.

Despite their importance, centromeres remain poorly understood and even a new released human genome sequence does not contain information on centromeric regions. Previously it was thought that this fraction of genome lacks genes and represents a junk DNA. Recent identification of functional copies of genes within centromeric regions of plant chromosomes raises the question that human centromeres spanning several megabases may be organized similarly. The main reason why centromeres remain poorly understood is that long stretches of tandemly repeated centromere-specific DNA sequences cannot be cloned by a standard technique and they are unstable during propagation in both bacterial and yeast hosts. We developed a novel approach for isolating of centromeric regions based on specific targeting of centromeric DNA and the following rescue of the targeting region as a set of large yeast artificial chromosomes (YACs). This new cloning

strategy was applied for studying several centromeric regions including a centromeric region of a minichromosome containing ~5 Mb of the human chromosome Y. William Brown and coauthors at the University of Oxford generated this minichromosome by two rounds of telomere-directed chromosome breakage leading to a loss of sequences from both arms of the chromosome. Despite the small size and loss of a significant part of centromeric repeats (only 140 kb of alphoid DNA was left), the minichromosome segregates accurately in mitosis, suggesting that this block of alphoid DNA alone or along with the short arm flanking sequence is sufficient for a centromere function. As a first step to construct Human Artificial Chromosome (HAC) the centromeric region of the minichromosome was TAR rescued in yeast as a circular YAC and its complete sequence was determined. For further functional analysis the YAC was retrofitted into a YAC/BAC with a mammalian selectable marker. When transfected into human cells, this circular DNA construct is stably maintained at 1 to 2 copies per cell. We are in progress to investigate the HAC as a vector for megabase-size genes and as a model for studying a human kinetochore.

The Organization and Expression of Human Diseases Genes

The mammalian gene function analysis is impeded by the lack of a convenient expression system. Attempts to express human genes using cDNA expression vectors frequently result in cell cycle arrest as a result of uncontrolled expression of the genes in transfected cells. It is obvious that a genomic copy of the genes with all regulatory elements (including potential regulatory elements in intron regions) instead of surrogate cDNA constructs would be preferential for gene expression studies. However, routine large insert size cloning techniques (BAC and YAC) produce clones with random genomic fragments and the gene of interest is either available only as a set of overlapping genomic fragments that form a contig or available as a large genomic fragment carrying other genes. A new approach referred to as TAR (Transformation-Associated Recombination) cloning has recently emerged, which allows entire genes and large chromosome regions to be specifically and accurately isolated from total genomic DNA. This nonenzymatic procedure for DNA cloning is based on in vivo recombination in the yeast *S. cerevisiae*, an organism that exhibits a high level of intermolecular recombination between homologous DNAs during transformation.

Over the last 2 years we successfully applied the new technique for isolation of entire copies of several human genes including breast cancer genes, BRCA1 and BRCA2, the metastasis-suppressor gene KAI1, the telomerase reverse transcriptase gene hTERT and a paternally expressed gene Ubiq. We are in progress to investigate regulation of expression of these genes using a recently developed Human Artificial Chromosome (HAC) as a gene delivery system.

Collaborators on this research are Carl Barrett and David Schlessinger, NIH; William Brown, University of Oxford; Andy Choo, Murdoch Institute for Research into Birth Defects; Pieter deJong, Roswell Park Cancer Institute; Bob Moyzis, University of California at Irvine; Mitsuo Oshimura, Tottori University, Japan; Lisa Stubbs, Livermore National Laboratory; and Peter Warburgton, Mount Sinai School of Medicine.

Recent Publications:

Annab L, et al. *Gene* 2000;250:201–8.

Kouprina N, et al. *Curr Proto Hum Genet* 1999;1:5.17:1–21.

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Cancer and Developmental Biology Laboratory

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The interests of the Cancer and Developmental Biology Laboratory are centered on the analysis of embryonic development, particularly the mechanisms of growth control and the regulation of differentiation through growth factors, cytokines, and their receptors.

The study of how embryos grow and develop from the fertilized egg is not only of intrinsic interest but also of relevance to understanding the origins and development of a wide variety of cancers. Many of the cellular processes involved in embryonic development (such as proliferation, adhesion, migration, and differentiation) are mediated by the actions of growth factors and their receptors. In many cancers, these same growth factors and receptors are frequently abnormally expressed or even mutated to form oncogenes. Understanding the functions of these factors in development will deepen our knowledge of the biology of cancer.

The laboratory at present consists of three sections. The Mammalian Developmental Biology Section, directed by Dr. Stewart, is analyzing the functions of cytokines in early development as well as the role of genomic imprinting in embryogenesis and its requirement in regulating cell proliferation and fetal growth. Emphasis is placed on studying the functions of genes of interest in mice via genetic approaches, particularly the derivation of novel strains carrying mutated genes that have been introduced by gene targeting. The other sections are Genetics of Vertebrate Development, headed by Dr. Lewandoski, who is studying the functions of growth factors in development of the limb and central nervous system, and Cell Signaling in Vertebrate Development, headed by Dr. Yamaguchi, who is analyzing the functions of the Wnt family of genes in development.

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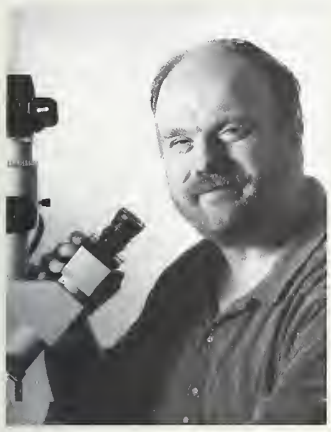

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Biography: *Dr. Stewart received his D.Phil. from the University of Oxford. He was a staff scientist at the European Molecular Biology Laboratory and a member of the Roche Institute of Molecular Biology before his current appointment as chief of the Cancer and Developmental Biology Laboratory.*

Cancer and Developmental Biology Laboratory
Cell Proliferation and Control of Growth in Mammalian Embryogenesis

Keywords:
animal models
embryonic stem cells
gene targeting
growth factors

Research: We are interested in understanding the molecular mechanisms underlying growth and cellular proliferation in the developing mammalian embryo.

Currently, we are studying two aspects of growth. The first is the function of leukemia inhibitory factor (LIF), a secreted cytokine previously shown to inhibit the differentiation of embryonic stem (ES) cells, the totipotent cells derived from the early mouse embryo. In adult mice, the principal site of LIF expression is in the uterus at the time of embryo implantation. By deriving mice deficient in LIF, we showed that this expression is essential for implantation. We are currently extending our studies on implantation and its regulation by LIF, as these will provide insights into cell-to-cell interactions between embryonic and maternal tissues and between epithelia and their mesenchyme, and how cell proliferation, both in the early embryo and in the uterus, is regulated by growth factors and the steroid hormones estrogen and progesterone.

Our second major area of interest is genomic imprinting. This is an unusual form of gene regulation, because only one parental allele of a particular gene is expressed, unlike the vast majority of genes that are expressed from both parental alleles. Among vertebrates, genomic imprinting occurs only in mammals, including humans, and it is unclear why this is so, although most of the current evidence indicates that imprinted genes are involved in regulating embryonic and postnatal growth. Genomic imprinting is essential to the normal development of mammals, since perturbing imprinted gene expression can lead to fetal lethality and has been associated with some congenital diseases in humans, including certain cancers. Our goals are to identify novel imprinted genes and to study their role in regulating fetal and tissue growth. Furthermore, imprinting is an epigenetic phenomenon, and we are also interested in determining the molecular basis of how imprinting is established during development of the germline. Much of our approach to studying both of these areas involves the generation of novel strains of mice carrying mutations in the genes of interest, using ES cells and gene-targeting strategies.

Recently, we have also started to analyze the functions of the nuclear lamins and nuclear organization in growth and disease. The nuclear lamins are intermediate filament proteins that regulate nuclear structure and chromatin organization. Mutations in the A type lamins are associated with the development of three congenital diseases, the autosomal dominant form of Emery-Dreifuss muscular dystrophy, dilated cardiomyopathy, and familial partial lipodystrophy. We are deriving mice carrying some of these mutations to understand how nuclear organization relates to cell function in development and disease processes.

Our collaborators on these studies include Brian Burke, University of Calgary; and Rachel Wevrick, University of Alberta.

Recent Publications:

Gérard M, et al. *Nat Genet* 1999;23:199–202.

Sullivan T, et al. *J Cell Biol* 1999;147:913–20.

Piras G, et al. *Mol Cell Biol* 2000;20:3308–15.

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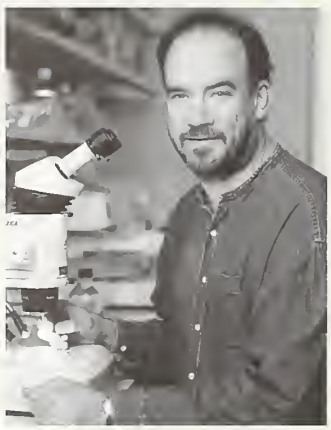

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Biography: *Dr. Lewandoski received his Ph.D. in microbiology from the New York University Medical Center in 1988. After completing postdoctoral research as an American Cancer Society Fellow under Dr. Gail R. Martin at the University of California–San Francisco (UCSF), he continued his work as a research anatomist in the Anatomy Department at UCSF. In 1999, Dr. Lewandoski established the Genetics of Vertebrate Development Section.*

Cancer and Developmental Biology Laboratory
The Role of Fibroblast Growth Factor Signaling During Mouse Development

Keywords:
Cre recombinase
development
eye
fibroblast growth factor
Flp recombinase
Gbx2 gene knockout
limb
neurogenesis
Rx

Research: To a certain degree, cancer can be thought of as disorganized growth. Our section is interested in understanding the molecules that sustain this growth by discerning their normal function during embryogenesis, which can be thought of as the opposite of cancer—organized growth. We mainly focus on the fibroblast growth factor (FGF) signaling pathway, using the mouse as a model system. In the mouse and human, 21 *Fgfs* genes have been classified thus far, based on conserved amino acid sequences encoding a domain necessary for receptor-ligand interactions. In the embryo, *Fgfs* are expressed in a variety of regions that may be termed "organizers," as defined in the pioneering work of Spemann and Mangold. An organizer is defined as a region of the embryo that is a source of signals that pattern and thus

"organize" the surrounding tissue. In order to maximize data from loss-of-function studies, Dr. Lewandoski helped devise the "allelogenic" strategy, along with Dr. Gail Martin (UCSF), in which an allelic series of mutations at a given locus is generated by producing only one targeted mouse line. This approach relies on altering the locus by strategically inserting the recognition sites for different site-specific DNA recombinases (Cre and Flp) such that various alleles can be generated by breeding the targeted mouse line to different Flp and Cre transgenic mice. Thus far the allelic series that we generated at the *Fgf8* locus has revealed a role for *Fgf8* during gastrulation, limb, and brain development.

One of the intriguing insights that has emerged from these studies is that at different stages of embryogenesis, FGF8 signaling plays different roles in cell migration, proliferation, patterning, and survival. How is this diversity of response achieved? To answer this question, our section is using several approaches to identify and study downstream targets of FGF signaling. Among the approaches we are using are various gene-trap strategies, which provide tagged insertional mutagenesis and microarray analysis. The logic that we are applying in adapting these mutagenesis strategies to isolate genes that lie specifically in the FGF signaling pathway can potentially be applied to any genetic pathway. The allelogenic strategy will be utilized in our analysis of the different genes that we isolate from these screens.

One target gene of FGF signaling is the homeobox gene *Gbx2*. *Gbx2* is expressed in all three germ layers of the late gastrula/early neurula stage of embryogenesis. However, in *Fgf8* null homozygotes (which fail to express another *Fgf* gene, *Fgf4*), *Gbx2* is not expressed, demonstrating that *Gbx2* is a downstream target gene of *Fgf8*, *Fgf4*, or both. Inactivation of the *Gbx2* gene has revealed that it is required for normal mid- hindbrain development, playing a role in the positioning of the "isthmic organizer," a region that is a source of signals that pattern these regions of the brain. We are currently extending this analysis by studying a hypomorphic (partial loss of function) *Gbx2* allele. We are also performing Cre-mediated tissue-specific inactivations of *Gbx2* to determine exactly where and when this gene is required for normal brain development.

Our collaborators include Peter Mathers, Ph.D., West Virginia University; and Wolfgang Wurst, Ph.D., GSF–Research Center, Institute for Mammalian Genetics and Max Planck Institute of Psychiatry Clinical Neurogenetics, Germany.

Recent Publications:

Meyers EN, et al. *Nat Genet* 1998;18:136–41.

Sun X, et al. *Genes Dev* 1999;13;1834–46.

Sun X, et al. *Nat Genet* 2000;25:83–6.

Lewandoski M, et al. *Nat Genet* 2000;26:460–3.

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Biography: *Dr. Yamaguchi received his Ph.D. from the University of Toronto where, with Dr. Janet Rossant, he studied the function of several growth factor receptor tyrosine kinases during mouse development. He was an International Human Frontier Science Program Fellow and Medical Research Council of Canada Fellow while completing postdoctoral research with Dr. Andrew P. McMahon at Harvard University. Dr. Yamaguchi joined the Cancer and Developmental Biology Laboratory in 2000, where he has established the Cell Signaling in Vertebrate Development Section.*

Cancer and Developmental Biology Laboratory
The Role of Wnts in Vertebrate Development and Cancer

Keywords:
armadillo/ß-catenin
ß-catenin
cell proliferation
cell signaling
development
differentiation
embryonic stem cells
gastrulation
gene targeting
growth factor
mouse
polarity
signaling molecules
stem cells
transgenic
wingless/WNT signaling
Wnt signaling

Research: In a developing multicellular organism, gradients of secreted signaling molecules coordinate growth and patterning along the body axes leading to the formation of specialized structures in precise locations. One such family of secreted signaling molecules, encoded by the *Wnt* genes, plays pivotal roles during both embryogenesis and tumorigenesis. Two different classes of Wnts have been identified, based on their activity in axis duplication and transformation assays. The primary focus of our research is to understand the molecular mechanisms by which Wnts regulate outgrowth and patterning during gastrulation.

All the cells of the embryo proper arise from a sheet of pluripotent cells termed the epiblast. Gastrulation converts the epiblast into the three primary germ layers: the ectoderm, from which the skin and nervous system arise; the mesoderm, which generates the skeletal system and internal organs; and the endoderm, which gives rise to the gut and associated organs. During his postdoctoral tenure, it became clear to Dr. Yamaguchi that members of both classes of *Wnts* were coexpressed during gastrulation in patterns that suggested roles in germ layer formation and patterning. Targeted mutations generated in representatives of both classes of Wnts revealed that although *Wnt8* is not essential, *Wnt5a* is required for proper gastrulation. Defects in the outgrowth of the embryonic trunk and tail are observed, and similar phenotypes are observed during the outgrowth of the face, tongue, limbs, external ear, external genitalia, and the gastrointestinal tract. The abnormal morphologies observed in these disparate structures bear a striking resemblance to each other, suggesting that *Wnt5a* plays a fundamental role in regulating tissue morphogenesis. Using a combination of embryological techniques (e.g., lineage tracing, transplantation, whole embryo electroporation and culture) coupled with molecular and cellular approaches, our current studies are directed towards understanding how *Wnt5a* may regulate stem cell proliferation. A second line of investigation is aimed at understanding the potential role of *Wnt5a* in regulating the cell-cell interactions that

lead to tissue polarity and convergent-extension movements during gastrulation. We are taking both proteomics and microarray approaches to identify new targets of the *Wnt5a* pathway.

In contrast to the *Wnt5a* mutants, embryos lacking *Wnt3a* completely lack posterior trunk and tail somites, forming ectopic neural structures at the expense of mesoderm. This observation suggests that *Wnt3a* regulates cell fate, acting as a switch between mesoderm and neural cell fates. *T* is a classic mouse mutation that, when homozygous, generates a paraxial mesoderm phenotype similar to the *Wnt3a* mutant phenotype. *T* disrupts the *Brachyury* gene, which encodes a highly conserved transcription factor expressed during gastrulation. We have shown that *Brachyury* is a direct transcriptional target of the *Wnt3a* but not the *Wnt5a* signaling pathway during the specification of mesoderm fates. Although both *Wnt3a* and *Wnt5a* are coexpressed during gastrulation, they clearly have unique functions. This begs the question of how Wnt signaling specificity is achieved. In this regard, we have identified a number of genes that may function downstream of Wnts to select specific signal transduction pathways. We are currently assessing their roles in these processes.

The transcriptional coactivator β-catenin is the primary effector of the canonical Wnt signaling pathway. Mutations in several components of the Wnt/β-catenin pathway have identified both oncogenes and tumor suppressors in this pathway that lead, in particular, to colorectal cancer. Embryos lacking *Wnt5a* display severe growth defects in the developing gastrointestinal tract. We are investigating whether aberrant activation of *Wnt5a* could lead to colorectal tumorigenesis. We are also assessing potential genetic interactions between *Wnt5a* and current mouse models of colorectal cancer. Ultimately, we hope to understand the role that Wnts play in the biology of intestinal stem cells.

Recent Publications:

Ciruna BG, et al. *Development* 1997;124:2829–41.

Yamaguchi TP, et al. *Genes Dev* 1999;13:3185–90.

Yamaguchi TP, et al. *Development* 1999;126:1211–23.

Yamaguchi TP. *Curr Biol* 2001;11:R713–24.

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The Laboratory of Cell Biology studies the processing transport and metabolism of proteins and small molecules. Seven principal investigators expert in molecular biology, genetics, biochemistry, structural biology, and cellular regulation of cell growth and metabolism, resistance to anticancer drugs, antigen processing, and pigment formation work with approximately 45 students and postdoctoral fellows on research projects related to regulation of cell growth and metabolism, the structure of membrane proteins, proteases and protein complexes, resistance to anticancer drugs, and pigment formation. The laboratory has four sections: the Molecular Cell Genetics Section has units focusing on the molecular basis of anticancer drug resistance and the biochemistry of energy-dependent transporters; the Pigment Cell Biology Section studies mechanisms involved in regulation of melanin synthesis; the Chemical Immunology Section studies antigen presentation and the role of p53 in cell cycle regulation; the Biochemistry of Proteins Section studies the role of AT-dependent proteolysis in multiple cellular processes. Two units work on x-ray and EM crystallography of proteins and protein complexes. Joint journal clubs, data presentations, and research seminars ensure sharing of expertise and extensive collaborations among members of this laboratory.

Current work in the laboratory is based on significant recent contributions it has made regarding important cellular processes involved in development, prevention, or treatment of cancer. For example, the multidrug transporter, an energy-dependent efflux pump for many different cytotoxic chemotherapeutic drugs that contributes to drug resistance of approximately half of all human cancers, was cloned and characterized in the laboratory. Two bacterial ATP-dependent proteases, LON and CLP, which have mammalian mitochondrial homologs, have been characterized and shown to share structural and catalytic features with mammalian proteosomes that are responsible for energy-dependent regulation of proteolysis in mammalian cells. The alternate metabolic pathways involved in melanin biosynthesis, a natural pigment protectant against UV-related DNA damage, have been defined. The structural requirements for binding of peptides to class I and class II major histocompatibility complex (MHC) molecules have been determined, and the information is being applied to the production of anticancer vaccines.

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Jan Rozycki	Postdoctoral Fellow

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Mario Bornia	Research Fellow

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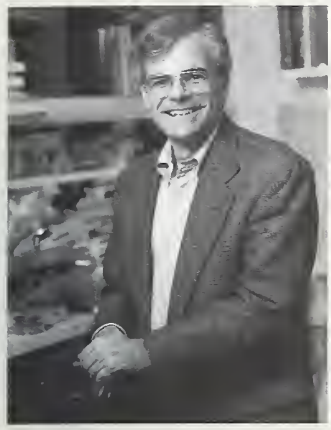

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Biography: *Dr. Gottesman obtained his M.D. from Harvard University Medical School, completed his internship and residency in medicine at the Peter Bent Brigham Hospital in Boston, and received his postdoctoral research training in molecular genetics with Martin Gellert at the NIH. After a year as an assistant professor in the Department of Anatomy at Harvard, he moved to the NIH in 1976. He currently serves as the NIH deputy director for intramural research as well as chief of the Laboratory of Cell Biology.*

Laboratory of Cell Biology
Analysis of Multidrug Resistance in Cancer

Keywords:
gene therapy
multidrug resistance
multidrug transporter
P-glycoprotein
vectors

Research: Success in treatment of some disseminated cancers with chemotherapy has led to intensified efforts to understand why many other cancers are intrinsically resistant to anticancer drugs or become resistant to chemotherapy after many rounds of treatment. Work in the Molecular Cell Genetics Section has revealed that a major mechanism of resistance of cancer cells to natural product anticancer drugs such as Adriamycin, etoposide, vinblastine, actinomycin D, and Taxol is expression of an energy-dependent drug efflux pump, termed "P-glycoprotein" (P-gp), or the multidrug transporter. This pump system contributes to drug resistance in about 50 percent of human cancers by preventing accumulation of powerful anticancer drugs in cancer cells. The sequence of the multidrug resistance (MDR1) cDNA determined in our laboratory has led to a model of the transporter as a pump with 12 transmembrane domains and 2 ATP sites; determination of the domains of P-gp responsible for substrate binding and coupling of ATPase activity to substrate transport are major goals of our work. Recent studies using affinity analogs of substrates of P-gp have identified regions around the fifth and sixth and eleventh and twelfth transmembrane domains as major drug interaction sites. Site-specific mutagenesis of these sites has confirmed that many different mutations within these regions alter substrate specificity of the transporter. Current studies are directed towards defining in more molecular detail how the various substrate interaction sites cooperate with each other, and how they stimulate the drug-dependent ATPase activity of P-gp. Model systems used for this analysis include expression in the yeast *S. cerevisiae*, MDR1 baculovirus expression vectors, retroviral expression systems, vaccinia vectors, and conditional expression systems such as a tet-off expression system for P-gp. The role of P-gp in mediating resistance to HIV infection by altering plasma membrane properties and in affecting pharmacokinetics of drug absorption, distribution, and excretion in the body are also being explored. While the studies on mechanism and function of P-gp will aid in the development of new chemotherapeutic regimens and the development of agents which reverse drug resistance, an alternative way to exploit information about the multidrug transporter is to use it to confer resistance

on drug-sensitive tissues such as bone marrow in patients undergoing intensive chemotherapy. This approach has been modeled in transgenic mice in which expression of the MDR1 cDNA in bone marrow makes this bone marrow resistant to toxicity of natural product anticancer drugs, and in animal models, including the mouse and dog, using retroviral vectors to confer selective advantage to transplanted, MDR1-transduced bone marrow in the presence of drugs such as Taxol.

In collaboration with other NIH scientists, we have also closely linked the MDR1 cDNA to other therapeutic genes, such as genes needed for treatment of Gaucher disease (glucocerebrosidase), Fabry disease (α-galactosidase), chronic granulomatous disease (various subunits of the NADPH oxidase complex), and severe combined immunodeficiency (adenosine deaminase and interleukin receptor gamma subunit). Bicistronic MDR1 vectors for gene therapy of AIDS are also being constructed. Tight linkage, in which selection for drug resistance invariably results in expression of the nonselected gene, can be achieved using bicistronic vectors in which the nonselected cDNA is translated from the same mRNA as the MDR1 cDNA under control of an internal ribosome entry site (IRES). Delivery of MDR1 and linked genes to target cells has focused on use of EBV, retroviral, and in vitro-packaged SV40 vectors. Ongoing projects in the laboratory are dedicated to elucidating other mechanisms of multidrug resistance in cancer cells. A model system for cross-resistance to cisplatin, methotrexate, and nucleotide analogs such as 5–FU has been developed in which accumulation of drugs in resistant cells is much reduced; identification of the mechanism of this phenotype is in progress.

Recent Publications:

Booth CL, et al. *Biochemistry* 2000;39:5518–26.

Lee CG, et al. *FASEB J* 2000;14:516–22.

Shen D-w, et al. *J Cell Physiol* 2000;183:108–16.

Gottesman MM. *Annu Rev Med* 2001; in press.

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Biography: *Dr. Ambudkar obtained his Ph.D. from Madurai Kamaraj University, Madurai, India, and received his postdoctoral training in membrane bioenergetics with Dr. Barry Rosen at the University of Maryland. He continued his postdoctoral work on biochemistry of membrane transport proteins with Dr. Peter Maloney at the Johns Hopkins University School of Medicine. In July 1995, after 5 years as an assistant professor in the departments of medicine and physiology at Johns Hopkins, Dr. Ambudkar joined the Laboratory of Cell Biology at the NCI.*

Laboratory of Cell Biology
Biochemistry of Multidrug Transporters

Keywords:
ABC transport proteins
anticancer agents
ATP hydrolysis
chemotherapy
drug transport
multidrug resistance

Research: ABC transporters such as P-glycoprotein (P-gp), the multidrug resistance-associated protein (MRP1), and the mitoxantrone-resistant protein (MXR1, also known as the breast cancer resistance protein BCRP, or ABCP), which function as ATP-dependent efflux pumps, play an important role in the development of multidrug resistance in most cancers. In addition, some of the other members of the MRP subfamily (MRP2–5) also transport anticancer agents in a conjugated form. Thus, these transporters also may contribute to the development of multidrug resistance in malignant cells. These transporters can recognize and transport a wide variety of amphipathic cytotoxic natural product anticancer drugs. Our studies are directed toward understanding the mechanism of action of the multidrug resistance-linked ABC transporters such as P-gp and MRP1. Recent studies with P-gp deal with the interaction between substrate and ATP sites and elucidation of the catalytic cycle of ATP hydrolysis. The kinetic analyses of ATP hydrolysis by reconstituted purified P-gp suggest that ADP release is the rate-limiting step in the catalytic cycle and the substrates exert their effect by modulating ADP release. In addition, we provide evidence for two distinct roles for ATP hydrolysis in a single turnover of P-gp, one in the transport of drug and the other in effecting conformational changes to reset the transporter for the next catalytic cycle. We have further exploited the vanadate (Vi)-induced ADP trapped transition-state conformation of P-gp to address the question of what are the effects of ATP hydrolysis on the nucleotide-binding site. We find that at the end of the first hydrolysis event there is a decrease in the affinity of nucleotide (ATP or ADP) for P-gp coincident with the impaired substrate binding. The kinetics of repeating succession of trapping and release of [α^{32}P]-8-azidoADP through an entire catalytic cycle was determined, and we also monitored the substrate binding at the beginning and end of each trapping event. Though the two hydrolysis events have different functional outcomes vis-à-vis the recovery of substrate binding and translocation, they show comparable kinetic properties for both incorporation and release of

nucleotide and the K_m for [α^{32}P]-8-azidoATP in the presence of vanadate is identical. These data demonstrate that both nucleotide sites behave symmetrically, and during individual hydrolysis events, the ATP sites are recruited in a random manner. Furthermore, only one site hydrolyzes ATP at any given time and the conformational change in this site that drastically decreases (>30-fold) the affinity of the second site for the ATP-binding. Thus, the blocking of ATP binding to the second site while the first one is in catalytic conformation appears to be the basis for the working model of an alternate catalytic cycle of ATP hydrolysis by P-gp, and this may be applicable as well to other ABC transporters linked with the development of multidrug resistance. Analyses of thermodynamic parameters indicate that 100–115 kJ/mole energy of activation is required for the drug-substrate stimulated ATP hydrolysis by P-gp and, consistent with its physiological role, P-gp appears to operate in the most energy efficient way in the ATP hydrolyzing mode as the formation of transition-state intermediate under nonhydrolysis condition requires three times higher energy of activation.

Collaborators on this research include Merritt Andrus, Brigham Young University, Provo.

Recent Publications:

Sauna ZE, et al. *Proc Natl Acad Sci USA* 2000;97:2515–20.

Kerr KM, et al. *J Biol Chem* 2001;276:8657–64

Sauna ZE, et al. *J Biol Chem* 2001;276:11653–61.

Sauna ZE, et al. *J Biol Chem* 2001;276:21198–208.

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Biography: *Dr. Appella obtained his M.D. from the University of Rome, Italy, and continued his research at Johns Hopkins and the NIH (National Institute of Diabetes and Digestive and Kidney Diseases) on dehydrogenases. Since 1965, he has been in the Laboratory of Cell Biology where he continues his research on tumor immunology, the p53 tumor suppressor protein, and the design of antiviral drugs against HIV.*

Laboratory of Cell Biology
MHC/Peptide Complexes Structure and Function Analysis

Keywords:
acetylation
antiviral drugs
HIV–1
MHC
NCp7
p53
peptides complexes
phosphorylation
Zn fingers

Research: Class I major histocompatibility complex (MHC) molecules are polymorphic cell surface proteins that assemble with peptides derived from the degradation of intracellular proteins. Cell surface class I/peptide complexes are ligands for T cell receptors (TCR) on CD8+ T lymphocytes. Our research is aimed toward understanding the biochemical and functional details of MHC-peptide and MHC-peptide-TCR interactions; these characterizations reveal fundamental details of the immune response and are a step toward principle-based development of immunotherapies. We anticipate that a detailed understanding of structure-activity relationships among immunologic ligands and receptors may lead to the effective use of peptides or peptidomimetics as specific modulators of immune responses. We are particularly interested in studying the factors that govern class I-restricted TCR specificity and cross-reactivity, as well as the qualitative responses observed to peptides that behave as agonists, or partial-full antagonists. Our approach features the extensive use of peptide-epitope analogs to probe recognition in model systems involving human and murine class I molecules. Our ongoing work is presently concentrated in three major areas: (1) analysis of epitope cross-recognition; (2) characterization of the CTL response to peptides derived from tumor-related proteins; and (3) application of mass spectrometric techniques to the analysis of immunologically relevant proteins and peptides.

The p53 Tumor Suppressor Protein

The human p53 tumor suppressor protein normally is present in a latent state and at a low level, but a variety of cellular stresses, including DNA damage, activate signaling pathways that transiently stabilize the p53 protein, cause it to accumulate in the nucleus, and activate it as a transcription factor. Activation leads either to growth arrest at the G_1/S or G_2/M transitions of the cell cycle or to apoptosis. The molecular mechanisms by which stabilization and activation occur are incompletely understood but are believed to be mediated by multiple posttranslational modifications to p53 itself and possibly to other proteins with which p53 interacts. We have prepared antibodies that recognize p53 only when it has been modified at a particular site. These autobodies were then used to characterize the responses to DNA

damaging agents. The analysis of p53 modified at individual sites revealed a complex and unexpected interdependency in site phosphorylation. However, regulation may be lost or altered in tissue culture experiments mainly due to genetic changes that occur upon prolonged passage in culture. Most of these problems can be ameliorated by working with mice; therefore, we are analyzing modification patterns in different mouse tissues after generating knock-in mutations for each site, especially for those tissues that show increased tumor development.

Design of Antiviral Drugs Against the HIV Virus

Development of drug-resistant HIV strains in response to nucleoside, nonnucleoside reverse transcriptase and protease inhibitors has necessitated the search for novel antiretroviral agents that target new structures for therapy of acquired immunodeficiency syndrome (AIDS). The involvement of HIV–1 NCp7 Zn fingers in multiple phases of the HIV–1 replication cycle and their mutationally nonpermissive nature have provided incentives for choosing this structure as a target for antiretroviral therapy. We have synthesized novel pyridinioalkanoyl thioester (PATE) derivatives that overcame the cellular toxicity associated with disulfide benzamides and showed superior antiviral activity and minimal cytotoxicity. Our ongoing work is concentrated on determining the mechanism of action of PATEs and determining the sites of modification on the NCp7 protein. For this purpose, a fluorescence-based assay using a zinc-specific fluorophore, Newport Green (NPG), mass spectrometry, and NMR are used to follow the kinetics of zinc ejection and to determine the site(s) of covalent modification on the target protein. Target selectivity, toxicology, and efficacy in a murine HIV model are also being studied in order to assess their potential for in vivo antiviral activity.

Recent Publications:

Kobayashi H, et al. *Cancer Res* 2001;61:4773–8.

Chao C, et al. *Proc Natl Acad Sci* 2000;97:19936–41.

Pearson M, et al. *Nature* 2000;406:207–10.

Basrur V, et al. *J Biol Chem* 2000;275:14890–7.

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Biography: *Dr. Hearing obtained his Ph.D. from the Catholic University of America and continued his studies on mammalian pigmentation initially as a postdoctoral fellow and then as a staff fellow in the Dermatology Branch at the NCI, moving to the Laboratory of Cell Biology in 1983. He has served as president of the PanAmerican Society for Pigment Cell Research and president of the International Federation of Pigment Cell Societies, and is currently the editor of the journal* Pigment Cell Research. *He serves as assistant chief of the Laboratory of Cell Biology and chief of the Pigment Biology Section at the NCI, where he continues his research.*

Laboratory of Cell Biology
Melanoma and Melanogenesis

Keywords:
melanin
melanoma
photoprotection
pigmentation

Research: This project is focused on characterizing parameters important to the growth, differentiation, and function of normal melanocytes and their significance to the outgrowth and metastasis of transformed melanocytes (malignant melanoma). These studies have evolved into an examination of the function and regulation of pigment-specific genes, i.e., genes that encode melanocyte-specific proteins. These proteins have generally been found to be localized in melanosomes, specific organelles that serve as the site of melanin pigment deposition; they function as catalytic and/or structural entities but perhaps more importantly serve as specific targets of natural and induced host immune responses to melanoma growth.

These studies have identified, isolated, and characterized melanogenic enzymes (including tyrosinase, TRP1, and TRP2) that regulate the quality and quantity of pigment produced within melanocytes, and thus influence normal melanocyte function, including its role in photoprotection. We have also characterized a melanosomal structural matrix protein (gp100/silver) that is important to the viability and proliferative capacity of melanocytes. These melanogenic proteins are encoded within a family of pigmentation-related genes that are specifically expressed by mammalian melanocytes. Interestingly, although expression of these genes is restricted to pigment-producing tissues, we have shown that they are independently regulated following stimulation or inhibition of differentiation. The phenotypic and functional properties of the melanins produced by these regulatory enzymes differ dramatically, and effects on the functional and photoprotective properties of those melanins are currently being examined.

Recent work has examined the expression of novel genes during the switch to pheomelanogenesis and a number of such genes have been identified. We are also continuing to characterize melanoma-specific antigens expressed by transformed melanocytes that play a role in immune responses to tumor growth; of interest is the fact that many (perhaps all) of those melanoma-specific antigens are normally expressed melanosomal proteins. Monoclonal antibodies to one of those antigens, termed "B700," specifically cross-react

with human melanoma and we have shown them to be useful as highly specific probes for malignant melanoma. Intravenous treatment of tumor-bearing hosts with those antibodies provides significant protection against metastatic growth. We have also shown that B700 constitutes the major immunodominant antigen in a melanoma vaccine that has proven to be efficacious for host survival in a novel model for spontaneous melanoma metastasis. Most recently, studies have been initiated to identify novel antigens expressed by less differentiated melanoma cells that might serve as immune targets in heterogeneous tumor populations.

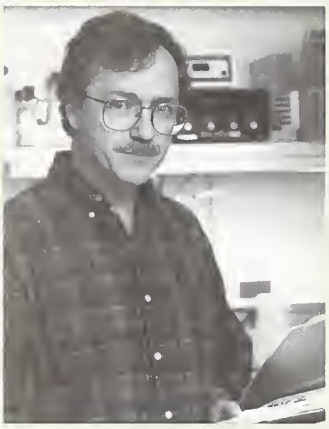
Recent Publications:

Virador MV, et al. *Exp Cell Res* 2000;259:54–63.

Abdel-Malek ZA, et al. *J Cell Sci* 2001;114:1019–24.

Furumura M, et al. *J Biol Chem* 2001; in press.

Toyofuku K, et al. *Biochem J* 2001;355:259–69.



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Biography: *Dr. Michael Maurizi received his Ph.D. in biochemistry from the University of Illinois and completed postdoctoral studies with Earl Stadtman and Ann Ginsburg in the National Heart, Lung, and Blood Institute at the NIH. Dr. Maurizi began his work on energy-dependent proteases in collaboration with Susan Gottesman in the Laboratory of Molecular Biology before joining the staff of this laboratory in 1991.*

Laboratory of Cell Biology
Structure and Function of ATP–Dependent Proteases Involved in Rapid Intracellular Protein Degradation in Prokaryotes and Eukaryotes

Keywords:
ATPase
chaperone
proteases
protein folding
proteolysis

Research: Intracellular protein degradation serves important regulatory and housekeeping functions, and major challenges are to identify the proteases responsible for degrading specific proteins or classes of proteins and to explain how appropriate proteins are targeted for degradation. Eukaryotic and prokaryotic organisms contain complex ATP-dependent enzymatic systems for the recognition of protein substrates, the marking of those proteins for degradation, and the rapid and extensive cleavage of the appropriate proteins. Our laboratory studies the structure and function of two ATP-dependent protease families from *E. coli*, Lon and Clp, which show remarkable parallels to eukaryotic energy-dependent proteases in structure, mechanism, and activity. Lon and Clp proteases are multimeric enzymes consisting of distinct ATPase and protease domains or subunits. Clp ATPases are evolutionarily related but can be distinguished on the basis of the proteolytic component with which they interact and by the particular

substrate that they target for degradation. ClpA and ClpX, which interact only with ClpP, direct the degradation of completely different protein substrates, while ClpY, which interacts only with ClpQ, directs degradation of yet other proteins. Electron microscopy reveals that Clp proteases are barrel-like structures consisting of a double ring of proteolytic subunits with rings of ATPase subunits axially aligned on both sides. The rings of the proteolytic component are slightly concave so that the junction of the two rings forms a hollow core, which is lined with the proteolytic active sites—creating a degradative chamber where proteins are cleaved multiple times. ClpA subunits have two distinct ATPase domains so that the ClpAP complex has two rings of ATPases on either side of ClpP. ClpX and ClpY subunits consist of a single ATPase domain and thus form shallower barrels.

An intriguing challenge will be to define the differences in activity or mechanism that explain the need for either one or two ATPases. Nucleotide binding alters the conformation of the ATPase domain and affects its interaction with the proteolytic domain. Both ClpA and ClpY hexamers are unstable in the absence of ATP, and formation of the ClpAP or ClpYQ complex requires ATP binding. Peptidase activity of both Clp and Lon proteases is activated by binding of nonhydrolyzable nucleotides, but protein degradation requires hydrolysis of ATP. The structure of Clp proteases is such that the ATPase blocks direct access to the proteolytic core, requiring that some work be done to get protein substrates into the proteolytic active sites. Clp ATPases display specific protein unfolding or remodeling activity and thus act in the manner of ATP-dependent molecular chaperones. This chaperone activity is used in binding and recognition of potential targets for degradation and in altering the conformation of protein substrates.

Recent studies with Lon, which, although having the ATPase and proteolytic domains in a single subunit, has an analogous mechanism of action, have shown that the need for ATP hydrolysis is related to the degree of secondary structure in the target protein. Thus, ATP hydrolysis is required to unfold a protein substrate so that it can have greater access to the proteolytic active sites. The Lon and Clp protease families are found throughout the bacterial kingdom and have been highly conserved in eukaryotic organisms. We have cloned human *LON* and *CLP* cDNAs and demonstrated that both proteases are encoded in the nucleus but occur exclusively in mitochondria. *E. coli* Lon is essential in cells with damaged DNA, and yeast Lon is apparently essential for the maintenance of functional mitochondria. The human *LON* gene maps to chromosome 19. The coding region includes ~13 kb of DNA and comprises 16 exons. Construction of dominant-negative mutants of human Lon is under way to study the physiological consequences of impaired Lon activity in human cells.

Collaborators on this research include Fabienne Beuron, Ann Ginsburg, Susan Gottesman, Martin Kessel, Alasdair Steven, Laurence Van Melderen, Sue Wickner, and Whi-Fin Wu, NIH; and Martine Courturier, Université Libre de Bruxelles, Belgium.

Recent Publications:

Kessel M, et al. *J Mol Biol* 1995;250:587–94.

Gottesman S, et al. *Genes Dev* 1997;91:815–23.

Grimaud R, et al. *J Biol Chem* 1998;273:12476–81.

Hilliard JJ, et al. *J Biol Chem* 1998;273:524–7.

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Biography: *Dr. Jacqueline Milne obtained her Ph.D. in biology from York University, Toronto, in 1991 and received her postdoctoral research training with Peter Devreotes in the Department of Biological Chemistry, The Johns Hopkins School of Medicine, and with Richard Henderson in the Laboratory of Molecular Biology, Medical Research Council, Cambridge, England. She joined the NCI's Laboratory of Cell Biology in 1999*

Laboratory of Cell Biology
Structural Analysis of Macromolecular Complexes by High Resolution Electron Microscopy

Keywords:
high-resolution electron microscopy
macromolecular complexes
three-dimensional reconstruction

Research: The main focus of our laboratory is the structure determination of large protein complexes by analysis of high-resolution images of single particles. In this method, large numbers of images of individual protein molecules are recorded using very low electron doses, sorted into distinct classes, and then added together to obtain distinct views of the molecules that have a high signal-to-noise ratio. The averaged views are then oriented with respect to each other, and used to reconstruct a model of the three-dimensional structure. The dramatic recent progress in this field on structure determination of the icosahedral core of the hepatitis B viral protein demonstrates that this approach of "crystallography without crystals" may have great potential to probe biologically-relevant complexes that are too large to be analyzed by NMR methods or that do not crystallize easily in the two- or three-dimensional arrays required for electron microscopic or x-ray crystallographic studies.

The catalytic core of the pyruvate dehydrogenase is an outstanding model system for refinement of single particle methods. Sixty copies of this enzyme assemble into a 1,800 kDa icosahedral complex that is readily purified and that shows a number of distinct orientations on electron micrographs. We are optimizing methods to accurately orient the single molecules and to correct distortions introduced during image collection on the electron microscope. Currently, processing of 3,500 individual molecular images has led to a three-dimensional model that has a resolution of 14+. The predicted envelope of this structure is in excellent agreement with the recently determined x-ray structure. Refinement of these methods will be useful for the analysis of

human P-glycoprotein and for related structural projects currently under way in the laboratory. In addition, this work lays the foundation to study complexes of the pyruvate dehydrogenase catalytic core when it is decorated with other proteins. Analysis of the catalytic core when it is complexed to the accessory enzymes E1 or E3 has already yielded a three-dimensional model that has a resolution of 35+. While single particle analysis is now the major focus of our laboratory, we have also been involved in an extensive analysis of the signal transduction pathways mediated by a seven-helix chemotaxis receptor that binds cAMP. Ligand binding activates both G-protein-dependent and G-protein-independent signal transduction events that, in turn, trigger a number of cell activities including chemotaxis. Distinct conformational intermediates of the receptor likely arise during the activation of these signal transduction pathways and during the return of the receptor to its unactivated state. Recent evidence suggests that seven other helix receptors can also activate multiple signal transduction pathways and interact with a number of distinct proteins other than G-proteins and arrestin. Continued development of single particle methods may provide a powerful tool to investigate the structural basis of signal transduction in these important macromolecular complexes.

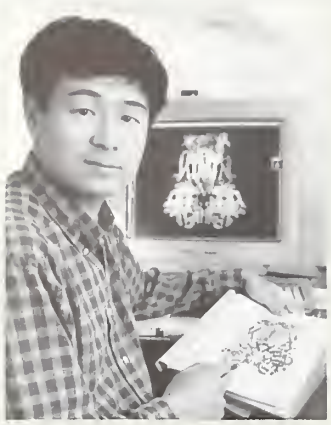
Recent Publications:

Kim JY, et al. *J Biol Chem* 1997;272:2060–8.

Milne JLS, et al. *Adv Second Messenger Phosphoprotein Res* 1997;31:83–104.

Milne JLS, et al. *J Biol Chem* 1997;272:2069–76.

Traynor D, et al. *EMBO J* 2000;19:4846–54.



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Biography: *Dr. Xia obtained his Ph.D. in the field of structural virology from the Department of Biological Sciences, Purdue University, Indiana, and received his postdoctoral training in membrane protein structural biology with Dr. Deisenhofer at the University of Texas, Southwestern Medical Center at Dallas, Texas. He joined the Laboratory of Cell Biology at the NCI as a senior investigator in 1998.*

Laboratory of Cell Biology
Structural Study of Biological Membrane Proteins

Keywords:
AAA protein
ATP-dependent protease
ClpAP
macromolecular complexes
membrane protein
multidrug resistance protein
P-glycoprotein
protein degradation
structural biology
x-ray crystallography

Research: Recent advances in genome research revealed that over one-third of open reading frames in yeast *(S. cerevisiae)* are predicted to be integral membrane proteins with 1 to 14 transmembrane segments, reflecting the importance of membrane proteins in the life cycle of this unicellular eukaryotic organism. In multicellular organisms, cellular communication and interaction require increased biological complexity and likely an increased fraction of membrane proteins. Membrane proteins provide vital cellular functions involved in cell-cell communication, recognition, adhesion, and membrane fusion; in material exchange, transportation and detoxication; and in processes of cellular energy conservation. Structural studies on a limited number of membrane proteins have contributed to our understanding of the function of these biological macromolecules. In the light of the increased number of membrane proteins being studied, the paucity of structural data of membrane proteins at atomic resolution creates a vacuum in our knowledge which is only being filled rather slowly. To date, only a few families of membrane protein structures have been determined; most of them are of bacterial origin. The situation arises mainly due to tremendous difficulty in purifying sufficient quantity of most membrane proteins, especially those of eukaryotic origin, needed for structural analysis and in producing diffraction quality crystals; and failure in overexpressing membrane proteins only makes the situation worse. In collaboration with both intramural and extramural laboratories, we try to explore the structure and function relations of polytopic membrane proteins crystallographically by examining a few carefully selected membrane proteins such as those involved in cellular multidrug resistance (human P-glycoprotein) and respiration (cytochrome *bc1* complex of both mitochondria and bacteria). We hope that these studies will result in deep understanding of membrane protein architecture in general, the mechanism of function of these important biological membrane proteins, and in potential development of therapeutics for the benefit of mankind.

Protein degradation is a major posttranslational mechanism for modulating the amounts of specific regulatory proteins and is involved in processes as diverse as timing of the cell cycle, the heat shock response, developmental changes, cell signaling pathways, metabolic adaptation to nutrient availability, response to DNA damage, and oncogenesis. Moreover, protein

degradation is an important mechanism of protein quality control, removing damaged or misfolded proteins as well as improperly synthesized peptides from cells. Almost all important cytosolic and nuclear protein degradations are carried out by ATP-dependent proteases that have been found in all organisms; loss of function mutations is usually lethal. Despite sequence divergence among them, there is a remarkable conservation in basic architecture and biochemical mechanisms in ATP-dependent proteases, such as prokaryotic ClpAP and Lon and eukaryotic proteasomes. The ClpAP protease, which we propose to study, is especially important for a number of reasons. First, ClpAP or its close homolog, ClpXP, are essential in many microorganisms. Second, ClpAP and ClpXP are highly conserved: ClpAP is found in the chloroplast of all plants and in photosynthetic bacteria, and ClpXP is found in the mitochondria of eukaryotes, including humans. Third, ClpA is the prototype of the Hsp100 molecular chaperones, which include yeast Hsp104, a chaperone shown to be involved in prion formation. Fourth, ClpA has significant sequence and structural similarity to AAA proteins, a broad class of protein conformation-transducing ATPases involved in a plethora of vital cellular functions. Last, Clp and other ATP-dependent proteases are structurally and mechanistically complex proteins, whose structure/function relationships reflect important biochemical principles that need to be understood at the submolecular level. Structural investigations have been conducted extensively on ClpA, ClpP, and ClpAP complexes, mostly by electron microscopic methods, and the structure of ClpP at atomic resolution is available. While the EM studies have provided rich information with respect to the subunit composition and molecular morphology, they are insufficient in elucidating the atomic details of these complexes. Atomic level structural information of ClpA would provide a high-resolution basis upon which models for function of this enzyme could be formulated, especially the binding of substrates and coupling of ATP hydrolysis to substrate folding/unfolding, about which very little is known. The structural solution would also provide an opportunity to explore the interaction between ClpA and ClpP and perhaps help to explain the functional implication of the symmetry mismatch at the interface of hexameric ClpA and heptameric ClpP rings in the ClpAP complex. We have initiated the project of structural investigation of ClpA by crystallographic method.

Recent Publications:

Yu L, et al. *J Bioenerg Biomembr* 1999;31:251–7.

Xia D, et al. *Biochem Cell Biol* 1998;76:673–9.

Xia D, et al. *Proc Natl Acad Sci* 1998;95:8026–33.

Xia D, et al. *Science* 1997;277:60–6.

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The Laboratory of Cell Regulation and Carcinogenesis (LCRC) continues its strong tradition of emphasis on the study of the family of key regulatory peptides known as transforming growth factor-β (TGFβ), with focus on understanding both its basic biology, molecular biology, and biochemistry, as well as its roles in disease pathogenesis. Major areas of current research include identification of underlying mechanisms of epithelial carcinogenesis in prostate, breast, gastric, and other cancers, with emphasis both on the dual tumor suppressive and oncogenic activities of TGFβ and its receptors and on hormonal regulation of prostate and breast cancer. The laboratory also studies mechanisms of signal transduction from the novel TGFβ receptor serine-threonine kinases, and the mechanisms of TGFβ action in regulation of immune cell function, particularly as they relate to disease pathogenesis.

The LCRC is organized into groups led by five principal investigators who cover a broad range of disciplines from biochemistry and molecular and cell biology to immunology, pathology, and oncology. The individual groups are strongly synergistic and interdependent even though their principal research areas are distinct, creating an interactive atmosphere that has been and continues to be a strong asset of the laboratory. In addition, the scope and breadth of the research in the laboratory provides a framework for integrating basic molecular and mechanistic studies with more complex animal model systems including transgenic and knock-out mice as well as conditional knock-out mice The laboratory prides itself on its mentorship of postdoctoral fellows and predoctoral interns and has a longstanding tradition of providing a congenial and supportive atmosphere in which to work.

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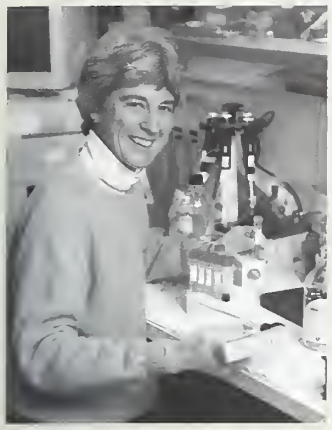

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Biography: *Dr. Roberts obtained her Ph.D. from the University of Wisconsin for the study of the metabolism of retinoic acid. Following postdoctoral research at Harvard University Medical School and teaching in the Chemistry Department at Indiana University, she joined the Laboratory of Chemoprevention in 1976 and became chief in 1995. She serves on numerous editorial boards, is a past president of the Wound Healing Society, and is a member of the Senior Biomedical Research Staff.*

Laboratory of Cell Regulation and Carcinogenesis
Mechanisms of TGFß Action In Vitro and In Vivo

Keywords:
animal models
bone morphogenetic proteins
cell signaling
chemotaxis
growth factors
inflammation
JNK
Jun
microarray
mouse embryo fibroblasts
signal transduction
transforming growth factor-ß (TGFß)

Research: The focus of our research group is on elucidation of mechanisms of TGFβ action, with the ultimate goal of identifying mechanisms common both to physiological processes such as wound healing and to disease pathogenesis. The approaches used range from basic biochemical studies of signal transduction and cellular energetics, to the study of mouse models.

Signal Transduction Pathways

Members of the TGFβ superfamily signal through a unique set of receptor serine-threonine kinases. Elucidation of the signal transduction pathways from this family of regulatory peptides and the mechanisms whereby these signals are integrated with and modulated by those from receptor tyrosine kinases is key to understanding regulation of cell growth and differentiation. A prominent signaling pathway is mediated by a set of novel proteins called SMADs, which participate in a direct signaling pathway involving their phosphorylation by the type 1 receptor kinase and subsequent translocation to the nucleus to participate in transcriptional complexes. A major challenge is to determine how a limited number of SMAD proteins selectively activates the broad spectrum of gene targets of members of the TGFβ superfamily, and to identify which transcriptional targets are regulated directly by specific SMAD proteins. We are now using a variety of approaches in microarray analysis to identify the relative importance of Smad2, Smad3, and MAPK signaling pathways in activation of specific target genes of TGFβ. Ongoing biochemical studies are focused on delineating the functions of specific domains of the SMAD proteins and on characterizing novel cytoplasmic and nuclear proteins which modulate TGFβ receptors and SMAD signal transduction pathways. Thus we have identified in the common mediator, Smad4, a unique proline-rich transcriptional activation domain which interacts with the coactivator p300/CBP and is required for activity of the Smad transcriptional complex. One of the interacting proteins, a nuclear transcriptional repressor which we have called SNIP1, interacts principally with Smad4 and with p300/CBP in such a manner as to block the activating interaction of Smad4 with these coactivators. Another protein, Trap1, identified by interaction cloning using an activated TGFβ receptor, appears to act as a shuttle between Smad2 and Smad4, possibly facilitating the association of

these two Smads in the cytoplasm. A third protein, SNX6, a member of the sorting nexin family of proteins, binds to the TGFβ receptors, possibly acting to regulate their intracellular trafficking. The characterization of these proteins and their functional activity suggests additional complexity in the Smad signal transduction pathways which may underlie certain cell-specific or target-specific effects.

Role of TGFβ in Wound Healing, Fibrosis, and Carcinogenesis

Many mechanisms are common to wound healing, fibrosis, and tumorigenesis, and tumors have been described as wounds which don't heal. TGFβ plays an important role in wound healing and is both released from degranulating platelets at the time of tissue injury and produced by fibroblasts and inflammatory cells migrating into a wound site. Numerous studies have shown that systemic or topical TGFβ can restore normal healing in models of impaired healing. We are now gaining new insights into the intrinsic role of TGFβ in wound healing by utilizing mice with a targeted deletion of either the TGFβ1 gene or one of its downstream signaling proteins, Smad3. Surprisingly, both of these models show improved wound healing and suggest that selective modulation of the responses of individual cell types to TGFβ may have therapeutic benefit. Based on these results and the common features between wound healing and fibrosis, we are now exploring the effect of loss of Smad3 in radiation injury and the secondary fibrotic sequelae. Results indicate that loss of Smad3 is protective against damage induced by radiation, possibly due to the diminished levels of TGFβ seen in lesions of the Smad3 null mice. The accumulating data suggest that selective inhibition of Smad3 activation would be beneficial in wound healing and protective against fibrosis.

Collaborators on this project are Chuxia Deng, Colin Duckett, James Mitchell, and Angelo Russo, NIH; Alain Mauviel, INSERM, Paris; Neil Perkins, University of Dundee, Scotland; Toshiro Shioda, Massachusetts General Hospital Cancer Center; and Tongwen Wang, Virginia Mason Research Center.

Recent Publications:

Kim RH, et al. *Genes Dev* 2000;14:1605–16.

Larisch S, et al. *Nat Cell Biol* 2000;2:915–21.

Wurthner J, et al. *J Biol Chem* 2001;276:19495–502.

Parks T, et al. *J Biol Chem* 2001;276:19332–9.

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Biography: *Dr. Green received his undergraduate degree in history and philosophy of science from Princeton University and was awarded a fellowship to continue his studies at the University of Munich. After receiving his M.D. from McGill University, Montreal, he completed a pediatric residency at the Children's Hospital of Philadelphia. Dr. Green came to the NCI as a clinical genetics fellow, studied gene regulation with George Khoury, and joined the Laboratory of Cell Regulation and Carcinogenesis as head of the Transgenic Carcinogenesis Group in 1997.*

Laboratory of Cell Regulation and Carcinogenesis
Transgenic Models for Prostate and Mammary Carcinogenesis

Keywords:
animal models
breast
breast cancer model
cancer cell growth regulation
cDNA
cell cycle control
chemoprevention
data mining
gene targeting
genetically engineered mouse models
hormones
mammary tumorigenesis
microarray
oncogenes
pathology of tumors in animals
prostate cancer
transgenic mouse
tumor angiogenesis

Research: Our primary focus is to determine molecular mechanisms involved in prostate and mammary tumorigenesis using transgenic mouse approaches, and to use these animal models as systems in which to test novel therapies. A primary question is understanding what molecular events are involved in tumor progression. To this end, we have concentrated our efforts on correlating the histogenesis of mammary and prostate lesions to molecular alterations that occur during the multistep process of carcinogenesis using the C3(1)/Tag transgenic model developed in our lab. Male C3(1)/Tag transgenic mice develop prostatic intraepithelial neoplasia (PIN) lesions very similar to those observed in humans, which often progress to invasive adenocarcinomas over several months. One hundred percent of female mice carrying the C3(1)/Tag transgene develop mammary adenocarcinomas over several months in a very predictable manner, demonstrating transition lesions similar to DCIS found during human breast cancer development. We are using this to compare mechanisms of tumorigenesis in two different hormone-dependent tissues within the same genetic background.

One focus of our work has been to determine what genetic changes in addition to the expression of SV40–T–Ag occur during mammary and prostate tumor progression in this model. Using comparative genomic hybridization, we have demonstrated that mammary tumor progression is associated with an amplification on chromosome 6 resulting in the amplification and overexpression of the ki-ras oncogene associated with an elevation of MAP kinase activity. Double transgenic mice lacking ki-ras have a delayed onset of mammary tumor formation, demonstrating the importance of ki-ras in tumor progression. Preliminary CGH analyses of prostate cancer indicate that other regions of amplification and deletions may be involved. We have demonstrated that Ha-ras mutations are rare in our transgenic mammary tumors but frequent in tumors of the prostate. Further genetic alterations are being identified by LOH at particular stages of tumor progression.

Important changes in the expression of genes that regulate the cell cycle have been identified, in particular the loss of p21. Recent gene therapy approaches in our lab have demonstrated that the restoration of p21 function can significantly reduce mammary tumor progression using this transgenic model. We have also demonstrated that *bax* expression is critical to protective apoptosis primarily during preneoplasia. Double transgenic mice lacking bax have a significantly accelerated progression of mammary tumors.

The role of sex hormones on both mammary and prostate tumor development are also being investigated. Hormone manipulations can lead to striking changes in the histopathologic phenotype of the mammary tumors. We have also studying how pregnancy may alter the natural history of tumor progression in this model. Our lab is also using in vitro and in vivo systems to explore how the androgen receptor may be involved in prostate cancer progression.

The laboratory is in the process of developing and evaluating several new prostate-specific promoters that may be useful for targeting expression to the prostate in transgenic mice, as well as for gene therapy. In addition, microarray technologies are being employed for gene discovery as well as to study how gene expression profiles change during both mammary and prostate tumor progression.

Recent Publications:

Shibata MA, et al. *Cancer Res* 1996;56:4894–903.

Liu ML, et al. *Oncogene* 1998;18:2403–11.

Shibata MA, et al. *EMBO J* 1999;18:2692–5701.

Cardiff RD, et al. *Oncogene* 2000;19(8):968–89.

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Biography: *Dr. Kim is a senior investigator in the Laboratory of Cell Regulation and Carcinogenesis. He obtained his Ph.D. in applied biochemistry at the University of Tsukuba, Japan, and he joined Michael Sporn's laboratory at the NCI in 1987. Dr. Kim was tenured in 1994 and now heads the Gene Regulation Group. His current research interests include the molecular basis for altered expression of TGFßs and their receptors in multiple pathologies, and the mechanisms of the development of resistance to TGFßs in the process of carcinogenesis.*

Laboratory of Cell Regulation and Carcinogenesis
Molecular Mechanism of Loss of TGFß Signaling During Carcinogenesis

Keywords:
apoptosis
carcinogenesis
Ewing's sarcoma
inflammation
oncogene
promoter
signal transduction
transcription
transcription factors
transforming growth factor-ß (TGFß)
transgenic mice
tumor suppressor

Research: The research objective of our group is to understand the molecular mechanisms responsible for resistance to TGFβ growth inhibitory activity during carcinogenesis. Recent findings demonstrate that the TGFβ receptor complex is a new addition to the family of human tumor suppressor genes and that inactivation of these receptors is important in multiple human malignancies. Because human cancer cells frequently demonstrate resistance to the normal growth inhibitory effects of TGFβ, it has been proposed that the development of such TGFβ resistance represents a significant step during carcinogenesis. We have demonstrated a strong correlation between the resistance to TGFβ and gross structural abnormalities in the TGFβ type 2 receptor (RII) gene in human gastric cancers. In addition, we have reported that mutation and transcriptional repression of the TGFβ type 2 receptor correlated with the loss of responsiveness of tumor cell lines to TGFβ.

Transcriptional regulation of the TGFβ RII gene. Several lines of evidence have suggested that the transcriptional repression of the TGFβ RII gene may be important in modulating TGFβ responsiveness. Our laboratory has cloned a novel transcription factor, ERT, that interacts with the purine-rich sequences in the TGFβ RII promoter region and regulates TGFβ RII expression. Recent studies have implicated members of the ets family of transcription factors as pathogenic mechanisms for multiple malignancies. We previously have identified multiple Ets binding sites in the TGFβ RII promoter, suggesting the functional importance of these sites in the transcriptional regulation of the RII gene. Ewing's sarcoma (ES)-specific chromosomal translocations fuse the EWS gene to a subset of the ets transcription factor family, the FLI1, ERG, or ETV1 gene. EWS–FLI1, EWS–EGR, and EWS–ETV1 are thought to act as aberrant transcription factors that bind DNA through their ETS DNA binding domains. We have shown that the EWS–Ets fusion proteins act as potent repressors of the TGFβ type 2 receptor gene, and that introduction of normal TGFβ RII into an ES cell line restores TGFβ sensitivity and blocks tumorigenicity. These results indicate that the transcriptional repression of TGFβ RII

is a major target of the EWS–Ets. We are currently investigating how EWS fusion proteins and various ets families of transcription factors regulate the expression of TGFβ RII gene.

Our overall research over the past years has suggested that a pharmacologic augmentation of TGFβ signaling pathways in human cancers such as stomach and Ewing's sarcomas may be a potential therapeutic strategy. Recently, we have demonstrated that a histone deacetylase inhibitor suppresses the proliferation of human breast cancer cells and specifically enhances TGFβ signaling by increasing the level of expression of TGFβ type 2 receptor. We are currently investigating the molecular mechanisms of induction of TGFβ RII by a histone deacetylase inhibitor.

The molecular mechanism of resistance to TGFβ signaling by viral oncoproteins. Many viral oncoproteins are known to modulate the TGFβ signaling. However, mechanisms are not well characterized. Because Hepatitis B virus (HBV) is closely associated with acute and chronic hepatitis, cirrhosis, and hepatocellular carcinoma, we have explored the potential role of TGFβ in the pathogenesis of fibrosis in chronic hepatitis and cirrhosis. We have discovered that the HBV-encoded oncoprotein pX amplifies and augments TGFβ signaling through a direct interaction with its signaling intermediate, Smad4. Since HTLV–1-infected T cell lines become resistant to TGFβ growth inhibitory activity, we are also investigating whether or not HTLV–1-encoded oncoprotein Tax alters TGFβ signaling.

Molecular mechanism of TGFβ-induced apoptosis. Although TGFβ is known to induce apoptosis in many cells, little information exists regarding the signaling pathways involved in apoptotic endpoints. In collaborative work, we have recently demonstrated that Cdc2 and Cdk2 kinase activity transiently induced by TGFβ1 phosphorylates retinoblastoma gene product (RB) as a physiological target in hepatoma cells and that this hyperphosphorylation of RB may trigger abrupt cell cycle progression, leading to irreversible cell death. We are currently investigating the underlying molecular mechanisms. In other collaborations, we have identified a novel factor, ARTS, which is a mitochondrial septin-like protein derived from an alternative splicing of the H5 gene. Overexpression of ARTS in cells commits them to the TGFβ-dependent apoptotic pathway. We are currently examining the role of the family of alternatively spliced variants of the human septin H5.

Role of loss of TGFβ responsiveness in gastrointestinal tract in vivo in transgenic mice. To study the role of the TGFβ signaling pathway during carcinogenesis in vivo, transgenic mice expressing a dominant-negative mutant form of the TGFβ RII (dnRII) targeted to the colon using the ITF promoter, and to the stomach and pancreas using pS2 promoter, have been generated. ITF-dnRII transgenic mice showed an increased susceptibility to inflammatory diseases. We are currently studying the role of the inactivation of the TGFβ signaling during colorectal and gastric tumorigenesis using ITF-dnRII, pS2-dnRII, and TGFβ type 2 receptor heterozygous mice.

Our collaborators include Yung-Jue Bang, Seoul National University Cancer Institute; Kyeong Sook Choi, Ajou University School of Medicine, Korea; and Sanford Markowitz, Case Western Reserve University.

Recent Publications:

Lee DK, et al. *Genes Dev* 2001;15:455–66.

Lee BI. *Cancer Res* 2001;61:931–4.

Park SH. *Oncogene* 2001;20:1235–45.

Larisch S. *Nat Cell Biol* 2000;2:915–21.

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Biography: *Dr. Letterio obtained his M.D. from the Ohio State University College of Medicine, where he received the Upjohn Award for Excellence in Research. He completed his clinical training in pediatrics at Case Western Reserve University, and a fellowship in hematology/oncology as a clinical associate in the Pediatric Branch of the NCI. He is currently a tenure track investigator in the Laboratory of Cell Regulation and Carcinogenesis, where his research has emphasized the importance of transforming growth factor-ß in hematopoietic and immune cell development and function.*

Laboratory of Cell Regulation and Carcinogenesis
The Role of TGFß in Hematopoietic Cell Development and Function

Keywords:
cytokine
hematopoiesis
immunology
lymphoid development
tumorigenesis

Research: The major focus of our research is on discovery of the critical roles of TGFβ in hematopoietic and immune cell function. The prototype of this family, TGFβ1, is expressed by all hematopoietic cell populations, regulates the proliferation and expansion of their progenitors, and plays an important role in controlling various aspects of their development and differentiated functions. Our primary goal is to gain a better understanding of the molecular and cellular events mediating these effects. We aim ultimately to translate this information into clinical trials that test the utility of TGFβ (or of TGFβ agonists) in disorders of hematopoietic and immune cell function, specifically those which are associated with altered expression or diminished response to this cytokine.

We have placed priority on the establishment of murine models to study the isoform-specific functions of TGFβ. This is essential, since it is difficult to duplicate in vitro the complex environment that is known to modulate hematopoiesis and immune cell function in vivo. Our work in several complimentary animal models is providing new information regarding the roles of TGFβ1 in the regulation of myelopoiesis, in the control of cell cycle progression, and also in the pathogenesis of both immune disorders and malignancies.

The central importance of this factor in immune cells is supported by the aggressive inflammatory and autoimmune phenotype that develops in mice in which expression of the type 1 TGFβ isoform is disrupted. We were first

to provide direct evidence that a TGFβ1-deficiency state predisposes to multiple pathogenic manifestations of autoimmunity, including the presence of specific, high-affinity, circulating IgG autoantibodies and progressive glomerular immune complex deposition. We provided the first direct evidence that endogenous TGFβ1 controls developmental expression of both class I and class II MHC antigens, and established a link between aberrant MHC antigen expression and the inflammation and autoimmunity resulting from TGFβ1 deficiency.

These models provide a unique view to the functions of TGFβ1 in normal development of specific hematopoietic cell lineages. Seminal studies in the NCI's Laboratory of Leukocyte Biology have suggested the importance of TGFβ in the control of hematopoietic stem cells and myelopoiesis. We provided the first direct in vivo evidence of this role by defining myeloid hyperplasia as a distinct, noninflammatory-driven phenotype of TGFβ1-deficiency. Analogous to myeloid metaplasia in humans, our class II MHC/TGFβ1-null mouse displays progressive extramedullary hematopoiesis, lymphadenopathy, splenomegaly, anemia, and increased cellularity within the marrow compartment. These mice also reveal the importance of TGFβ in the differentiation of specialized antigen-presenting cells, a concept supported by the absence of epidermal dendritic cells, or Langerhans cells (LC), in their skin.

The tumor suppressor functions of TGFβ are also clearly relevant in the regulation of hematopoietic cell growth and transformation. Insensitivity to the growth inhibitory effects of TGFβ has been demonstrated for many lymphoid and myeloid malignancies. We have recently identified loss of cell surface TGFβ receptors as a mechanism contributing to the genesis of plasma cell tumors in mice, and are investigating the function of this pathway in human B cell tumors, including numerous TGFβ-resistant lymphomas. The importance of this tumor suppressor activity is greatly emphasized by our work in the TGFβ1-null mouse system. New lines carrying additional inactivating mutations in other cell cycle regulatory genes have shown that the absence of TGFβ1 increases susceptibility to spontaneous tumorigenesis in both gastrointestinal and mammary epithelia, in addition to hematopoietic lineages.

In summary, our emphasis on murine model systems has provided new insights into the autocrine effects of TGFβ1 and the role of those effects in autoimmune and hematopoietic manifestations of TGFβ1 deficiency. We have linked the loss of the tumor suppressor functions of this pathway to both epithelial and hematopoietic malignancies in our model systems. Our current research aims are to define both the cellular and molecular mechanisms that mediate these events, particularly those which govern cell cycle progression and the growth inhibitory responses to TGFβ.

Collaborators include Chuxia Deng, Jonathan Keller, Ashok Kulkarni, Ian Magrath, Michael Potter, Francis Ruscetti, Mark Udey, NIH; and Andrew Koff, Memorial Sloan-Kettering Cancer Center.

Recent Publications:

Letterio JJ, et al. *Annu Rev Immuno* 1998;16:137–61.

Amoroso S, et al. *Proc Natl Acad Sci USA* 1998;95:189–94.

Letterio JJ, et al. *EMBO J* 1999;18(5):1280–91.

Shigetoshi K, et al. *J Immunol* 1999;163(7):4013–9.

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Biography: *Dr. Wakefield obtained her D.Phil. in biochemistry at the University of Oxford, England, for studies on the bioenergetics of the chromaffin granule. In 1983, she joined the Laboratory of Chemoprevention (now the Laboratory of Cell Regulation and Carcinogenesis) at the NCI to work on TGFßs and their relation to tumorigenesis. Dr. Wakefield was tenured in 1989, and now heads the Tumor Suppressor Group.*

Laboratory of Cell Regulation and Carcinogenesis
Tumor Suppressor and Oncogenic Activities of Transforming Growth Factor-ßs in Breast Cancer

Keywords:
animal models
breast cancer
cancer cell growth regulation
metastasis
mouse
transforming growth factor-ß (TGFß)
transgenic mice
tumor suppressor

Research: Transforming growth factor-βs (TGFβs) are potent inhibitors of epithelial cell growth. Recently, components of the TGFβ response path have been shown to be diminished or absent in a number of human cancers, implicating loss of TGFβ function as one mechanism contributing to tumor development. However, TGFβ expression is often upregulated in advanced human cancers, suggesting that the role played by the TGFβ system may be complex. We propose that TGFβ function as "conditional tumor suppressors," with suppressor activity dependent on (1) the levels of the TGFβs and their receptors, (2) the stage of tumorigenesis, and (3) the nature of cooperating oncogenic events. We are studying this hypothesis in mouse and xenograft models of breast cancer.

The effects of TGFβ are highly context-dependent, so we have chosen to address these questions in vivo in the intact organism, where all the complex contextual cues such as cell-cell interaction, hormonal milieu, and appropriate extracellular matrix are maintained. Our approach has been to generate genetically-engineered mice in which TGFβ function is experimentally compromised in target organs. To address the role of TGFβ in breast cancer, we have generated transgenic animals overexpressing antagonists of TGFβ action in the mammary gland. To date we have used two classes of antagonist: (1) a dominant-negative mutant form of the type 2 TGFβ receptor (DNR), which functions as a cell-autonomous antagonist, and (2) a soluble TGFβ antagonist consisting of the extracellular domain of the type 2 TGFβ receptor fused to an immunoglobulin Fc domain. Using this approach, we have shown that loss of TGFβ response by overexpression of the DNR causes abnormal mammary gland development and

an increased susceptibility to tumorigenesis induced by chemical carcinogens. This proves that TGFβ can function as a tumor suppressor in the mammary gland in the early stages of tumorigenesis. Conversely, our mice overexpressing a soluble TGFβ antagonist are protected against experimental metastasis, confirming a pro-oncogenic role for TGFβ in late-stage disease.

We are developing regulatable transgenic models that will allow us to control the stage at which we inactivate the TGFβ system. The regulatable models will also allow us to pursue the question of the importance of TGFβ dose in determining whether TGFβ will act as a tumor suppressor or an oncogene. We have already worked with mice with a genetically reduced TGFβ1 dosage (the heterozygous TGFβ1 knock-out mice), and we have shown that TGFβ is a novel form of tumor suppressor whose function is critically dose-dependent, which distinguishes it from classical tumor suppressors such as the retinoblastoma gene product. These mouse models will provide useful tools for analyzing the molecular mechanisms underlying the tumor suppressor and oncogenic functions of TGFβs in the mammary gland.

We complement the transgenic work with experiments in which we genetically modify cell lines representing different stages of the tumorigenic process, and then assess tumorigenicity in a nude mouse xenograft system. Most recently, we have used a retroviral approach to introduce the cell-autonomous TGFβ antagonists into a panel of human breast-derived epithelial cell lines of varying degrees of malignancy. This approach provides a complementary experimental tool for dissecting out the differing roles of TGFβs at different stages of cancer development. Results from all these experiments should give clinically useful insights into the functions of TGFβs during tumor initiation, promotion, and progression, and illuminate how the system could be most effectively used in novel chemopreventive and therapeutic strategies.

Our collaborators include Miriam Anver, SAIC–Frederick; Erwin Böttinger, Albert-Einstein College of Medicine, NY; Chuxia Deng, Glenn Merlino, Jim Mitchell, Angelo Russo, and Jo Anne Zujewski, NIH; Fred Miller, Barbara Karmanos Institute, MI; and Monica Tsang, R&D Systems, Inc., MN.

Recent Publications:

Tang B, et al. *Nat Med* 1998;4:802–7.

Tang B, et al. *Cancer Res* 1999;59:4834–42.

Wakefield LM, et al. *Breast Cancer Res* 2000;2:100–6.

Zujewski J, et al. *Breast Cancer Res* 2000;3:66–75.

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The Laboratory of Cellular and Molecular Biology investigates the role of growth factors, growth factor receptors, immunoreceptors, and downstream signaling molecules in normal cell growth, and aberrations that occur in these signal transduction pathways during oncogenesis. Our recent studies have concentrated on understanding physical interactions of different ligands with their cognate receptors, mechanisms that modulate receptor activation and identification, and analysis of molecules that comprise signal transduction pathways. The growth factor receptor systems that are being investigated most extensively include the keratinocyte growth factor receptor, fibroblast growth factor receptors, hepatocyte growth factor receptor (the *c-met* proto-oncogene), and frizzled receptor. The immunoreceptors under investigation are the T cell antigen receptor and mast cell FcεRI receptor.

Projects under way involve study of previously unknown ligand-receptor interactions, requirements for accessory molecules in mediating ligand binding, the generation and characterization of transgenic mice overexpressing certain growth factors, analysis of the regulatory role of receptor extracellular domains and intracellular tyrosine residues in transformation, and isolation of receptor variants that are specifically expressed in tumor cells. Studies involving signaling molecules activated in different receptor pathways include the characterization of several guanine nucleotide exchange factors, the role of Stat molecules in proliferative and functional responses, the function of adaptor molecules, and factors controlling plasma cell proliferation and transformation. Several of these effector proteins are being analyzed for their ability to induce, enhance, or inhibit transformation in vitro and to determine if their expression is altered in human tumors.

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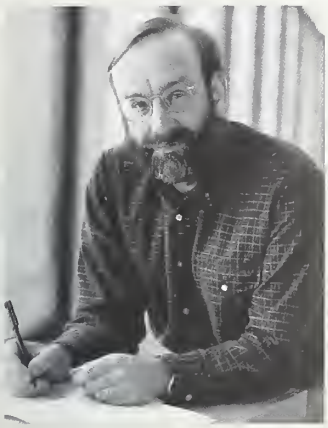

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Biography: *Dr. Samelson received his M.D. degree from Yale University in 1977. Following training in internal medicine at the University of Chicago, he carried out postdoctoral research at the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases. He joined the Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, in 1985, became senior investigator in 1988, and chief of the Section on Lymphocyte Signaling and deputy branch chief in 1995. He became chief of the Laboratory of Cellular and Molecular Biology, NCI, in 1999.*

Laboratory of Cellular and Molecular Biology
Biochemical Basis of T Cell Activation

Keywords:
cell signaling
immune response
lymphocyte
protein phosphorylation
protein-tyrosine kinase
receptor signaling
signal transduction
T cell activation
T cell receptor
T lymphocyte development

Research: T lymphocytes (T cells) are responsible for distinguishing between foreign and self-antigens, and thus for the initiation of the complex immune response that rids the body of pathogens. In clinical settings, the T cell response must be controlled in autoimmune diseases or following allograft transplantation. In both cases, T cells are inappropriately activated with pathologic consequences. In contrast, clinical oncologists hope to enhance the T cell response to tumor antigens in order to recognize and reject malignant cells. T cell recognition of foreign and self-antigens is mediated by the T cell antigen receptor (TCR). This multicomponent structure is comprised of clonally defined as well as nonpolymorphic subunits. Antigen recognition is mediated by a disulfide-linked heterodimer whose two chains each contain variable and constant region domains. Additional subunits mediate TCR signal transduction, proper receptor assembly, and transport.

Engagement of the TCR by antigenic peptide bound to a histocompatibility molecule (in humans, an HLA molecule) leads to a complex set of biochemical events in which the initial activation of protein phosphorylation is critical. Following binding of the antigen-HLA complex to the TCR, receptors aggregate, associated protein tyrosine kinases (PTK) are activated, and TCR subunits and a number of linker or adapter molecules are phosphorylated on tyrosine residues. These initial events lead to the generation of multiple protein complexes that localize at the TCR and the plasma membrane. These complexes contain enzymes that are regulated by protein phosphorylation and whose substrates are located at the membrane. Activation of protein serine/threonine kinases and pathways coupled to small G proteins, and elevation of intracellular calcium, are examples of the events that follow. These changes regulate cellular movements and lead to activation of transcriptional events that induce production of T cell lymphokines and result in differentiative events.

This laboratory has investigated many of the elements of this activation pathway. Initial studies focused on characterization of the multiple TCR subunits and discovery of TCR tyrosine phosphorylation. Protein tyrosine

kinases of the Src family, Fyn and Lck, are associated with the TCR and TCR coreceptors, respectively. The ZAP–70 PTK binds to phosphorylated TCR subunits upon receptor activation. Extensive biochemical studies on these PTKs have been performed in the laboratory. These studies characterized ZAP–70 binding to the TCR and showed how activation of this enzyme is regulated. More recently we have used conjugates of ZAP–70 and the green fluorescent protein to study the dynamics of PTK recruitment to the TCR and PTK activation.

Many molecules are substrates for the PTKs activated by TCR engagement. The tyrosine phosphorylation of some of these molecules creates binding sites for additional molecules. These linker or adaptor molecules include LAT, Grb2, Crk, SLP–76, and Cbl. LAT, initially characterized by this laboratory, is an integral membrane protein with multiple tyrosine residues. After phosphorylation these residues provide binding sites for other critical linkers, listed above, and enzymes that activate Ras and hydrolyze phospholipids. The discovery of the many proteins involved in T cell activation, the description of how these proteins interact, and characterization of the multiple signaling pathways involved in T cell activation remain ongoing, stimulating pursuits for this laboratory.

Collaborators include Robert Abraham, Duke University; Ettore Appella, Jennifer Lippincott-Schwartz, Paul Love, and Juan Rivera, NIH; and Roy Mariuzza, University of Maryland.

Recent Publications:

Samelson LE, et al. *Cold Spring Harb Symp Quant Biol* 2000;64:259–63.

Saitoh S, et al. *Immunity* 2000;12:525–35.

Zhang W, et al. *J Biol Chem* 2000;275:23355–61.

Bunnell S, et al. *Immunity* 2001;14:315–29.

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Biography: *Dr. Parent obtained B.Pharm. and M.Sc. degrees from the Université of Montréal in 1985 and 1987, respectively. She received a Ph.D. degree from the University of Illinois at Chicago in 1992. Dr. Parent then joined the laboratory of Dr. Peter N. Devreotes in the Department of Biological Chemistry of the Johns Hopkins University School of Medicine for postdoctoral training. In 1996, she was promoted to the rank of Instructor in the same department. She joined the Laboratory of Cellular and Molecular Biology at the NCI in May 2000.*

Laboratory of Cellular and Molecular Biology
Signal Transduction and Cell Migration

Keywords:
adenylyl cyclase
chemotaxis
Dictyostelium discoideum
G protein-coupled signaling
genetic analysis
inflammation

Research: All living cells can sense their environment. The term directional sensing refers to the ability of a cell to determine the direction and proximity of an extracellular stimulus. Directional sensing is needed to detect morphogens that control differentiation and attractants that direct cell migration, as in chemotaxis. This fascinating response is critical in embryogenesis, angiogenesis, neuronal patterning, wound healing, and immunity. Chemotaxis is strikingly exhibited during the life cycle of the social amoebae, Dictyostelium discoideum. During growth, these cells track down and phagocytose bacteria. When starved, they move towards secreted adenosine 3Æ–5Æ cyclic monophosphate (cAMP) signals, form aggregates, and differentiate into spore and stalk cells. The fundamental role of chemotaxis in this simple eukaryote provides a powerful system for its analysis at both genetic and biochemical levels.

Both amoebae and mammalian leukocytes use G protein-linked signaling pathways to respond to chemoattractants. Binding of the attractants to receptors of the seven transmembrane helix class leads to the dissociation of the G proteins into a and b/g-subunits. Chemotaxis is likely mediated through the b/g-subunits. In both leukocytes and amoebae, chemoattractants elicit a variety of rapid responses including transient increases in Ca2+ influx, in the intracellular messengers IP3, cAMP and guanosine 3Æ–5Æ cyclic monophosphate (cGMP), and in the phosphorylation of myosins I and II. Chemoattractants also induce actin polymerization, most likely through the activation of the Rho family of small guanosine trisphosphatases. All these events rapidly subside in the presence of persistent stimulation. This rapid inhibition may allow a migrating cell to "subtract" the ambient concentration of attractant and more accurately sense the direction of a gradient.

This laboratory is interested in studying how specific G protein-coupled signaling events translate into complex cellular responses such as cell migration. We have shown that the essential regulator of adenylyl cyclase called CRAC, a pleckstrin homology (PH) domain-containing protein, rapidly and transiently translocates to the plasma membrane upon chemoattractant

addition. Using the GFP technology, we have shown that this association occurs selectively at the leading edge of chemotaxing cells. These results suggest that the activation of adenylyl cyclase is spatially and temporally restricted. The exact mechanism of action by which CRAC binds the plasma membrane in such a specific fashion and activates adenylyl cyclase is unknown. In D. discoideum and mammalian cells, PKB—another PH domain-containing protein—also transiently associates with the leading edge of chemotaxing cells. We propose that cells target these PH domain-containing proteins to the plasma membrane in order to spatially regulate signal transduction pathways. Our goal is to identify the molecules involved in the targeting of these proteins and define the molecular mechanisms cells use to spatially activate signaling pathways as observed in chemotaxis.

Recent Publications:

Jin T, et al. *Science* 2000;287:1034–6.

Parent CA, et al. *Cell* 1998;95:81–91.

Parent CA, et al. *J Biol Chem* 1996;271:18333–6.

Parent CA, et al. *Science* 1999;284:765–70.

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Biography: *Dr. Rubin received his M.D. and Ph.D. in molecular biology from Washington University, St. Louis, MO, in 1983. Following an internal medicine residency program at the Jewish Hospital of St. Louis, he joined the Laboratory of Cellular and Molecular Biology at the NCI in 1986 as a biotechnology fellow, where he is now a senior investigator. Since 1998, Dr. Rubin has been an editorial board member of the Journal of Biological Chemistry.*

Laboratory of Cellular and Molecular Biology
Keratinocyte Growth Factor and Hepatocyte Growth Factor/Scatter Factor

Keywords:
frizzled
HGF
KGF
sFRP
Wnt

Research: From 1986 to 1997, our research primarily dealt with the purification and biological activities of two heparin-binding mitogens, keratinocyte growth factor (KGF, also known as FGF–7) and hepatocyte growth factor/scatter factor (HGF/SF). These proteins are mediators of mesenchymal-epithelial communication that can stimulate cell migration, differentiation, proliferation and tissue morphogenesis. Through collaborative studies, we have explored the role of these factors in development, tissue repair, reproductive tract biology, and neoplasia. We and others have shown that KGF has remarkable cytoprotective effects consistent with the hypothesis that it functions as a homeostatic factor to maintain epithelial barrier function. This has led to its use in clinical trials to reduce the mucositis

associated with chemoradiotherapy. We also identified two truncated HGF/SF isoforms, designated HGF/NK1 and HGF/NK2, which bind with high affinity to Met (the HGF/SF tyrosine kinase receptor), and we demonstrated that they act as partial agonists or antagonists of HGF/SF activity. We determined that the amino-terminal domain of HGF/SF retains the heparin-binding properties of the full-length protein, and established an important role for proteoglycan in HGF/SF isoform signaling.

Our ongoing KGF research is being carried out through collaborations and concerns its potential direct and indirect effects on development and function of the immune system. Collaborative studies of HGF/SF involve the definitive mapping of its heparin-binding site, which could result in the production of more potent agonists or antagonists that might be of benefit in a variety of clinical settings.

Secreted Frizzled-Related Proteins

For the past 5 years, the major focus of our research has been a soluble protein we identified that has a cysteine-rich domain homologous to the putative Wnt-binding site of Frizzleds, components of the cell surface Wnt receptor complexes. We have shown that this secreted Frizzled-related protein (sFRP–1) can bind directly to Wnt protein and modulate its activity. Thus, we believe it functions to regulate Wnt-dependent developmental processes and might also have an impact on Wnt signaling associated with neoplasia. Having generated an abundant source of recombinant sFRP–1, we are currently studying its structure/function properties and biological activity in a variety of settings. Gene targeting and transgenic projects also are under way to assess sFRP–1 function in vivo. With the support of an NCI Intramural Research Award, we have begun to screen peptide phage display combinatorial libraries to identify motifs responsible for binding to sFRP–1. Such information could lead to the development of analogs that would modulate Wnt and sFRP–1 activities. In another series of experiments, we have characterized the promoter region of the human *sfrp-1* gene and identified several potential binding sites for transcription factors.

In summary, our work has centered on the discovery and analysis of soluble polypeptide factors involved in the regulation of growth and differentiation. The projects have been highly interactive, involving several collaborations on and off the NIH campus, and have the potential to generate reagents of therapeutic relevance.

Our collaborators are Bruce Blazar, University of Minnesota; Donald Bottaro, Entremed; R. Andrew Byrd, Alan Perantoni, Stuart Rudikoff, Jane Trepel, and Paul Wingfield, NIH; Andrew Farr, University of Washington; Matthew Gillespie, St. Vincent's Institute of Medical Research, Melbourne, Australia; and Brian Kay, University of Wisconsin.

Recent Publications:

Finch PW, et al. *Proc Natl Acad Sci USA* 1997;94:6770–5.

Üren A, et al. *J Biol Chem* 2000;275:4374–82.

Yoshino K, et al. *Mech Dev* 2001;102:45–55.

Fukumoto S, et al. *J Biol Chem* 2001;276:17479–83.

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Biography: *Dr. Rudikoff obtained his Ph.D. from the University of Miami in 1970 and subsequently joined the NCI where he pursued his interest in the structure and function of antibodies. More recently, his attention has turned to cancers associated with the very cells that produce antibodies.*

Laboratory of Cellular and Molecular Biology
Biological Aspects of Plasma Cell Tumor Development

Keywords:
animal models
cancer cell growth regulation
myeloma
signal transduction

Research: Plasma cell tumors, which represent neoplasms of terminally differentiated cells in the B lymphocyte lineage, are, in humans, largely associated with an invariably fatal form of cancer known as multiple myeloma. Both treatment and patient survival for this disease have remained largely unchanged over the last 25 years. Part of the difficulty in dealing with multiple myeloma stems from a lack of understanding of the basic biology and biochemical lesions associated with both initiation and progression of these tumors. This laboratory is involved in the study of myeloma with the goal of understanding the biology of plasma cell tumor development and simultaneously assessing new therapeutic approaches. Recent studies have focused on the characterization of signal transduction pathways that contribute to the maintenance and/or progression of myeloma cells. Studies over the past several years have implicated two signaling pathways, interleukin 6 (IL–6) and insulin-like growth factor-I (IGF–I) in the development of myeloma. Activation of either of these pathways produces both a proliferative and antiapoptotic effect on myeloma cells. The importance of IL–6 has been most convincingly demonstrated in animal models wherein IL–6-null mice fail to develop plasma cell tumors. Examination of the IGF–I pathway in murine plasma cell tumors revealed constitutive activation and we have subsequently pursued characterization of this pathway in human lines. Recent studies have demonstrated that IGF–I activates two distinct signaling pathways. The first of these, the MAPKinase cascade, leads to a proliferative response. In the second, IP–3K is activated followed by downstream targets Akt and Bad, leading to an antiapoptotic effect. The relevance of IGF–I stimulation has been demonstrated in vivo wherein tumors grow larger and more rapidly in mice receiving IGF–I than in untreated controls. Current studies are aimed at identification of further downstream targets and defining their roles in proliferation/apoptosis as well as examining crosstalk between the MAPK and PI–3K pathways.

One of the more promising clinical developments in myeloma treatment is the discovery that 30 to 40 percent of patients resistant to standard therapy are responsive to thalidomide. To pursue this potential treatment regimen, we are examining the biological effects of a number of thalidomide

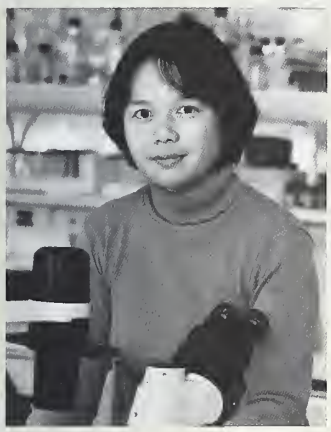
derivatives on myeloma lines. Effective compounds will be further evaluated in animal models. The mechanism of thalidomide action in myeloma is largely unknown as experiments are being initiated to address this question.

Recent Publications:

Li W, et al. *Cancer Res* 2000;60:3909–15.

Ge N, et al. *Blood* 2000;96:2856–61.

Ge N, et al. *Oncogene* 2000;19:4091–5.



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Biography: *Dr. Ying Zhang received her Ph.D. degree from University of Wisconsin–Madison in 1995. She carried out her postdoctoral training in the laboratory of Dr. Rik Derynck at University of California, San Francisco. She was an assistant research investigator in the Department of Growth and Development at UCSF before joining the Laboratory of Cellular and Molecular Biology in 2000.*

Laboratory of Cellular and Molecular Biology
Molecular Mechanisms of TGFß Signaling Pathway

Keywords:
protein phosphorylation
protein-protein interaction
serine/threonine kinase
Smad
TGFß signaling
ubiquitination

Research: Transforming growth factor-β (TGFβ) is a multifunctional growth factor with a wide range of physiological activities. These include inhibition of proliferation of a variety of cell types, negative regulation of the immune system, and positive regulation of extracellular matrix deposition. TGFβ and related factors also serve as inductive signals during development to direct tissue patterning and cell fate determination. The signaling response of TGFβ and TGFβ-related factors is mediated by a complex of two types of serine/threonine kinase receptors at the cell surface and their substrates, the Smad proteins. Following activation by the receptors, the Smads move into the nucleus to activate target gene transcription in association with other transcription factors. Alterations of TGFβ signaling pathways underlie various forms of human cancer and developmental diseases.

We have previously identified four Smad proteins and demonstrated that Smad3 associates with the ligand-activated receptor complex and is a substrate of the TGFβ receptor kinase, thus providing the first evidence for a direct biochemical link between Smads and the receptors. Upon ligand-induced phosphorylation and activation by TGFβ receptor kinases, Smad3 then forms complex with Smad4 and translocate into the nucleus where they activate transcription of target genes to mediate TGFβ-induced gene expression and growth inhibition. Transcription regulation by Smads is a complex process that involves crosstalk between different DNA responsive

elements and transcription factors to achieve maximal promoter activation and specificity. For instance, in a subset of TGFβ immediate-response target promoters, including most of the TGFβ-responsive extracellular matrix genes, AP–1 binding sites have been found to be involved in mediating the TGFβ signal. We have shown that at these promoter sites, the Smad3 and Smad4 complex physically interacts with both the DNA and c-Jun/c-Fos and functionally cooperates with c-Jun/c-Fos to mediate the TGFβ response. In collaboration with colleagues in the laboratory of Dr. Rik Derynck at UCSF, we have also characterized CBP/p300 as an obligatory coactivator in Smad-mediated transcription. These findings provide a general mechanism for how Smads induce transcription in cooperation with a variety of transcription factors.

In the future, we will continue to work on the signal transduction mechanism of the TGFβ, particularly the mechanisms that regulate the activity of the Smad proteins, and the physiological role of their function. Using combinational molecular and biochemical approaches, we have identified a novel ubiquitin E3 ligase, Smurf2, as an interacting partner for Smads. We demonstrated that Smurf2 selectively interacts with the receptor-activated Smads—e.g., BMP pathway-specific Smad1 and Smad5—thereby triggering their ubiquitination and degradation. We are currently extending these findings to assess how selectively targeting Smads' degradation is regulated and how the ubiquitin-mediated proteolysis of Smads affects cell growth, tissue differentiation, and other biological functions regulated by the TGFβ superfamily signaling.

In addition, we are also interested in how TGFβ signaling converges with other pathways in response to growth factors and consequent activation of mitogen-activated protein kinase (MAPK) pathways. We would like to understand the role of this crosstalk in controlling TGFβ-regulated gene transcription, cell proliferation, extracellular matrix production, and tumor progression. In the meantime, a long-term research program using mouse genetics to address the physiological and pathological roles of the TGFβ/Smad signaling in tumorigenesis has also been initiated.

Recent Publications:

Zhang Y, et al. *Nature* 1996;383:168–72.

Zhang Y, et al. *Nature* 1998;394:909–13.

Zhang Y, et al. *Trends Cell Biol* 1999;9:274–9.

Zhang Y, et al. *Curr Biol* 1997;7:270–6.

Laboratory of Cellular Carcinogenesis and Tumor Promotion

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The Laboratory of Cellular Carcinogenesis and Tumor Promotion (LCCTP) conducts an integrated research program designed to elucidate the cellular and tissue changes associated with specific stages of carcinogenesis, to define the molecular mechanisms involved, and to develop rational approaches for cancer prevention. Studies are performed in vivo in experimental animals, in vitro in cell and organ culture, and on tissues and cells obtained from human volunteers and cancer patients. Senior members of the laboratory staff have received considerable recognition in the fields of molecular pharmacology, experimental carcinogenesis, and biochemical epidemiology.

The breadth of expertise and interests in the laboratory provides a unique environment for a comprehensive research program exploring cancer causation in cell, animal, and human models, and for translating research findings into advances for diagnosis, prevention, and treatment of cancers. The laboratory is composed of eight principal investigators in five sections, and each is charged with major responsibility for a segment of the laboratory goals. The laboratory also manages two CCR Core facilities, the Germline Mutation Core and the Flow Cytometry Core.

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Molecular Mechanisms of Tumor Promotion Section

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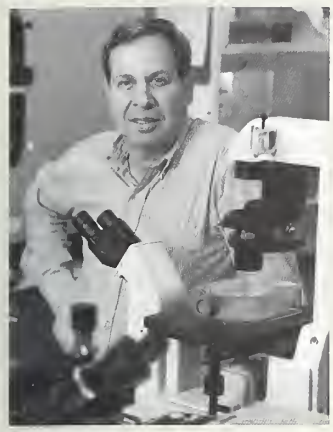
Daniel Schoeffner	Postdoctoral Fellow
Makoto Takahama	Adjunct Investigator
Masaharu Yamazaki	Postdoctoral Fellow



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Biography: *Dr. Yuspa received his B.S. from Johns Hopkins University and his M.D. from the University of Maryland Medical School. He completed his internship and residency at the Hospital of the University of Pennsylvania and has been a senior investigator at the NCI since 1972. Among his honors are the Lila Gruber Award of the American Academy of Dermatology and the Clowes Award from the American Association for Cancer Research. Dr. Yuspa is author of more than 300 publications in the fields of carcinogenesis and epithelial differentiation.*

Laboratory of Cellular Carcinogenesis and Tumor Promotion
The Pathogenesis of Squamous Cell Cancer

Keywords:
carcinogenesis
oncogenes
protein kinase
transcription factors
tumor suppressor genes

Research: The elucidation of specific genetic changes associated with early events in human cancer pathogenesis has focused efforts to relate these changes to specific characteristics of the neoplastic phenotype. The mouse skin carcinogenesis model lends itself well to these analyses. Mutations of the *ras* gene family and p53 are frequently detected in human squamous cell carcinomas and mouse skin carcinomas. To analyze the consequences of oncogenic *ras* in keratinocytes, we have used a combined in vivo-in vitro approach, culturing wild-type or genetically modified keratinocytes, producing additional genetic or biochemical modifications in vitro, and often grafting modified cells as skin grafts in nude mice to assess the consequences of the alteration in vivo. In keratinocytes, activation of the *ras* gene initiates an autocrine loop through the epidermal growth factor receptor (EGFR). Keratinocytes from EGFR null mice also form tumors after *ras* activation. While these tumors are small, they have a very high growth fraction. In the absence of EGFR, proliferating tumor cells migrate into the suprabasal compartment prematurely, enter the differentiation program, and experience cell cycle blockade. The *ras* oncogene also causes activation of protein kinase Cα and modification of AP–1 transcriptional activity, producing characteristic aberrant expression of keratinocyte genes. *Ras* activation also leads to inactivation of PKCδ through tyrosine phosphorylation mediated by src family kinases. We now understand that PKCδ is a component of the keratinocyte death pathway, and its inactivation results in defective terminal cell death, a requirement for squamous tumor formation. Current studies reveal that PKCδ targets mitochondria, alters mitochondrial membrane potential, and causes caspase activation. We are now evaluating pharmacologic and genetic methods to overexpress or reactivate PKCδ in tumors to restore a death pathway.

Inactivation of *p53* in conjunction with *ras* gene activation is associated with rapid premalignant progression and anaplastic mouse skin tumor formation. *p53*-dependent transcriptional activity increases during keratinocyte differentiation. Therefore, we exploited *p53* null keratinocytes to identify novel genes that are *p53* dependent in maturing keratinocytes using

differential display. A chloride channel protein of mitochondria that is upregulated by both *p53* and TNFα was discovered by this approach. When overexpressed, this protein (mtCLIC) causes apoptosis mediated by cytochrome c release and caspase activation. MtCLIC is upregulated in keratinocytes during differentiation and after DNA damage, and antisense mtCLIC prevents *p53*-mediated apoptosis. MtCLIC expression is decreased during experimental tumor progression and in a variety of human epithelial cancers. One of the compelling reasons for focusing studies in a model where genetic changes accelerate premalignant progression is to identify tumor markers associated with premalignant lesions at high risk for progression and to define the pathways involved. Such information is essential to address intervention strategies designed to prevent malignant conversion.

Recent Publications:

Fernandez-Salas E, et al. *J Biol Chem* 1999;274:36488–97.

Glick A, et al. *Proc Natl Acad Sci USA* 1999;96:14949–54.

Hansen LA, et al. *Cancer Res* 2000;60:3328–32.

Rutberg SE, et al. *Cancer Res* 2000;60:6332–8.

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Biography: *Dr. Peter Blumberg received both his A.B.,* summa cum laude, *and his Ph.D. from the Department of Biochemistry and Molecular Biology, Harvard University. He was Helen Hay Whitney fellow at the Cancer Center, Massachusetts Institute of Technology, and was assistant and associate professor of pharmacology at Harvard Medical School. Since 1981, Dr. Blumberg has been chief of the Molecular Mechanisms of Tumor Promotion Section.*

Laboratory of Cellular Carcinogenesis and Tumor Promotion
Mechanism of Action of Phorbol Esters and Related Derivatives

Keywords:
capsaicin
pain
protein kinase C
signal transduction
tumor promotion

Research: The central focus of the program of the Molecular Mechanisms of Tumor Promotion Section is to elucidate the pathways of signal transduction for the lipophilic second messenger sn-1,2-diacylglycerol, as well as for the exogenous, ultrapotent analogs, the phorbol ester tumor promoters. Protein kinase C (PKC), the major receptor for these compounds, receives particular emphasis, but attention is also directed at identifying factors responsible for differences in regulation between protein kinase C, protein kinase D, the chimaerins, and RasGRP. As part of an extensive collaborative effort, we seek to understand the nature and consequences of phorbol ester binding to the regulatory domain of PKC and its related receptors. An important milestone in this ongoing program is the development of synthetic ligands with nM

affinities and with unique selectivity. Another is the identification of novel structural classes of ligands. The iridals, for example, are natural products derived from *Iris spp.* that possess modest selectivity for the RasGRP family of receptors. A second aspect of our program is to understand how the behavior of the binding domain is modified in the context of the intact receptor molecule, the membrane, and the cell. Using site-directed mutagenesis, we are analyzing the relative contributions of specific hydrophobic residues in the C1 (ligand-binding) domain to ligand binding and to interaction of the C1 domain with the lipid bilayer. Using fusion constructs between green fluorescent protein and protein kinase Cδ, we find that different ligands can translocate the protein kinase C to different intracellular compartments. Since localization will determine access to substrates, determining the structural basis for differential localization is of great interest. Using homologous ligands of varying lipophilicity, we find that lipophilicity is one important determinant. A third aspect is to understand the role and ligand selectivity of the classes of phorbol ester receptors other than protein kinase C. Here, particular attention is being directed toward RasGRP, a family of receptors that directly links diacylglycerol signaling with Ras activation, and toward protein kinase D, a receptor family that appears to be regulated both directly by phorbol esters as well as indirectly by activation by protein kinase C.

Our second general area of focus is on the mechanism of action of capsaicin. These studies developed from our finding that resiniferatoxin, a natural product structurally related to the phorbol esters, acts not on protein kinase C but rather as a unique, ultrapotent analog of capsaicin, the pungent constituent of red pepper. These studies are driven by the potential of such analogs to desensitize capsaicin-sensitive neurons, which are involved in pain transmission, and thus to represent a novel class of nonnarcotic analgesics. Using resiniferatoxin, we have been able to develop a ligand-binding assay for vanilloid receptors. As part of a collaborative effort with medicinal chemists, we seek to identify more potent, synthetic agonists, to design antagonists, and find molecules that function as partial agonists. At the biological level, we wish to understand how ligand binding and cellular assays of activity translate into whole animal response. At the mechanistic level, we seek to understand both the structural basis for ligand interaction and the regulatory processes responsible for desensitization of pain pathways.

Our collaborators include Chaya Brodie, Bar-Ilan University, Israel; Marcelo Kazanietz and Jeffrey Winkler, University of Pennsylvania; Alan Kozikowski, Georgetown University; Jeewoo Lee, Seoul National University, Korea; Franz-Josef Marner, Mainz University, Germany; Victor Marquez and Stuart Yuspa, NIH; George Pettit, Arizona State University; James Stone, University of Alberta, Canada; and Shaomeng Wang, University of Michigan.

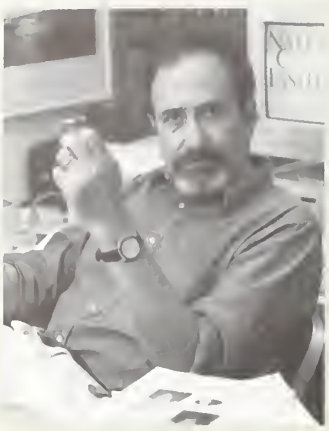
Recent Publications:

Lorenzo PS, et al. *Cancer Res* 2001;61:943–9.

Mandil R, et al. *Cancer Res* 2001;61:4216–619.

Pak Y, et al. *J Med Chem* 2001;44:1690–701.

Wang QJ, et al. *J Biol Chem* 2001;276:19580–7.



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Laboratory of Cellular Carcinogenesis and Tumor Promotion
The Involvement of Retinoids in Carcinogenesis and Differentiation

Keywords:
breast cancer
chemical carcinogenesis
chemoprevention
lung cancer
retinoids

Research: There is general agreement that carcinogenesis results from alterations in normal differentiation pathways. It is also well documented that retinoids are essential for the maintenance of normal epithelial differentiation and function. This research effort tests the hypothesis that something goes fundamentally wrong in the metabolism and function of retinoids during the process of carcinogenesis in general and that this may significantly contribute to malignant progression.

Expression of a smaller lecithin:retinol acyl transferase transcript and reduced retinol esterification in MCF–7 cells compared to normal epithelial human breast cells. The metabolic pathway responsible for the synthesis of retinoic acid (RA) starts with the hydrolysis of the retinyl esters to generate retinol, which is eventually oxidized to RA. Accumulation and availability of retinyl ester is, therefore, fundamental to warrant continued supply of RA. Retinyl ester concentration is homeostatically regulated by RA through an autoregulatory loop, which acts on lecithin:retinol acyl transferase (LRAT). We used normal human mammary epithelial (HMEC) cells and a human breast cancer cell line (MCF–7) engineered to stably express a truncated retinoic acid receptor (RARα403) with strong RAR dominant-negative activity. These cells were used to study the involvement of the RARs on RA-stimulated transcription of the LRAT gene. HMEC cells expressed a retinoid-upregulated 5 Kb LRAT transcript and synthesized retinyl esters from ^{3}H-retinol. In sharp contrast, human carcinoma MCF–7 cells failed to express the 5 Kb LRAT transcript and to synthesize retinyl esters. Instead, they expressed a 2.7 Kb LRAT transcript. Interestingly, both transcripts were upregulated by inclusion of retinoic acid in the culture medium. Stable expression of the dominant-negative construct RARα403 blunted the upregulation of LRAT mRNA by RA. In conclusion, LRAT activity is strongly downregulated in MCF–7 cells compared to normal mammary cells. The decline in retinyl ester synthesis, due to impaired LRAT activity, is accompanied by lack of expression of the 5 Kb mRNA in these cells and the expression of a shorter (2.7 Kb) transcript, suggesting a fundamental change in retinoid homeostasis in the human breast cancer cell line MCF–7.

Inhaled 13-cis-retinoic acid is an effective chemopreventive agent against lung adenomas in the AJ mouse. In the past year inhalation technology was utilized as an approach to lung cancer chemoprevention by 13-cis-retinoic acid (isotretinoin). This was based on the hypothesis that inhaled isotretinoin might provide sufficient drug to the target cells for efficacy, while avoiding systemic toxicity. To test this hypothesis, carcinogen-treated A/J mice were exposed to isotretinoin aerosol. Male A/J mice were given single intraperitoneal (IP) doses of urethane, a common experimental lung carcinogen, or benzo(a)pyrene (BaP) or 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), putative major carcinogens in tobacco smoke. The next day, exposures to isotretinoin aerosols were started. After 10 to 16 weeks, the mice were sacrificed to count areas of pulmonary hyperplasia and adenomas. For all carcinogens, the mice exposed to isotretinoin showed reduction of tumor multiplicity ranging from 56 to 80 percent ($p<0.005$). Inhaled 13-*cis* RA had a significant stimulatory activity on TGase II in lung ($P<0.001$), but not liver tissue ($P<0.544$). Further, retinoic acid receptors RARα, β, and γ were found to be highly sensitive biomarkers of retinoid exposure in rats and mice. Inhaled 13-*cis* RA (at a daily deposited dose of 6.4 mg/kg/day in rats) was effective in upregulating the expression of lung tissue RARα, β, and γ at day 1 (RARα by 3.4-fold; RARβ by 7.2-, and RARγ by 9.7-fold), and at day 17 (RARα by 4.2-fold; RARβ by 10.0-, and RARγ by 12.9-fold). A daily deposited dose of 1.9 mg/kg/day was also effective, but required longer exposures. At day 28 of exposure, lung RARα was induced by 4.7-fold; RARβ by 8.0-, and RARγ by 8.1-fold. Inhalation of the same aerosol concentration in graded exposures, for durations from 5 to 240 min daily for 14 days, induced all RARs from 30.6- to 74-fold at the shortest exposure time. By contrast, long-term feeding of a diet containing pharmacological RA (30μg/g diet) failed to induce RARs of SENCAR mouse lung tissue, though it markedly induced liver RARs (RARα by 21.8-fold; RARβ by 13.5-, and RARγ by 12.5-fold). A striking increase of RARα expression was evident in the nuclei of hepatocytes from these mice. A time-course study revealed that pharmacological dietary RA stimulated RARα, β, and γ already at day 1 by 2-, 4-, and 2.1-fold respectively over physiological RA (3μg/g diet) without any measurable effect on lung tissue RARs. These data demonstrate that 13-*cis* RA delivered to the lung tissue of rats is a potent stimulant of lung RARs, but has no effect on liver RARs. Conversely, dietary RA stimulates liver RARs, but fails to affect lung tissue RARs. These data support the concept that epithelial delivery of chemopreventive retinoids to lung tissue may be a more efficacious way for the upregulation of the retinoid receptors and chemoprevention of lung carcinogenesis.

Our collaborators include Alan Dahl, A. Imondi, and Michael Placke, Battelle Pulmonary Therapeutics, Columbus, Ohio; and Robert Dedrick and James Mulshine, NIH.

Recent Publications:

Okamoto K, et al. *Cancer Chemother Pharmacol* 2000;46:128–34.

Dahl AR, et al. *Clin Cancer Res* 2000;6:3015–24.

Wang D, et al. *Clin Cancer Res* 2000;6:3636–45.

Andreola F, et al. *Biochem Biophys Res Commun* 2001;279:920–4.

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Biography: *Dr. Glick received his B.A. from Oberlin College and a Ph.D. from the Biology Department of Yale University in 1987. He was a postdoctoral fellow in the Laboratory of Chemoprevention at the NCI from 1987 to 1990, then joined the Laboratory of Cellular Carcinogenesis and Tumor Promotion in 1990 as a senior staff fellow. He is currently a tenure track investigator.*

Laboratory of Cellular Carcinogenesis and Tumor Promotion
The Role of TGFß in the Pathogenesis of Squamous Cell Cancer

Keywords:
cancer cell growth regulation
keratinocyte
senescence
skin carcinogenesis
tetracycline control
TGFß
transgenic mice

Research: TGFβ1 is a member of a large family of multifunctional secreted polypeptides that regulate proliferation, differentiation, apoptosis, and matrix production. Most epithelial cancers in humans and animal models have some type of disruption of the TGFβ signaling pathway ranging from alterations in ligand expression to mutational inactivation of signaling proteins involved in the growth inhibitory response. Paradoxically, overexpression of TGFβ acts to enhance the malignant phenotype. The research focus of our laboratory is to understand the mechanism of TGFβ action in the pathogenesis of epithelial cancers, using multistage carcinogenesis of the mouse epidermis as a model system. We utilize a combined in vitro and in vivo approach with a variety of transgenic and gene knock-out mouse models to study the dual role of TGFβ in multistage carcinogenesis.

Our group was the first to demonstrate that loss of autocrine TGFβ1 expression accelerated premalignant progression of benign tumors with compromised genomic stability as a potential mechanism driving tumor progression. Keratinocytes with defects in either autocrine TGFβ1 production or response do not become senescent but rapidly become aneuploid, have an elevated number of chromosome breaks, and undergo malignant transformation with high frequency. More recently we have shown that TGFβ1 expression and signaling are necessary for replicative senescence induced by an activated *ras* oncogene. Since Smad proteins mediate TGFβ signal transduction, our current focus is understanding the contribution of each Smad to the senescence response. Using a unique set of nonneoplastic TGFβ1 null and control keratinocyte cell lines developed in our group, we have found that the TGFβ1 null cells are deficient in the DNA repair enzyme methylguanine methyltransferase (MGMT), and this is due to hypermethylation of CpG islands in the MGMT promoter. Interestingly, hypermethylation of the MGMT promoter is a frequent event which can be detected in vivo in premalignant squamous lesions. These results suggest a novel role for TGFβ signaling in the hypermethylation of promoters that occurs during progression of human and animal cancers.

To examine the pathological and molecular effects of overexpression of TGFβ1 at defined stages of tumor progression, we have generated a new bitransgenic model for conditional expression of TGFβ1 using the Tet-on and Tet-off regulatory system. Doxycycline-dependent overexpression of TGFβ1 in normal adult epidermis causes a progressive alopecia and dermatitis accompanied by follicular hyperplasia. We are currently analyzing the molecular basis for this pathology using cDNA microarray technology. These studies should lead to a clearer mechanistic understanding of the disparate actions of TGFβ1 in carcinogenesis and provide new therapeutic targets for human cancers in which TGFβ1 is overexpressed.

Postdoctoral positions for any of these projects are currently available.

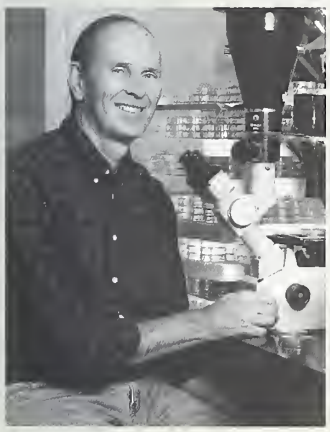
Recent Publications:

Tremain R, et al. *Oncogene* 2000;19:1698–709.

Diamond I, et al. *J Invest Dermatol* 2000;115:788–94.

Yamada H, et al. *J Biol Chem* 2001; in press.

Liu X, et al. *Proc Natl Acad Sci USA* 2001; in press.



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Biography: *Dr. Hennings received his B.S. from Ohio State University and obtained his Ph.D. in experimental oncology from the McArdle Laboratory at the University of Wisconsin. As the recipient of a postdoctoral fellowship from the American Cancer Society, he trained at the University of Oslo. Since 1971, Dr. Hennings has been a member of the In Vitro Pathogenesis Section, now in the Laboratory of Cellular Carcinogenesis and Tumor Promotion. In 1996, Dr. Hennings became the assistant chief of this laboratory.*

Laboratory of Cellular Carcinogenesis and Tumor Promotion
Genetic Studies of Susceptibility to Skin Carcinogenesis

Keywords:

cancer genetics
DNA repair
keratinocyte
K-*ras*
methylation
mutated *ras* oncogenes
phorbol ester
protein kinase C
skin carcinogenesis

Research:

The Genetics of Susceptibility to Development of Skin Tumors

The major emphasis of our research is to identify genes involved in determining susceptibility and resistance to skin carcinogenesis. We have shown that three inbred strains of susceptible mice, designated SENCARA/Pt, SENCARB/Pt, and SENCARC/Pt, develop both benign and malignant skin tumors when treated by standard initiation-promotion protocols. Resistance to skin tumor development was semidominant in the F1 progeny of the SENCARA/Pt x BALC/cAnPt cross. After initiation by a low-dose of 7,12-dimethylbenz[a]anthracene (DMBA) and promotion by repeated exposures to 12-0-tetradecanoylphorbol-13-acetate (TPA), tumors developed in all of the SENCARA/Pt mice, none of the BALB/cAnPt mice, and only

18 percent of the F1 progeny. When tumors were induced in the (BALB/cAnPt x SENCARA/Pt)F1 x SENCARA/Pt backcross mice, only 16.5 percent displayed the susceptible phenotype, indicating that multiple genes determine susceptibility to skin carcinogenesis. A genome scan to associate chromosomal regions with susceptibility and resistance loci indicated that a locus on chromosome 5 is important in the control of susceptibility of SENCARA/Pt mice, with potential additional susceptibility genes on chromosomes 9, 11, and 12. Congenic mice bred with the putative susceptibility region of chromosome 5 from SENCARA/Pt mice on a BALB/cAnPt background did not develop more tumors than BALB/cAnPt mice, indicating that this locus alone is not sufficient to account for susceptibility. In future studies, we will compare the genes involved in the development of benign tumors with the genes involved in the conversion of benign to malignant skin tumors.

Mechanism of Action of Tumor Initiators and Promoters

Thapsigarin, a weak tumor promoter for mouse skin, and ionomycin, a Ca^{2+} ionophore with no promoting activity, act by different mechanisms to increase intracellular free Ca^{2+} levels in keratinocytes. Neither thapsigarin nor ionomycin activate protein kinase C, but each displayed synergism with the phorbol ester tumor promoter TPA in keratinocyte culture assays for promotion and terminal differentiation. In order to determine whether agents that elevate intracellular Ca^{2+} are synergistic with TPA for promotion of skin tumor formation, DMBA-initiated mice were treated with either thapsigargin or ionomycin 5 mins before each promoting application of TPA. At sub-optimal promoting doses of TPA, a synergism for tumor promotion (resulting in a two- to three-fold increase in number of tumors per mouse) was found for both agents. This increase in tumor induction could result from the elevation of intracellular Ca^{2+} coupled with activation of protein kinase C.

Mezerein, a relatively weak promoter for DMBA- or urethane-initiated tumors, induces the rapid appearance of a novel subpopulation of papillomas in mice initiated by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). These tumors appear after only 4 weeks of promotion and some convert to malignancy within 10 weeks after initiation. More than 40 percent of the early-arising tumors show codon 12 K-*ras* mutations, a rare mutation in mouse skin tumors induced by other protocols. The mechanism of the selective action of mezerein in promoting a subpopulation of MNNG-initiated tumors bearing mutated K-*ras* is being explored in keratinocytes in cell culture.

A Possible Role for O^6-Methylguanine DNA Methyltransferase in Skin Carcinogenesis

The O^6-methylguanine adducts formed as a result of interaction of carcinogenic alkylating agents with DNA are relevant to carcinogenesis and are repaired by the DNA repair enzyme O^6-methylguanine DNA methyltransferase (MGMT). The expression of MGMT is controlled by methylation of CpG islands in the *MGMT* promoter. In a large sample of skin tumors, we have found that (1) nearly half of benign and malignant skin tumors display hypermethylation of the *MGMT* promoter, (2) methylation of the *MGMT* promoter reduced expression of the MGMT protein, and (3) methylation of the *MGMT* promoter increased with time after initiation,

suggesting a role in tumor progression. We are currently examining whether the *p53* tumor suppressor gene may be involved in the mechanism of the loss of MGMT expression in skin tumors.

Our collaborators are Beverly Mock and Michael Potter, NIH.

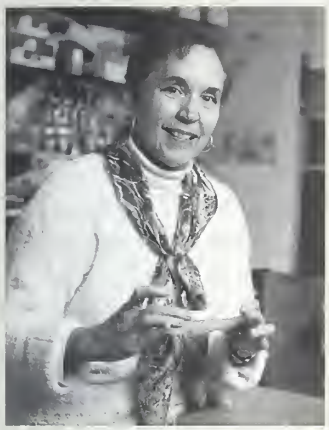
Recent Publications:

Hennings H, et al. *Mol Carcinog* 1997;20:143–50.

Mock BA, et al. *Carcinogenesis* 1998;19:1109–15.

Weinberg WC, et al. *Cancer Res* 1999;59:2051–4.

Rehman I, et al. *Mol Carcinog* 2000;27:298–307.



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Biography: *Dr. Miriam Poirier received a B.Sc.* magna cum laude *in chemistry. She then obtained an M.Sc. in experimental oncology from the McArdle Laboratories at the University of Wisconsin under Drs. James A. and Elizabeth C. Miller, and she received her Ph.D. from Catholic University, Washington, D.C. Since 1997, Dr. Poirier has been the head of the Carcinogen-DNA Interactions Section of the Laboratory of Cellular Carcinogenesis and Tumor Promotion.*

Laboratory of Cellular Carcinogenesis and Tumor Promotion
Carcinogen-DNA Interactions: Their Extent, Mechanisms, and Biological Consequences

Keywords:
chemical carcinogenesis
DNA adducts
HIV–1
human biomonitoring
mitochondria

Research: This research effort has concentrated on mechanisms of interactions between chemical carcinogens, some of which are commonly used drugs, and DNA. Topics under study include both the extent of DNA adduct formation and persistence, and the biological consequences of DNA damage in cultured cells, animal models, and human tissues. DNA adduct processing in specific structural (mitochondrial, telomeric) genomic regions, and in functional (transcribing, nuclear matrix) regions, are also subject to investigation. The DNA adduct data are correlated with specific effects of carcinogen/drug exposure, including tumorigenesis, clinical response, specific toxicities, and functional impairment of target organs and organelles. We are particularly interested in searching out themes that appear to be common in both animal models and human subjects with the intention of applying the knowledge gained to either enhance or reduce a specific effect in humans.

The work encompasses issues relevant to human toxicity as well as human clinical response. The compounds of intensive investigation include anti-retroviral nucleoside analogs (zidovudine or AZT and lamivudine or 3TC),

polycyclic aromatic hydrocarbons (PAHs), and tamoxifen. The nucleoside analog drug, AZT, which incorporates into DNA causing arrest of the replicating DNA strand, is currently used to prevent fetal HIV–1 transmission in infected pregnant women. However, it is a moderately strong transplacental carcinogen in mice and becomes incorporated into the DNA of newborn mouse organs. AZT–DNA incorporation has also been observed in cord blood DNA of human infants, and in organ DNA of fetal *Erythrocebus patas* and *Macaca mulatta* monkeys exposed transplacentally for either long (last 50 percent of gestation) or short (4 hr before birth) periods of time, respectively. Documentation of AZT incorporation into mitochondrial DNA (mtDNA) of fetal patas monkeys is consistent with the mtDNA depletion observed in cardiac and skeletal muscle, and abnormal mt morphology revealed by electron microscopy (EM). In the same organs, dose-related abnormalities in oxidative phosphorylation enzyme activities associated with transplacental AZT and 3TC exposure include a severe depletion of Complex I and increases in Complexes II and IV.

A second drug carcinogenic in rodents is tamoxifen (TAM), which is administered in the clinic to prevent breast cancer. Oral administration of TAM causes liver tumors and DNA adduct formation in rats, and increased incidence of endometrial cancers in women. However, TAM–DNA adduct formation in human tissues has been controversial and unresolved due to lack of sufficiently specific and sensitive methods. We have elicited an antiserum against TAM–DNA, and established and validated a highly sensitive immunoassay able to detect three TAM–DNA adducts in 10^9 nucleotides. The TAM–DNA antiserum has been used in quantitative and immunohistochemical assays to detect TAM–DNA adduct formation in human endometrium. Molecular dosimetry studies of individuals in China exposed to high levels of PAHs are in progress. An immunohistochemical method to quantitate PAH–DNA adduct levels in human esophageal biopsies has been developed using the Chroma Vision ACIS. Microdensitometry of PAH–DNA nuclear signal (fast red color intensity) has been quantified in human keratinocytes exposed to different levels of benzo[a]pyrene (BP), and nuclear color intensity increase with BP dose gives a correlation coefficient of 0.99. Staining of human esophageal samples is positive and the method is being applied to correlate PAH–DNA levels with esophageal cancer risk.

Among our collaborators are Sanford Dawsey, Mark Roth, Nathaniel Rothman, Gene Shearer, and Rashmi Sinha, NIH; Bhalchandra Diwan, SAIC–Frederick; Andrea Kovacs, UCLA; Bernadette Schoket, National Institute of Public Health, Budapest, Hungary; Vernon Walker, Lovelace Respiratory Research Institute, Albuquerque, NM; and Ainsley Weston, NIOSH–CDC, Morgantown, WV.

Recent Publications:

Olivero OA, et al. *Ann NY Acad Sci* 2000;918:262–8.

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Meng Q, et al. *Proc Natl Acad Sci USA* 2000;97:12667–71.

Olivero OA, et al. *Exp Biol Med* 2001;226:446–9.

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Biography: *Dr. Shen received her B.A. in biology from East China Normal University and her Ph.D. degree in molecular biology from Columbia University in 1992. She was a postdoctoral fellow with Dr. Andrew McMahon of Harvard University in mouse genetics and mammalian development. From 1996 to 1999, she was a National Research Council (NRC) associate with Dr. Heiner Westphal at the National Institute of Child Health and Human Development, NIH. In October 1999, she joined the Laboratory of Cellular Carcinogenesis and Tumor Promotion as a tenure track investigator and director for the Germline Mutation Core Facility in the Center for Cancer Research.*

Laboratory of Cellular Carcinogenesis and Tumor Promotion
Establishment and Characterization of Animal Models for Complex Human Diseases Using Genetically Modified Mouse Strains

Keywords:
animal model
c-Ret
GDNF
genetic susceptibility
haploinsufficiency
Hirschsprung disease
knockout
mouse genetics
peptide growth factor

Research: We have been using gene-targeting strategy to study the in vivo function of genes involved in mammalian development and to establish animal models for human diseases. These mouse models offer important insights into the mechanisms for disease etiology and pathogenesis in humans. One of the genes we are studying is glial cell line-derived neurotrophic factor (GDNF), a distant member of the TGFβ superfamily. *Gdnf* knock-out mice display severe defects in kidney organogenesis and enteric nervous system development, leading to perinatal lethality. The complete absence of kidney and enteric neurons in the gastrointestinal (GI) tract in *Gdnf*–/– mice provides molecular etiology for human congenital diseases such as bilateral renal aplasia and Hirschsprung disease. Strikingly similar defects found in *Gdnf*–/– and *c-ret*–/– mutant mice also suggest a direct functional link between GDNF and c-RET, an orphan receptor tyrosine kinase. *c-RET* mutations are found in several human cancer syndromes and HSCR. Biochemical studies demonstrate that c-Ret and GFRα1, a glycosylphosphatidylinositol (GPI)-linked cell surface protein, form a receptor complex for GDNF. Binding of GDNF to GFRα1 activates c-Ret tyrosine kinase and downstream signaling.

While mutations in *c-RET* locus are currently estimated to account for up to 25 percent of all Hirschsprung cases, approximately 70 percent of the patients have unknown genetic defects. Most of these cases are defined as sporadic, i.e., without a family history. Our study using *Gdnf* mutant mice suggests that GDNF may be one of the candidate genes for Hirschsprung disease susceptibility. Using a population genetics approach, we show that GDNF insufficiency confers Hirschsprung disease susceptibility in *Gdnf* heterozygous (*Gdnf*+/–) offspring. These *Gdnf*+/– mice exhibit a reduction in the number of enteric neurons in the GI tract, varying degrees of gut motility deficits, and early onset lethality. The mortality of the *Gdnf*+/– mice is

remarkably similar to the sibling risk of Hirschsprung disease in humans. A combination of tissue recombination study and a transgenic marking strategy that labels all enteric neurons in the gut demonstrates that GDNF plays a critical role in the colonization of enteric neurons in the GI tract. These results establish a mouse model that is in closest resemblance to human Hirschsprung disease and provide insights into the developmental mechanism for disease pathogenesis. The combined gene targeting technology and population study strategy developed in our laboratory may be generally applicable to dissect the genetic basis of disease susceptibility for many complex, late onset human diseases.

In the future, we will continue to use the mouse as a major research tool to study the functions and dysfunctions of GDNF and other signaling molecules during normal development and in disease processes. We are developing conditional expression systems to manipulate the levels of GDNF during development and in adult mice. We will establish technical conditions to study how small changes in expression levels of a rate-limiting molecule during development may lead to susceptibility of individuals to diverse diseases later in life, from congenital Hirschsprung disease to cancer. Moreover, we are characterizing downstream molecular targets of the GDNF/c-Ret signaling pathway. These research efforts will provide us with novel insights into the molecular basis for human disease susceptibility and progression, and for developing effective diagnosis and therapeutics for human diseases.

Recent Publications:

Messer CJ, et al. *Neuron* 2000;26:247–57.

Granholm AC, et al. *J Neurosci* 2000;20:182–90.

Oppenheim RW, et al. *J Neurosci* 2000;20:5001–11.

McCright B, et al. *Development* 2001;128:491–502.

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Laboratory of Cellular Carcinogenesis and Tumor Promotion

Regulation of Breast Cancer Development and Angiogenesis by Vascular Endothelial Growth Factor and Tissue Inhibitor of Metalloproteinases-1

Keywords:
breast cancer
neuropilin-1
TIMP–1
tumor angiogenesis
VEGF

Research: Growth of solid tumors is dependent on the development of neovessels which both provide nutrients and serve as an entry route for disseminating tumor cells into the systemic circulation. Tumor neovascularization can be suppressed by disrupting one or more of the angiogenic steps which include adhesion, proteolytic degradation of the extracellular matrix, migration, proliferation, and organization of endothelial cells into functional capillaries. Our research is focused on breast cancer with the main emphasis on vascular endothelial growth factor (VEGF) and tissue inhibitor of metalloproteinases-1 (TIMP–1) and their significance in the regulation of tumor development and neovascularization.

VEGF is a potent endothelial mitogen that is produced by various types of cancer cells. It also increases vascular permeability to plasma proteins and, as such, it may indirectly promote tumor growth through stimulation of stromal formation. This is particularly relevant in human breast carcinomas where the stromal component often occupies more than half of the tumor mass. We used a one-vector retroviral Tet system to demonstrate the importance of VEGF overexpression in promoting growth of human breast carcinoma xenografts in nude mice, even at the prevascular stage of tumor development. In contrast, antisense-mediated suppression of VEGF gene expression from the time of tumor cell inoculation resulted in growth arrest in the same model. To better understand the role of VEGF in mammary tumor development and progression, we employ transgenic mouse models with either overexpression or conditional knockout of the VEGF gene in mammary epithelial cells.

As an inhibitor of matrix metalloproteinases (MMPs), TIMP–1 plays an important role in both physiological and pathological tissue remodeling. It is also a growth factor for different cell types in vitro and was previously identified as an erythroid potentiating activity (EPA) based on its ability to simulate growth of erythroid progenitor cells. Recombinant TIMP–1 protein has been shown to inhibit tumor growth in vivo but there is emerging

literature associating TIMP–1 overexpression with malignancy and poor prognosis. These apparent paradoxical effects of TIMP–1 on tumor growth are incompletely understood. We established transgenic mouse models to evaluate the effects of TIMP–1 on different stages of mammary carcinogenesis with special emphasis on tumor neovascularization. These include mice with liver targeting of the transgene, resulting in high circulating levels of biologically active TIMP–1 protein, and mice with mammary epithelial targeted expression of the TIMP–1 transgene. Mammary carcinogenesis studies so far have shown a significant decrease in primary tumor growth but not metastatic incidence in the mice with high circulating TIMP–1 levels. Ongoing studies place particular emphasis on the effect of TIMP–1 on the initiation of neovascularization during the early stages of mammary tumor development in the TIMP–1 transgenic models.

Collaborators include Napoleone Ferrara and Hans Peter Gerber, Genentech; Glenn Merlino and Lothar Hennighausen, NIH; and Kay-Uwe Wagner, University of Nebraska.

Recent Publications:

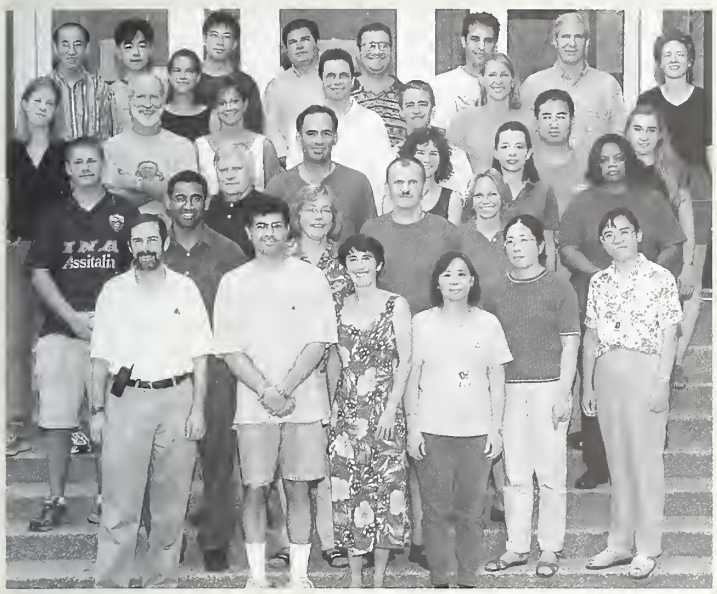
Okajima E, et al. *Biochem Biophys Res Commun* 2000;270:108–11.

Takayama S, et al. *Toxicol Sci* 2000;53:33–9.

Harris SR, et al. *Anticancer Res* 2000;20:2249–54.

Yoshiji H, et al. *Hepatology* 2000;32:1248–54.

Laboratory of Cellular Oncology



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The Laboratory of Cellular Oncology (LCO) conducts fundamental and applied research on the cellular and molecular basis of neoplasia. The LCO is composed of six interacting groups, headed by Drs. Douglas R. Lowy, John T. Schiller, Wayne C. Anderson, Linda Wolff, Jeffrey E. DeClue, and Paul Randazzo. Their specific research programs are described on the pages that follow.

The research in the LCO focuses on normal and abnormal growth regulation, cell signaling, and papillomaviruses. Fellows develop their own projects in discussion with the principal investigators. There is a great deal of interaction within each research group and between the groups. Most investigations utilize a molecular genetic and a biochemical genetic approach. The research is hypothesis driven and mechanistically oriented. The projects vary from fundamental aspects of biology to applied clinical research. Current investigations include biological and biochemical studies of the *ras* and *myb* oncogenes and their protein products, the *NF1* (type 1 neurofibromatosis) and *TSC2* (tuberous sclerosis) tumor suppressor genes and their encoded proteins, the protein kinase C family, and regulation of the ARF proteins. The papillomavirus research is concerned with mechanisms of virus assembly, cell transformation by the viral oncogenes and their protein products, the epidemiology and natural history of papillomavirus infection, the development of a vaccine against genital papillomavirus infection, and cervical cancer.

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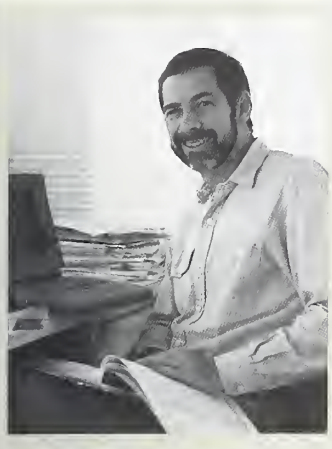

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Biography: *Dr. Lowy received his M.D. from New York University School of Medicine in 1968. Between 1970 and 1973, he was a research associate in the Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, NIH. He trained in internal medicine at Stanford University and dermatology at Yale University, and started his laboratory at the NCI in 1975. He has been chief of the Laboratory of Cellular Oncology since 1983 and deputy director of the Division of Basic Sciences since 1996. He has received the Wallace Rowe Award for Virus Research and has been a member of many scientific advisory boards, grants committees, and editorial boards.*

Laboratory of Cellular Oncology
Regulation of Cell Growth and Tumor Viruses

Keywords:
biochemical genetics
cancer cell growth regulation
cancer prevention
cancer vaccines
cell growth regulation
cell signaling
cervical cancer
cervical dysplasia
clinical trial
G-proteins
guanine nucleotide exchange factor
HPV
humoral immunity
immune response
oncogenes
papillomaviruses
proto-oncogenes
Ras
transformation
tumor suppressor genes
vaccine

Research: This laboratory has had a long-term interest in the molecular biology of tumor viruses and growth regulation. Much of the work is concerned with fundamental aspects of normal and abnormal cell growth, but some of the research has direct clinical application. The systems studied include papillomaviruses and signaling through the Ras oncoproteins.

Papillomaviruses

Papillomaviruses are associated with benign and malignant tumors in humans and animals. More than 70 different human papillomavirus genotypes (HPV types) have been described. A subset of HPV types infect the genitalia, and some of these types have been etiologically linked to cervical cancer, which is the second most common cancer among women worldwide. The papillomavirus research is carried out in collaboration with John Schiller's laboratory, which is also in the LCO.

We have examined biochemical and genetic aspects of the papillomavirus oncogenes and their protein products. In addition, we have developed techniques for large-scale production of virus-like particles (VLPs) composed of papillomavirus structural proteins. The VLPs are providing insight into basic aspects of papillomavirus biology as well as serving as the basis for a candidate subunit vaccine against papillomaviruses. Our vaccine studies in animals have shown that immunization with VLPs can induce substantial antibody-dependent protection against experimental challenge with papillomaviruses that induce cutaneous or mucosal lesions. Early phase human trials with a candidate HPV–16 L1 VLP have shown the vaccine was well tolerated, induced a consistent antibody response, and at high doses was as immunogenic without adjuvant as with adjuvant. We have developed in vitro assays for HPVs which suggest that little cross-immunity exists between different HPV types. This information has important implications for developing a polyvalent vaccine to protect against genital papillomaviruses associated with benign and malignant disease. Refinements in the VLP technology has enabled us to incorporate other proteins into the VLPs, thereby giving the VLPs additional properties. We have also been able to

study the formation of infectious virus in vitro, leading to the unexpected observation that the minor structural viral protein L2 recruits the other components required for assembly of infectious particles. This analysis has also unexpectedly implicated the nonstructural viral E2 protein, which binds to specific sites in the viral genome, in the specific encapsidation of the viral genome.

Regulation of Normal and Abnormal Growth

Most of our recent growth regulation research has been concerned with the *ras* oncogene and its positive regulators, the Ras-specific guanine nucleotide exchange factors (Ras-GNEFs). These molecules play a key role in signal transduction, and *ras* is mutationally activated in many human and animal tumors. The analysis of two closely related, widely expressed Ras-GNEFs, *sos1* and *sos2*, has identified important differences in the stability of their encoded proteins and in the duration of their signaling properties. The Sos2 protein carries out long-term signaling less efficiently than Sos1 to a degree that physiologic levels of Sos2 cannot support transformation by oncoproteins such as tyrosine kinases, which rely on Sos-dependent activation of Ras for their transforming activity. In addition, the Sos2 protein is a physiologic substrate for degradation by the ubiquitin-proteasome system, while Sos1 is not subject to such regulation. Other studies on signaling through Sos indicate that coordinate signals at the N terminus and C terminus of the protein are required for its activation. We are also studying GRF1 and GRF2, two closely related brain-specific Ras-GNEFs, that are activated by calcium. We have found that the calcium-dependent activation of GRF stimulates Raf via two signals, one that is Ras-dependent and another that is Ras-independent. Also, GRF1 and GRF2 form homo- and heterodimers through their Dbl domains. Dimerization may be required for propagating the Ras-independent signal from GRF that stimulates Raf. Further studies are planned to elucidate the mechanisms underlying the regulation of Sos and GRF signaling.

Collaborators on this research include Françoise Breitburd and Gerard Orth, Pasteur Institute, Paris; Allan Hildesheim, Eugenio Santos, and Mark Schiffman, NIH; Reinhard Kirnbauer, University of Vienna; and Klaus Nielsen and Berthe Willumsen, University of Copenhagen.

Recent Publications:

Lowy DR, et al. *Biochim Biophys Acta* 1998;1423:M1–M8.

Day PM, et al. *J Virol* 1998;72:142–50.

Anborgh PH, et al. *Mol Cell Biol* 1999;19:4611–22.

Qian X, et al. *EMBO J* 2000;19(4):642–54.

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Biography: *Dr. Wayne Anderson received his Ph.D. from the Department of Biochemistry, University of North Dakota. He then served as a postdoctoral fellow with Dr. Earl Stadtman at what was then the Heart and Lung Institute, NIH, and as a senior staff fellow with Dr. Ira Pastan in the Laboratory of Molecular Biology, NCI. In 1975, Dr. Anderson assumed the position of senior investigator within the NCI. He joined the Laboratory of Cellular Oncology in 1989.*

Laboratory of Cellular Oncology
Role of Protein Kinases in Modulating Cell Growth and Malignant Transformation

Keywords:
cell proliferation
oxygen free-radicals
phosphate transporter
protein kinase C
signal transduction
viral receptor

Research: The overall objective of this project is to better understand the role(s) of specific protein kinases in cell growth regulation and malignant transformation. Techniques in molecular biology, cell biology, and biochemistry have been utilized to better elucidate the regulatory properties of specific protein kinases and to help identify potential cellular targets modulated by these kinase activities. Included have been studies on the regulatory and biological properties of protein kinase C (PKC), cyclic AMP-dependent protein kinase (PKA), and Raf protein kinase, as well as studies to identify biological activities specifically regulated by the different isoforms of PKC. These protein kinases have been shown to play a crucial role in cellular signal transduction triggered by a variety of growth factors, hormones, and neurotransmitters, and to regulate a number of physiological functions including secretion and exocytosis, gene expression, cell proliferation, and tumor promotion. Thus, it is important to characterize the regulatory and biological properties of these protein kinases and to define their role(s) in different transmembrane signaling pathways.

Previous studies in our laboratory have established that altered subcellular distribution of proteins, as well as oxygen free-radical-induced oxidation of proteins, may play significant roles in the regulation of transmembrane signaling pathways. As a continuation of studies to define the specific regulatory and biological roles of the different PKC isotypes, investigations have been carried out to determine biological activities specifically regulated by the different PKC isotypes, particularly PKCε. Previously, it was found that PKCε was involved in regulating Golgi functions, including protein secretion, and also that PKCε could serve as a negative regulator of phorbol ester tumor promoter (PMA)-stimulated phospholipase D activity. In other studies, we showed that the membrane receptors for the gibbon ape leukemia virus (Pit-1) and the amphotropic murine retrovirus (Pit-2) both serve normal cellular functions as sodium-dependent phosphate transporters, and also established that PMA-induced activation of PKC enhanced sodium-dependent Pi uptake by these receptor/transporters.

In recent studies we have determined that Pit-2 is the form of the transporter/viral receptor regulated by PKC. To determine which PKC isoform is involved in regulating Pit-2 mediated Pi uptake, experiments were carried out with inhibitors of different PKC isoforms, with overexpression of different PKC isotypes in NIH 3T3 cells, and with PKC isotype-selective antisense oligonucleotides. Taken together, the results of these studies indicated that Pi uptake by the Pit-2 phosphate transporter/viral receptor is specifically regulated by PKCε. The mechanism responsible for the PKCε-mediated upregulation of Pit-2 Pi transport remains to be elucidated. Further studies also are required to determine if PKCε-mediated activation of the Pit-2 transporter/viral receptor might influence recognition of the viral envelope protein and viral entry into the cell.

Oxygen free-radicals have been suggested to play a significant role in regulating transmembrane signaling pathways to modulate cell proliferation, differentiation, and the actions of certain hormones. In addition, oxidants have been implicated in a number of pathological disorders, including aging, atherosclerosis, and cancer. However, there is relatively little information available on the specific cellular proteins oxidatively modified in response to oxygen free-radicals. Previously, we have shown that oxidative modification may be an important regulatory parameter of PKC. Results of recent studies now indicate that the reversible oxidation of cysteine residues within the C1 cysteine-rich zinc finger domain may be involved in the oxidative regulation of PKC. In other studies, we have established that PKA activity, as well as cyclic AMP binding to the RI and RII regulatory subunits, is decreased in cells isolated from patients with the hyperproliferative disease psoriasis. It was determined that this decrease in PKA activity in psoriatic cells was due to oxidative modification. Studies now have determined that treatment of cytosolic and purified preparations of PKA in vitro with γ-irradiation and other reactive oxygen species generating systems also resulted in decreased PKA activity and decreased cyclic AMP binding to the RI and RII regulatory subunits of PKA. In addition, a significant increase in the level of oxidized protein (as determined by an increased level of carbonyl residues) was observed in psoriatic fibroblasts, indicating an elevated oxidation state in psoriatic cells. These results suggest that oxidative modification may be an important regulatory mechanism for PKA, and that an altered oxidative state may be involved in mediating the decrease in PKA activity and cyclic AMP binding noted in cells from psoriatic patients.

Phorbol 12-myristate, 13-acetate (PMA) has been reported to be a mitogenic cofactor for many cell types, including NIH 3T3 cells. We have observed, however, that at very low cell population density PMA significantly inhibited the growth of NIH 3T3 cells. Higher cell population density (above 10 percent confluency) provided protection from the growth inhibitory effect of PMA, possibly through events mediated by cell-cell contact or cell-matrix attachment. This PMA-induced growth arrest was accompanied by an elevation in the level of p21cip1 protein, along with cell cycle arrest at the G1/S transition. To examine the possible involvement of the different PKC isoforms in mediating this PMA growth inhibitory response, studies were carried out with PKC isotype-specific inhibitors, as well as by overexpression of active holo and dominant-negative mutant

PKC constructs in these cells. Taken together, results of these studies suggested that PMA has a cell population density-dependent, bimodal effect on the growth of NIH 3T3 cells, and that this PMA growth inhibitory effect is mediated through PKCε. Related studies also were carried out with PC–12 rat pheochromocytoma cells to examine the role of specific PKC isoforms in mediating a mitogenic versus a differentiation signal in response to the growth factors NGF and EGF. Protein kinase Cε was found to be involved in mediating both growth arrest and differentiation in response to NGF. These findings suggest that altering the relative levels of the different PKC isoforms can markedly alter the biological effect noted in response to agents which regulate PKC activation, such as hormones, growth factors, and PMA.

Investigations into the specific regulatory and biological properties of these different protein kinases, and their selective modulation by physiological and pharmacological agents, are crucial to a better understanding of the role(s) these important enzymes play in cell growth regulation, malignant transformation, tumor formation, and multidrug resistance. In turn, this will help to identify environmental and nutritional factors that may act by altering these parameters to implement their carcinogenic and anticarcinogenic effects.

Collaborators on this research include Peter Blumberg, NIH; Maribeth Eiden and Rayudu Gopalakrishna, University of Southern California–Los Angeles; and Daniele Evain-Brion and Francoise Raynaud, University Rene Descartes, Paris.

Recent Publications:

Jobbagy Z, et al. *J Biol Chem* 1999;274:7067–71.

Brodie C, et al. *Cell Growth Differ* 1999;10:183–91.

Hong D–H, et al. *Am J Physiol* 1999;277:G1041–7.

Jobbagy Z, et al. *J Virol* 2000;74:2847–54.

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Biography: *Dr. DeClue graduated from Brown University in 1983. He attended the University of California–Berkeley from 1983 to 1988 and earned a Ph.D. in zoology, working with Dr. G. Steven Martin. The focus of his graduate work was the mechanism of transformation by avian retroviruses encoding protein-tyrosine kinases as their oncogenes (e.g., erbB, fps, src). Dr. DeClue joined NCI's Laboratory of Cellular Oncology in 1989, focusing on the regulation of Ras. In 1992, Dr. DeClue served as visiting professor at the University of Copenhagen, Denmark.*

Laboratory of Cellular Oncology
Molecular Analysis of Growth Regulation

Keywords:
cancer cell growth regulation
erbB receptor family
tumor suppressor genes

Research: The studies in the laboratory are focused on the molecular pathology of two human genetic diseases: neurofibromatosis type 1 (NF1) and tuberous sclerosis complex (TSC). Both conditions result in the development of benign and occasionally malignant tumors in a wide variety of organs. We are studying the function of the proteins encoded by the disease genes, and exploring other changes that occur during tumor development. In our studies, we have utilized *NF1* knock-out mice and a rat strain with a germline mutation in *TSC2*. Our studies also involve the function and regulation of the low molecular weight GTPases Ras and Rap1. The *NF1* product neurofibromin functions as a negative regulator of Ras activity, while the *TSC2* product tuberin has a similar function for the closely related Rap1.

Our current studies of NF1 have focused on the role of the epidermal growth factor receptor (EGFR) in the development of benign and malignant peripheral nerve sheath tumors. While Schwann cells do not normally express EGFR, we found that a portion of Schwann cells in benign NF1 patient tumors and many or all cells in malignant NF1 tumors express EGFR, indicating that it may play a role in the pathology of this disease. Furthermore, we observed similar results using Schwann cells from the mouse model system.

Our studies of TSC are based on examining the interaction between tuberin and the product of the *TSC1* gene, hamartin. These proteins form a tight complex in cells that appears to regulate the stability of tuberin. We are determining the contact regions for this interaction and exploring the mechanism(s) by which hamartin inhibits cell growth. One possibility is that tuberin has a direct impact on cellular signaling pathways through its interaction with Rap1, while hamartin acts as a cofactor or stabilizer for tuberin.

Our collaborators include Marilyn Glassberg, University of Miami; Nancy Ratner, University of Cincinnati; and Julian Sampson, University of Cardiff, Wales.

Recent Publications:

Anborgh PH, et al. *Mol Cell Biol* 1999;19:4611–22.

Kleymenova E, et al. *Genet Res* 1999;74:139–44.

DeClue JE. *Lung Biol in Health and Dis* 1999;131:419–40.

DeClue JE, et al. *J Clin Invest* 2000;105:1233–41.

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Biography: *Ramanujan Hegde received his M.D. and Ph.D. degrees from the University of California–San Francisco. He joined the Laboratory of Cellular Oncology as an NCI scholar in 1999.*

Laboratory of Cellular Oncology
Regulation of Secretory and Membrane Protein Biogenesis

Keywords:

membrane protein complexes
membrane traffic
protein folding
protein transport
translocation
transport proteins

Research: Our laboratory is interested in the mechanisms by which nascent secretory and membrane proteins are translocated across or integrated into the endoplasmic reticulum (ER) membrane, appropriately modified, folded, and assembled prior to subsequent transit to various parts of the cell. The current understanding of the molecular components that direct the translocation of a limited subset of simple secretory proteins has revealed a remarkably (and probably deceptively) simple picture. As the lessons learned from these studies are being extended to more complex substrates, it is becoming increasingly obvious that our understanding is neither complete nor clear.

One largely unexplored concept is the idea that certain aspects of translocation can be regulated or misregulated in the pathogenesis of disease. This notion has been difficult to explore in the absence of a mechanistic understanding of the basic processes involved. With the recent insights into simple secretory protein translocation, the stage is now set to ask whether more complex substrates can be subject to regulation in some aspect of their biogenesis. Thus, the long-term objectives of our laboratory are to reveal the biogenesis of secretory and membrane proteins as a novel site of regulation in the cell, identify the regulatory mechanisms and factors involved, and define roles for this type of regulation in cell biology and disease pathogenesis.

The prion protein (PrP), a brain glycoprotein involved in various neurodegenerative diseases, has proven to be an incredibly instructive and dramatic example of complex and highly regulated translocation. In addition to its notoriety as the putative "protein-only" infectious agent in prion diseases, it had been noticed many years ago that PrP behaved unusually when synthesized in a cell-free translation and translocation system. An initially homogeneous cohort of nascent PrP chains would give rise to three distinct populations of PrP: a fully translocated form (termed ^{sec}PrP), and two transmembrane forms that spanned the membrane in opposite orientations (^{Ntm}PrP and ^{Ctm}PrP). Recent studies have revealed that even a slight overrepresentation of the ^{Ctm}PrP topologic form results in the development of neurodegenerative disease in both mouse model systems and naturally occurring human disease. Additionally, it appears that ^{Ctm}PrP–mediated neurodegeneration is a final common pathway in the pathogenesis of both genetic and infectious forms of prion disease. Thus, understanding translocational regulation, using PrP as a model system, is of substantial interest from both a general cell biological and medical perspective.

At present, the mechanisms of how a homogeneous cohort of nascent PrP can be directed toward multiple topologic outcomes remains largely unknown. We are taking two complementary approaches to this problem. First, specific sequence elements within PrP that are involved in determining its topology are being identified and dissected. Second, we are identifying and characterizing the components of the translocation apparatus that are involved in directing PrP topology.

Using the first approach, we have recently discovered that the N terminal signal sequence is critical to the determination of topology. This function of the signal sequence was found to be independent of its role in targeting PrP to the ER, and thus represents a novel function for signal sequences. The mechanism by which an ordinarily cleaved N terminal signal sequence can direct protein topology is presently being investigated. In the second approach, we have employed solubilization, fractionation, and reconstitution of ER membrane proteins to demonstrate that regulatory trans-acting factors are absolutely required for PrP to be synthesized in the proper ratio of its topologic forms. We are taking several approaches to identify and characterize such translocation accessory factors that are likely to be involved in currently mysterious aspects of translocation and topology determination.

Finally, we are applying lessons learned from the study of PrP to explore the translocational regulation of other substrates. For example, are other proteins synthesized in multiple folded forms, each of which might have a different function? Do signal sequences encode posttargeting information critical to the biogenesis of other substrates? What is the importance of translocation accessory factors for other complex secretory and membrane proteins? It is envisioned that the answers to these and related questions will reveal a richly complex point of regulation that has direct implications for physiology and disease.

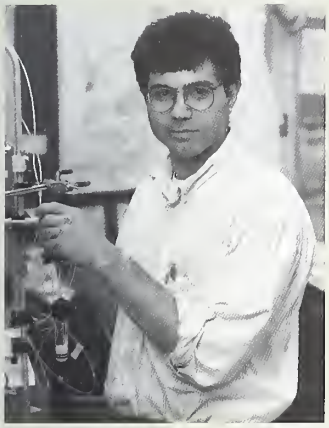
Recent Publications:

Hegde RS, et al. *Science* 1998;279:827–34.

Hegde RS, et al. *Mol Cell* 1998;2:85–91.

Hegde RS, et al. *Trends Cell Biol* 1999;9:132–7.

Hegde RS, et al. *Nature* 1999;402:822–6.



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Biography: *Paul Randazzo received his M.D. and Ph.D. from Brown University. He received residency training in anatomic pathology at the Hospital of the University of Pennsylvania. He joined the NCI in 1990 as a senior staff fellow.*

Laboratory of Cellular Oncology
Regulation of ADP–Ribosylation Factor

Keywords:
adhesion molecules
biochemistry
cell signaling
membrane traffic
protein chemistry
signal transduction

Research: Membrane traffic is integral to cellular functions including protein secretion, cell migration, and signal transduction. Although the molecular machinery of membrane traffic has been elucidated, the mechanisms linking the changes in membrane traffic with other cellular events are not well described. The ADP-ribosylation factors (Arfs) are a family of GTP-binding proteins that regulate membrane traffic at multiple sites and could be a point of integration.

Arfs were first identified as a cofactor for cholera toxin, which was the basis for the name. Later, Arf proteins were found to be necessary for efficient membrane traffic in normal physiology. Function of Arf proteins requires the controlled binding and hydrolysis of GTP, which require specific exchange factors and GTPase activating proteins (GAPs). We have been studying the interaction of Arf with these regulators and the influence of small molecules on those interactions.

Arf Activity Is Modulated by Phosphoinositides

Because Arf functions at membranes, we investigated the influence of membrane composition on Arf activity. We found that Arf has two lipid-binding sites. One, comprised of an N terminal α-helix and covalently bound myristate, interacts nonspecifically with hydrophobic surfaces when GTP is bound. This domain also interacts with target proteins. A second site interacts specifically with PIP_2, a phospholipid important for signal transduction. PIP_2 binding to Arf directs the interaction with one identified Arf GAP. Arf has also been found to affect phospholipid metabolism. This has led us to propose that Arf in a target of phosphoinositide signaling and, through a system of feedback loops involving lipid metabolites, orders membrane traffic events.

Arf GTPase-Activating Proteins (GAPs) Are Targets of Phosphoinositides and Tyrosine Kinases

We have been studying Arf GAP as a potential means of transducing changes in membrane traffic in response to established signal transduction pathways. We have found a family of GTPase-activating proteins (GAPs) for Arf, the centaurin betas, which contain PH, Arf GAP, and ANK repeat domains. All tested members of the family function as GAPs in vitro and are dependent on two phospholipids, phosphatidic acid and phosphatidylinositol 4,5-bisphosphate. The PH domain regulates activity by a mechanism not previously described for this motif. Two members of the family have been found to bind Src and FAK family proteins, and one of these has been shown to regulate the cycle of events that result in cell migration. Current work is aimed at identifying the specific membrane traffic events affected by the phospholipid-dependent Arf GAPs, the influence of phosphoinositides Src and FAK on those events, and the cellular functions of other members of the centaurin family of proteins, including a possible role of one member, which is found on a chromosomal locus linked to neuroblastoma, in malignant transformation.

Collaborators include Jonathan Cooper, Fred Hutchinson Cancer Research Center, Seattle; Julie Donaldson, NIH; and Joseph Schlessinger, New York University.

Recent Publications:

Randazzo PA, et al. *J Biol Chem* 1994;269:10758–63.

Randazzo PA. *J Biol Chem* 1997;272:7688–92.

Randazzo PA. *Biochem J* 1997;324:413–9.

Brown MT, et al. *Mol Cell Biol* 1998;18:7038–51.

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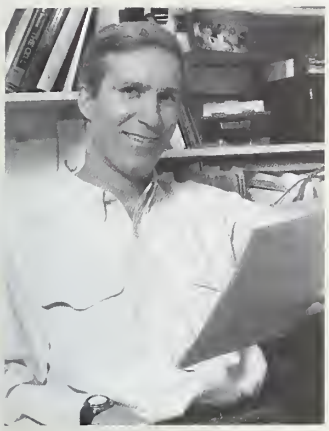

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Biography: *Dr. Schiller graduated from the University of Wisconsin–Madison with a B.S. in molecular biology in 1975. In 1982, he received a Ph.D. from the Department of Microbiology of the University of Washington in Seattle, then joined the Laboratory of Cellular Oncology as a National Research Service Award postdoctoral fellow in 1983. Dr. Schiller became a senior staff fellow in the Laboratory of Cellular Oncology in 1986 and a senior investigator in 1992. In 1998, he became chief of the Neoplastic Disease Section of the lab.*

Laboratory of Cellular Oncology
Papillomaviruses: Basic Biology and Vaccine Development

Keywords:
antibody
autoantibodies
cancer immunotherapy
cancer vaccines
CCR5
cervical cancer
HPV
immunotherapy
TNF
tumor antigens

Research: Papillomaviruses (PVs) infect the squamous epithelia of a wide variety of animals and man. Infections are species-specific and generally induce localized benign proliferation. However, the lesions induced by certain PVs can undergo malignant progression. There is a strong association between malignant progression of human genital lesions and certain human (H) PV types, most frequently HPV–16. The major goals of the laboratory have been to elucidate the role of HPVs in the genesis of human cancers and to develop safe and effective vaccines to prevent genital HPV-induced disease.

Difficulties in studying PV virions, due to the lack of efficient in vitro propagation methods, were partially overcome by our recent demonstration that the virion proteins can self-assemble into virus-like particles (VLPs) and the VLPs can encapsidate the viral genome to generate infectious virions when overexpressed in eukaryotic cells. Three types of studies based upon the VLP technology are being conducted.

First, questions into the basic biology and biochemistry of virion assembly and infection are being examined. We have determined that the L1 major capsid protein alone has the capacity to self-assemble into structures that closely resemble authentic virions. The L2 minor capsid protein is required for infection and genome packaging but is not required for interaction with the primary virus cell surface receptor. L2 appears to function in generating infectious virions by inducing the colocalization of the capsid proteins and genome to nuclear organelles called PML oncogenic domains (PODs). We have determined that the viral transcription/replication factor, E2, is also required for genome encapsidation and colocalizes to PODs if L2 is present. This suggests that specificity for genome encapsidation is mediated by the strong binding of E2 to specific sequences conserved among all PVs, coupled with an interaction between E2 and an intermediate in capsid assembly at the PODs.

Second, HPV VLP-based vaccines are being developed to prevent cervical and other genital HPV-associated cancers. We and our colleagues have demonstrated type-specific protection from high-dose experimental infection after VLP vaccination in rabbit and cattle models. To facilitate the evaluation of immune responses in human vaccine trials, we have developed in vitro infectivity and neutralization assays based on in vitro-generated HPV pseudovirions. The possibility that VLPs of one type could cross protect against infection by other types has been evaluated using these assays. The results strongly suggest that protection in VLP-based vaccines in humans will primarily be type specific. A phase I trial of an HPV–16 L1 VLP vaccine has recently been completed. Induction of high titers of neutralizing antibodies, even without adjuvant, and low reactogenicity were observed. A phase II trial is currently in progress. To increase the therapeutic potential of a VLP-based vaccine, we have incorporated other viral or cellular polypeptides into the VLPs as L2 fusions. HPV–16 E7 chimeric VLPs, where shown to retain their ability to elicit high titers of neutralizing antibodies and also elicit potent CD8, restrict CTL responses that protected mice from experiment challenge with E7-expressing tumor cells, even in the absence of adjuvant. A phase I trial of an HPV–16 L1/L2–E7–E2 chimeric VLP is in the planning stage.

Third, VLP-based vaccines for the induction of autoantibodies to self proteins are being developed. These vaccines are based on the hypothesis that high-titer, high-affinity antibodies to self antigens can be generated if they are presented to the immune system in a context that resembles the ordered repetitive array of virion surface epitopes. As a proof of concept, high-titer antibodies to the chemokine receptor CCR5 have been generated by replacing a dominant neutralizing epitope of L1 with the first external loop of CCR5. These antibodies blocked M-tropic HIV infection in vitro. The results suggest a general method for inducing autoantibodies for basic science and therapeutic applications. Similar vaccines targeting other self antigens, including TNFα, VEGF, and HER–2/neu, are currently under development.

Recent Publications:

Day PM, et al. *J Virol* 1998;72:142–50.

Greenstone HL, et al. *Proc Natl Acad Sci USA* 1998;95:1800–5.

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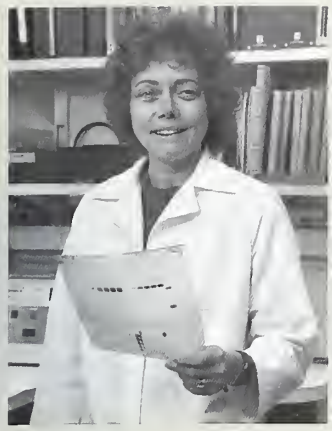

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Biography: *Dr Wolff obtained her Ph.D. from Ohio State University where she carried out research on the immunological and oncogenic aspects of the feline leukemia virus. When she first joined the NCI in 1981 in Edward Scolnick's Laboratory of Tumor Virus Genetics, she continued investigations in retroviral pathogenesis under the mentorship of Sandra Ruscetti. Her ongoing research focuses on transformation and differentiation of hematopoietic cells of the myelomonocytic pathway. She is currently on the editorial board of the journal* Apoptosis.

Laboratory of Cellular Oncology
Oncogene Involvement in Murine Myeloid Leukemia

Keywords:
c-myb
c-myc
differentiation
insertional mutagenesis
leukemia
transcriptional regulation

Research: Acute leukemias of the myeloid system are a common form of hematopoietic neoplasia in man. Since only a limited number of genes involved in this disease have been identified, animal models for identification and characterization of other oncogenes that participate in formation of these leukemias are essential. For this purpose, our laboratory has developed a murine model for acute monocytic leukemia in which neoplasias arise as a consequence of both peritoneal chronic inflammation and retroviral insertional mutagenesis. Genetic loci found to be activated by retroviral insertion in these leukemias include the proto-oncogene c-myb, and a newly identified loci Mml1-4.

Investigations into the role of the c-myb gene in this leukemia model have led to a series of discoveries that contribute to our understanding of this disease as a multistep process. The c-myb gene, which encodes a nuclear transcription factor involved in proliferation and differentiation of hematopoietic cells, is mutated by the virus early after infection in the hematopoietic organs of the mouse. Following this preleukemic event, the cells migrate to the peritoneal cavity, differentiate, and undergo clonal expansion. Recently, we found leukemogenic c-Myb protein, which is found in leukemic cells and is truncated due to insertional mutagenesis by the virus, is more resistant to proteolysis by the ubiquitin-26S proteasome pathway than the normal protein. The truncated protein is missing sequences that target it for degradation. Therefore, it has a longer existence in the cell compared to that of the normal protein, which could account for its ability to drive proliferation at inappropriate times. Because of this observation, we are now investigating mechanisms that regulate proteolysis of the normal c-Myb and determining how these are altered in the case of the leukemogenic form. Very recently, we localized in c-Myb determinants that are involved in targeting the protein for degradation. One important site in the middle of the protein overlaps a predicted protein-protein interaction region that has a partial leucine zipper motif and the other site is at the extreme C terminus. Other data suggests that phosphorylation of the protein is required for degradation since treatment of cells with an inhibitor of phosphatases causes more rapid degradation by the proteasome.

Another active form of research in the laboratory involves the characterization of the functional role of c-Myb in leukemic cells and the target genes regulated by this transcription factor and involved in promoting the neoplastic disease. To this end, we have recently conditionally expressed Myb and a dominant-negative form of c-myb in myeloid leukemia cell lines. Using these systems we have been able to show that c-*myc* is positively regulated by c-Myb. This is an important target that that can explain c-Myb's ability to cause cells to proliferate.

Presently, major focuses of the laboratory are the characterization of new common loci of viral integration, the identification of genes that are regulated by c-Myb using microarray technology, and the development of transgenic models for leukemia.

A collaborator on this research is Juraj Bies, Slovak Academy of Sciences, Bratislava, Slovakia.

Recent Publications:

Wolff L. *Biochim Biophys Acta* 1997;1332:F67–F104.

Bies J, et al. *J Virol* 1999;73:2038–44.

Bies J, et al. *Oncogene* 2000;19:2846–54.

Schmidt M, et al. *Mol Cell Biol* 2000;20:1970–81.

Laboratory of Comparative Carcinogenesis

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Our research interests focus on the normal and aberrant organismal and cellular events and signaling pathways that can enhance or inhibit tumor development or other disease processes. For example, we have found that the von Hippel-Lindau (VHL) tumor suppressor protein, implicated in cancers of the kidney, adrenal gland, and brain, localizes to the mitochondria in normal kidney epithelial cells, implicating for the first time this organelle in the normal functioning of VHL protein.

Our studies of renal organogenesis in vitro have led to the discovery of multiple cytokines critical to induction of tubulogenesis in rat metanephric mesenchyme, and evaluations of downstream signaling events in response to these factors have implicated specific regulatory molecules in this process. Since childhood renal tumors caricature normal development with the expansion of blastemal mesenchymal stem cell populations, our efforts may lead to strategies for designing noncytotoxic therapies in treating these neoplasms.

We have shown how metals known to be human carcinogens can inflict promutagenic oxidative DNA base damage through interaction with DNA-binding proteins such as histones and protamines and/or inhibition of DNA repair. We have found that exposure of male mice to chromium (III), a common industrial exposure chemical, results in increased tumors in adrenal medulla, lung, Harderian gland, and thyroid tissues of their offspring, and that this effect is in spite of minimal penetration of the chromium into the germ cells of the testis. A possible transgenerational effect on gene expression is being explored by use of DNA microarray analysis.

Arsenic is a known human carcinogen, but the mechanism of action of this important environmental contaminant is undefined. In cells, arsenic undergoes mono- and dimethylation and consumes methyl groups in the process. We recently found during malignant transformation induced by low level chronic arsenic exposure that genomic DNA becomes hypomethylated. The probable cause for this undermethylation is arsenic-induced chronic depletion of available S-adenosyl-methionine, the methyl donor cofactor for many methyltransferases, including DNA methyltransferases. Thus, arsenic carcinogenesis is linked to DNA hypomethylation, a state associated with aberrant gene activation and connected to acquisition of malignant phenotype.

Studies on the multifaceted signaling agent nitric oxide (NO) have shown it to have promutagenic potential, but experiments to date have failed to confirm its postulated carcinogenicity. Indeed, NO-releasing drugs under development in this laboratory have shown promise in anticancer applications. Collaborations with investigators in other disciplines have shown our drugs to promote healing of injured arteries, protect the liver from toxic injury, induce penile erections, and reduce clot formation.

Livers of mice infected by the tumorigenic bacterium *Helicobacter hepaticus* have been studied for changes in the levels of the ras oncogene protein p21, mitogen-activated protein kinase, and several isoforms of PKC. In contrast to upregulation of growth factors EGF and TGF, and the cell cycle control protein cyclin D1 early in the tumorigenic process, ras and PKCs increased only late, and no change in activated MAPK was noted. Thus, some other transcytoplasmic signaling pathway links the growth factors with nuclear cell cycle control.

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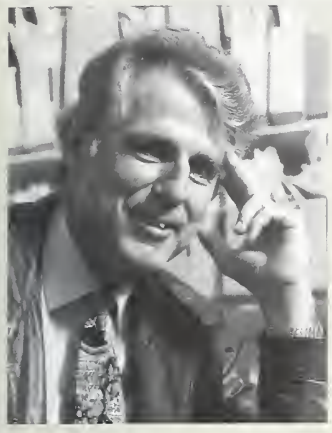

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Laboratory of Comparative Carcinogenesis
Chemistry and Biology of Nitric Oxide

Keywords:
drug design
nitric oxide

Research: Nitric oxide (NO) is a potent and multifaceted bioregulatory agent. This project is aimed at (1) finding ways to target NO to specific sites in the body for important research and/or therapeutic applications, and (2) characterizing the possible role of NO as a determinant of cancer risk.

Our strategy in pursuing these goals is to begin by characterizing the fundamental chemistry of the NO-releasing diazeniumdiolates (compounds containing the [N(O)NO] functional group). We then attempt to exploit our accumulating knowledge in this area as a platform for solving problems in biomedical research and clinical medicine. We are currently pursuing basic research investigations into the structure, spectra, dissociation to NO, alkylation, arylation, and photodegradation of the diazeniumdiolate functional group with an eye toward designing prodrugs that are stable at physiological pH but that can be activated to generate NO by enzymatic action. An example is AcOM–PYRRO/NO, an esterase-sensitive diazeniumdiolate that penetrates the cell and generates NO within the cytoplasm on esterase-induced hydrolysis; AcOM–PYRRO/NO has proved to be two orders of magnitude more potent as an inducer of apoptosis in HL–60 leukemia cells in culture than the spontaneously dissociating parent ion, PYRRO/NO. A second achievement has been the design of agents that can be activated for NO release by enzymes of the glutathione S-transferase (GST) family. The pi isoform of GST is overexpressed in some tumor cells, rendering them resistant to several lines of anticancer therapy, while the alpha isoform is vital to proper cell function. By exploiting the growing knowledge about the structures of the GSTs' active sites, we have gone from an initial drug candidate whose alpha:pi rate ratio was 100 to a third generation analog with a ratio of 0.3. If enough additional pi selectivity can be designed into this series, a drug capable of irreversibly inhibiting the pi enzyme while sparing the alpha may be forthcoming.

We will place continuing emphasis on designing additional drugs and devices capable of targeting NO release to selected tissues for use as research tools and for possible therapeutic benefit. At the same time, we will continue to characterize NO's potential activity as a pro- or anticarcinogen.

Collaborators in these efforts include Timothy Billiar, University of Pittsburgh; Scott Bohle, University of Wyoming; Keith Davies, George Mason University; Emanuela Felley-Bosco, University of Lausanne; Xinhua Ji, NIH; Mark Meyerhoff, University of Michigan; Paul Shami, VA Medical Center, Salt Lake City; Shivendra Singh, Mercy Hospital of Pittsburgh; and John Toscano, Johns Hopkins University.

Recent Publications:

Fitzhugh AL, et al. *Free Radic Biol Med* 2000;28:1463–9.

Saavedra JE, et al. *Bioorg Med Chem Lett* 2000;210:751–3.

Mowery KA, et al. *Biomaterials* 2000;21:9–21.

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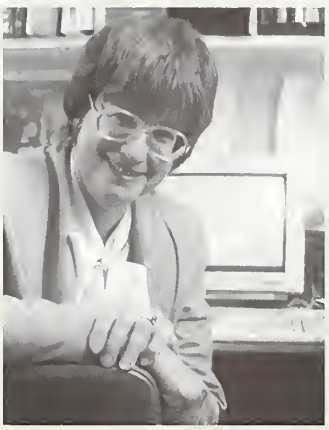

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Biography: *Dr. Anderson received her A.B. from Bryn Mawr College and her Ph.D. from the University of Pennsylvania in biology, with postdoctoral work in biochemistry at the University of Minnesota Medical School. She developed a program in carcinogen metabolism and perinatal carcinogenesis at the Memorial Sloan-Kettering Institute. She joined the NCI in 1982 and has continued studies of the mechanisms of tumorigenesis.*

Laboratory of Comparative Carcinogenesis
Cellular Pathogenesis in Animal Models of Human Cancer Risk Mechanisms

Keywords:
chromium
corticosterone
glucose
insulin-like growth factor 1
K-ras protein
lung adenocarcinoma
preconceptional
reactive oxygen species
transforming growth factor-α

Research: Useful insights into the mechanisms of causation and development of cancers, and possible strategies for prevention and therapy, may be provided by studies of homologous risk situations in experimental animals. Adenocarcinomas are the most common form of human lung cancer, and these are well modeled by the comparable neoplasm in mice. The K-*ras* gene is frequently mutated in both human and murine lung adenocarcinomas. We are investigating the role of normal and mutated K-ras protein in growth control and malignancy of the lung. Secondly, increasing incidences of certain childhood cancers have led to interest in exposures of parents, including preconceptional exposures, as part of the etiology. We are investigating the mechanism of preconceptional carcinogenesis.

Cellular Mechanisms in the Development of Lung Adenocarcinomas
This year we have contributed to a mounting body of evidence that the K-ras protein has a tumor suppressive, rather than a proliferative, role in lung type 2 cells, the target tissue for adenocarcinoma development. In mouse lung tumors caused by the environmental and tobacco smoke carcinogen, N-nitrosodimethylamine, we have found that K-ras protein is downregulated

relative to control lung, especially in the membrane fraction, the site of action of the protein. Furthermore, in lung tumors after treatment with the promoting agent 2,3,7,8-tetrachlorodibenzo-p-dioxin, a human lung carcinogen, the downregulation of total and membrane-localized K-ras protein was exacerbated, suggesting a possible mechanism for the promotive effect. Currently research is based on three aims. (1) If K-ras protein has a role in controlling cell growth and differentiation, how does this work? What extracellular signals activate it, and how is the message passed downstream? We are utilizing microarray analysis (Incyte Genomics system) to characterize genes with altered expression after K-ras activation and in malignant cells, and Ciphergen ProteinChips® to discover proteins interacting with K-ras protein. (2) How does mutation in K-ras protein contribute to tumorigenesis? In addition to possible abnormality in downstream signal passage, mutant K-ras protein, which is in a permanently activated state, increases intracellular reactive oxygen as measured by three different methods. These may cause clastogenic damage and/or lead to abnormal function of signaling pathways influenced by reactive oxygen. We are pursuing this hypothesis by development of an inducible vector system and use of specific inhibitors. (3) If K-ras protein is not driving proliferation in tumor development, what is? With the discovery that transforming growth factor-α is secreted by malignant but not nontransformed mouse lung type 2 cells, an autocrine signaling pathway involving the ErbB3 and 4 receptors and phosphatidyl inositol 3-kinase was implicated. The generality of this pathway is under study.

Mechanisms of Preconceptional Carcinogenesis

Pursuing the hypothesis that the mechanism of preconceptional carcinogenesis is epigenetic, we have found in several studies that exposing male mice 2 weeks before mating to the occupational metal and nutritional supplement chromium(III) has led to marked, highly significant alterations in average serum corticosterone and glucose in the offspring. Serum insulin-like growth factor 1 (IGF–I) has also shown significant changes in some studies. Microarray analysis of gene expression changes in livers of the offspring has revealed a strong downregulation of expression of IGF binding protein 1 correlated with reduced serum corticosterone. Study of other target tissues is under way with this technique. Chromium(III) was found to be absent from sperm and from the germinal epithelium of the testis by several highly sensitive techniques. This confirms an indirect effect. The nature of this effect is under study. Other paternal exposures and treatments are being investigated for their potential to alter serum hormones and glucose. In general, this highly novel phenomenon has considerable potential to influence the physiology of offspring and the risk of many conditions in addition to cancer including asthma, obesity, and diabetes.

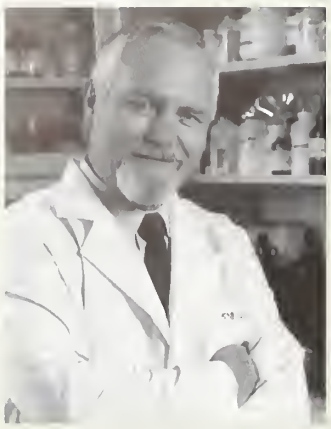
Recent Publications:

Ramakrishna G, et al. *Exp Lung Res* 2000;26:659–71.

McKenna IM, et al. *Mutat Res* 2001;490:57–65.

Khan Q, et al. *Toxicol Appl Pharmacol* 2001;173:105–113.

Singh R, et al. *Carcinogenesis* 2001;22:1281–7.



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Biography: *Dr. Kasprzak received his M.S. and Ph.D. in chemistry from the University of Poznan, and his D.Sc. in metal toxicology from the University Medical School of Poznan, Poland. He held several positions at academic medical schools both in Poland and in the United States, and served in the International Union of Pure and Applied Chemistry Divisions of Clinical and Analytical Chemistry as well. He joined the NIH in 1982 to study the carcinogenicity of metals.*

Laboratory of Comparative Carcinogenesis
Mechanisms of Transition Metal-Induced Carcinogenesis

Keywords:
8-oxo-dG
8-oxo-dGTPase
carcinogenesis
DNA adducts
DNA damage
Fhit
histones
metal carcinogenesis
nickel
oxidative DNA damage
protamines

Research: Certain transition metals, including nickel, chromium, cadmium, and copper, are carcinogenic to humans and/or animals. Their effects include cancer in the progeny of fathers exposed to welding fumes and other metal dusts. However, mechanisms of the carcinogenic activity of these metals remain obscure. In recent years, we have been testing a hypothesis that one such mechanism would involve metal-mediated oxidative damage to DNA and nuclear proteins. In 2000 and 2001, we continued mechanistic studies of that hypothesis. Our investigations were focused on transition metals' interactions with protamine HP2, core histone H2A, and selected antimutagenic and tumor suppressor "nudix" hydrolases.

The results of our recent investigations of protamine HP2 allowed us to propose two physiological binding sites for Zn(II) (an essential sperm constituent) in the sperm chromatin. These binding sites were different from that found for toxic metals, Ni(II) and Cu(II). Toxicity of the latter has been associated with redox catalysis. Ni(II) and Cu(II) coordinated by the N terminal Arg-Thr-His- motif of HP2 are redox active and mediate oxidative damage to the peptide. The major target for oxidation is Tyr-8 seemingly located away from the binding site. A 2D NMR study revealed that the reason for this specificity is a peculiar structuring effect of the N terminally coordinated Ni(II) on the rest of the molecule that brings Tyr-8 close to the metal center.

Using synthetic model peptides, we continued to investigate Ni(II) interactions with the core histones. The formation of a strong complex at pH 7 and above with the TESHHK motif in the C terminal "tail" of histone H2A and hydrolysis of this motif by Ni(II) was confirmed in longer peptides, modeling the entire "tail," and in whole bovine H2A. Ni(II) bound to the entire tail lacks significant oxidation-mediating activity towards DNA and the peptide itself. However, a secondary complex formed between Ni(II) and the cut-off SHHKAKGK product of hydrolysis is redox-active and is capable of enhancing oxidative promutagenic damage to DNA (single-strand breaks and base oxidation). Also, this complex is degraded by hydrogen peroxide,

especially at its histidine and serine residues. Since the physiological roles of the histone "tail" include locking interactions with other histones and DNA, the observed truncation of this "tail" by Ni(II) may affect gene expression.

The investigations of "nudix" hydrolases have been focused on MTH1, an 8-oxo-dGTPase preventing incorporation of promutagenic 8-oxo-dGTP into DNA, and on Fhit, a tumor suppressor protein having diadenosine triphosphate (Ap_3A) phosphohydrolytic activity. Using our newly developed in vivo assay for 8-oxo-dGTPase, we confirmed the inhibition of its activity by Cd(II) in the testes of rats exposed to Cd(II), the rat testicular carcinogen. Immunohistochemical staining revealed for the first time presence of MTH1 in cell nuclei and in the acrosomic vesicles of spermatocytes and sperm. Possible inhibition of MTH1 in the sperm may be relevant to preconception carcinogenesis. Several metals, Cu(II)>>Ni(II)>Zn(II)>Cr(III)>Cd(II)>>Co(II), were found to inhibit recombinant human Fhit with efficiency decreasing in the given ranking. The inhibition is caused by metal binding to the His and Cys residues in the enzymatic active center of Fhit. The exceptionally high inhibitory potential of Cu(II) (IC_{50} = 0.4 μM) is apparently due to oxidation by Cu(II) of the Cys-39 residue and the resulting dimerization of Fhit molecule through –S–S– bond. Also, Fhit expression was found to be significantly lower, or even absent, in nickel-induced mouse sarcomas. Thus, both MTH1 and Fhit may participate in the mechanisms of metal-induced mutagenesis and carcinogenesis.

Collaborators on this research are: Wojciech Bal, University of Wroclaw, Poland; Miral Dizdaroglu, National Institute of Standards and Technology; and Yusaku Nakabeppu, Kyushu University, Japan.

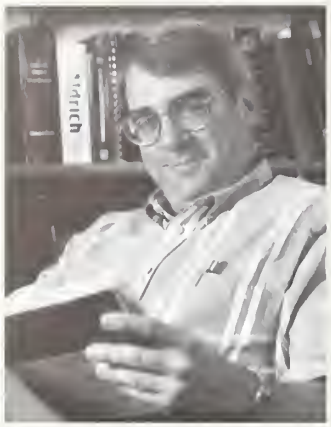
Recent Publications:

Bal W, et al. *Chem Res Toxicol* 2000;13:616–24.

Bal W, et al. *Chem Res Toxicol* 2000;13:823–30.

Bialkowski K, et al. *Nucleic Acids Res* 1998;26:3194–201.

Bialkowski K, et al. *Carcinogenesis* 1999;20:1621–4.



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Biography: *Dr. Moschel received his Ph.D. in biochemistry from Ohio State University in 1973. He conducted postdoctoral research for 3 years in organic chemistry at the University of Illinois before joining the Molecular Aspects of Chemical Carcinogenesis Section. Dr. Moschel was promoted to staff scientist in 1987 and head of the Carcinogen-Modified Nucleic Acid Chemistry Section in 1992. He serves on the editorial advisory board of Chemical Research in Toxicology and is a member of the American Cancer Society's Peer Review Committee on Carcinogenesis, Nutrition and the Environment.*

Laboratory of Comparative Carcinogenesis
Chemistry and Mutagenicity of Carcinogen-DNA Damage; Chemotherapy Adjuvants

Keywords:
chemotherapy
DNA repair
mutagenesis
nucleic acid chemistry

Research: The Carcinogen-Modified Nucleic Acid Chemistry Section uses site-specific mutagenesis techniques to focus on understanding the chemical and biological effects of carcinogen damage to DNA and the role played by DNA repair mechanisms in modulating these effects. The section also develops compounds that can inactivate the human DNA repair protein, O^6-alkylguanine-DNA alkyltransferase (alkyltransferase). These compounds are used to improve the effectiveness of chemotherapeutic drugs that modify the O^6-position of DNA guanine residues.

We developed a shuttle vector system that allows us to study mutagenesis by carcinogen-modified bases in human cells. We used this system to examine the role of alkyltransferase and mismatch repair in modulating mutagenesis by O^6-methyl-, O^6-ethyl- and O^6-benzylguanine as well as O^4-methylthymine in human kidney cells (293) or colon tumor cells (SO) or in the mismatch repair defective human colon tumor cell lines H6 and LoVo. These are the types of modified bases produced by potent alkylating carcinogens. O^4-methylthymine mutagenesis was high and unaffected by DNA repair status of the cells. In contrast, all the O^6-substituted guanines were subject to alkyltransferase repair in these cells. Furthermore, both O^6-methyl- and O^6-ethylguanine were subject to mismatch repair processing in the 293 and SO cells. These results are significantly different from those for studies with these same bases in *Escherichia coli*. The findings emphasize the importance of studying site-specific mutagenesis by carcinogen-modified bases in human cells.

O^6-benzylguanine is the prototype alkyltransferase inactivator that is currently in clinical trials in combination with the chemotherapeutic drugs 1,3-*bis*(2-chloroethyl)-1-nitrosourea and temozolomide. However, O^6-benzylguanine is not an ideal drug, since it is only sparingly soluble in water and it is not specific for alkyltransferase inactivation in tumor cells. Therefore, we are developing additional inactivators that are more water

soluble, better formulated, or more tumor cell-specific than O^6-benzylguanine. Of the more water soluble derivatives we have prepared, oligodeoxyribonucleotides containing O^6-benzylguanine have proven to be extremely effective inactivators of the human protein. Related DNA segments bearing methylphosphonate linkages are also effective in cells although uptake may limit the usefulness of these derivatives. Nevertheless, if uptake can be improved, oligodeoxyribonucleotides containing O^6-benzylguanine residues may prove to be very useful drugs for potentiation of alkylating agent chemotherapy.

In collaborative experiments with Dr. Eileen Dolan of the University of Chicago Medical Center, 8-substituted O^6-benzylguanine derivatives, i.e. O^6-benzyl-8-trifluoromethyl-, –8-bromo-, –8-oxoguanine, and 8-aza-O^6-benzylguanine were compared to O^6-benzylguanine for their ability to inactivate alkyltransferase in various tissues of nude mice bearing human lung and brain tumor xenografts. Although O^6-benzylguanine proved to be superior to the 8-substituted derivatives at depleting alkyltransferase in xenografts, 8-aza-O^6-benzylguanine and O^6-benzyl-8-bromoguanine exhibited superior alkyltransferase inactivating ability in brain and kidney tissues, suggesting that these analogs might be more effective than O^6-benzylguanine at inactivating alkyltransferase in tumors of these organs.

Recent Publications:

Pauly GT, et al. *Chem Res Toxicol* 2001;14:894–900.

Pegg AE, et al. *J Pharmacol Exp Ther* 2001;296:958–65.

Ewesuedo RB, et al. *Cancer Chemother Pharmacol* 2001;47:63–9.

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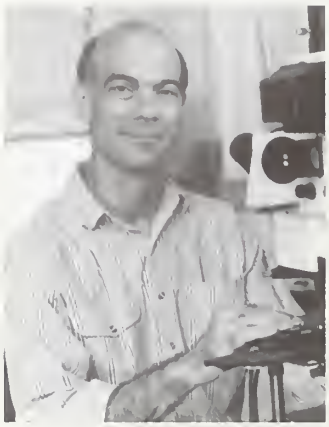

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Biography: *Dr. Perantoni received his Ph.D. in cell biology from Catholic University in 1983, having conducted his thesis research at the NCI. After serving as an assistant professor in the Pathology Department, University of Colorado Medical School, he returned to the NCI in 1992.*

Laboratory of Comparative Carcinogenesis
Growth/Differentiation Factors in Organogenesis and Their Roles in Tumorigenesis

Keywords:
cell signaling
childhood tumors
differentiation
growth factors
induction
kidney
organogenesis
Wilms' tumors

Research: Our efforts are focused on characterizing the inductive signaling pathways involved in metanephric blastemal cell specification/patterning and those regulatory molecules, both secreted and nuclear, that modulate this process in an effort to define potential targets of carcinogenesis.

The regulation of epithelial morphogenesis is critical for the coordinate development of many adult organ structures. In the differentiating metanephros, this process is regulated by reciprocal interactions between ureteric bud epithelia and the metanephric mesenchyme. The ureteric bud invades the overlying mesenchyme and induces conversion of the mesenchyme into stromal and epithelial elements, giving rise to the polarized tubular and glomerular epithelial structures of the adult nephron. At the same time, the mesenchyme stimulates the ureteric bud to grow, branch, and eventually form the collecting duct system of the adult kidney. The Developmental Biology Working Group has focused on the elucidation of mechanisms of inductive signaling in metanephric development, seeking the ligands responsible for nephronic differentiation, other noninductive regulatory factors of nephrogenesis, and the molecular targets of induction. Previously, we reported the establishment of a cell line derived from the renal inductor, ureteric bud, and have now purified two soluble secreted inductive factors from medium conditioned by these bud cells. We have determined that they are leukemia inhibitory factor (LIF) and transforming growth factor-β2 (TGFβ2), that they can function independently of each other to induce tubular differentiation, and that in combination with FGF2 they accelerate this process to yield tubules with kinetics comparable to those found in vivo. We also found that signaling by these inductive cytokines correlates with activation of the Wnt pathway. Concurrently, we analyzed the developmental regulatory effects of two members of the secreted Frizzled-related protein (sFRP) family, which are expressed in the metanephros. These soluble receptors for Wnts can modulate Wnt protein interaction with and activation of membrane-bound Frizzled receptors. In studies of metanephric development, we reported that sFRP–1 is a potent inhibitor of Wnt signaling and tubulogenesis, while sFRP–2 can partially block the inhibitory activity of sFRP–1. Finally, in our search for molecular targets of induction, we have

identified some 72 sequences, of which 36 are novel, by differential display. One of the cDNA sequences that is downregulated with tubule formation, i.e., CITED1/melanocyte-specific gene 1, seems to play a pivotal role in specification of metanephric mesenchyme. It is specifically expressed in blastemal populations of the metanephros and nephroblastoma but not in differentiated epithelia, and appears to function through modulation of both Wnt and BMP signaling pathways in blocking tubulogenesis. This would suggest that it functions as a gatekeeper for metanephric blastemal cell differentiation. These findings provide insight into the dynamics and complex interactions that modulate stem cell specification in the kidney, and perturbations of these dynamics, at least in part, may explain the accumulation of stem cell populations in neoplasms such as Wilms' tumor/nephroblastoma.

Collaborators on this research include Mark de Caestecker, Michael Lerman, and Jeffrey Rubin, NIH; Sergei Ivanov, SAIC–Frederick; and Toshi Shioda, Harvard University.

Recent Publications:

Karavanova I, et al. *Development* 1996;122:4159–67.

Plisov SY, et al. *Genesis* 2000;27:22–31.

Plisov SY, et al. *Development* 2001;128:1045–57.

Yoshino K, et al. *Mech Dev* 2001;102:45–55.

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Biography: *Dr. Waalkes received his Ph.D. degree in pharmacology and toxicology in 1981 from West Virginia University where he studied perinatal toxicology of cadmium. He then completed postdoctoral work at the University of Kansas where his studies focused on the cellular and molecular mechanisms of acquired tolerance to metal toxicity. He has been with the NCI since 1983.*

Laboratory of Comparative Carcinogenesis
Molecular Mechanisms of Inorganic Carcinogenesis

Keywords:
apoptosis
arsenic
cadmium
chemical carcinogenesis
cytotoxicity
genomics
in vivo models
lead
metalloprotein
metallothionein
proto-oncogenes
rodent
transformation

Research: Some of the highest priority hazardous substances are inorganics. The EPA annually lists agents posing the greatest hazard to the U.S. population and for many years inorganics, such as arsenic, cadmium, chromium and lead, have consistently topped this list. These inorganics are particularly hazardous as they cannot be metabolized into less toxic subunits and often are bioaccumulated in organisms. Inorganics are also an important class of human and rodent carcinogens, but their modes of action are as yet undefined. Our work has focused on defining the molecular mechanisms of inorganic carcinogenesis.

Arsenic has been known to be carcinogenic to humans for over 100 years. Despite this, the mode of carcinogenic action for this important environmental contaminant is still unknown. Arsenic undergoes mono- and dimethylation and this is known to consume cellular methyl groups in the process. We recently found that in cells having undergone malignant transformation by low level chronic arsenic exposure, the genomic DNA is hypomethylated. Assessment of global DNA methylation showed that DNA from cells transformed with arsenic was undermethylated. The probable cause for this undermethylation is an arsenic-induced chronic depletion of available S-adenosyl-methionine, the methyl donor cofactor for many methyltransferases, including methyltransferases responsible for DNA methylation. The extent of undermethylation in these cells is highly correlated with tumorigenicity of the cells upon inoculation into nude mice. Thus, arsenic transformation is linked with DNA hypomethylation, a state often associated with gene activation and likely connected to acquisition of malignant phenotype. The genetic effects of arsenic-induced DNA hypomethylation are now being assessed by gene array techniques. We find that, indeed, arsenic-induced transformation is associated with the activation of many oncogenes and the reduced expression of tumor suppressor genes.

We have found that cadmium can malignantly transform human prostate epithelial cells. This is important confirmatory evidence since cadmium has been associated with prostate malignancies in humans and since both

incidence and mortality of prostate cancer in the United States have been increasing recently. Additionally, we find that cadmium blocks apoptosis induced by many genotoxic agents in several cell lines including human prostatic epithelial cells. The blockage of apoptosis by cadmium occurs in the absence of an effect on genotoxicity so it is probable that cadmium is allowing genetically damaged cells to escape this important cellular checkpoint and become transformed. This could be an epigenetic mechanism by which cadmium can be carcinogenic. This could have an important impact in tumor initiation and/or progression in the prostate by cadmium.

Recent Publications:

Achanzar WE, et al. *Cancer Res* 2001;61:455–8.

Waalkes MP, et al. *Toxicol Appl Pharmacol* 2000;166:24–35.

Chen H, et al. *Mol Carcinog* 2001;30:79–87.

Lu T, et al. *Toxicol Sci* 2001;59:185–92.

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The activities of this laboratory fall into several broad areas: macromolecular structure and function; membrane structure and function; immunology; pharmacokinetics; and computational and modeling methodology. The work is both theoretical and experimental. Application of theoretical understanding to these biological systems is accomplished through the use of advanced computing. The laboratory is exceptional in that all of its elements, including the experimental groups, use computers extensively.

Another major role of the laboratory is to provide scientific motivation and direction to the Advanced Biomedical Computing Center (ABCC), under contract to the NCI at the NCI–Frederick. Many of the theoretical studies are possible only through use of the supercomputing facilities at the ABCC, which was created and sponsored through this laboratory's initiative.

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Deborah Eyler	Support Staff
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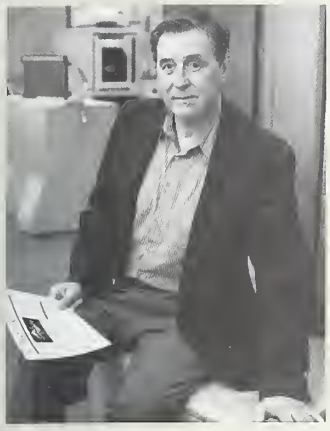

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Biography: *Dr. Maizel obtained his Ph.D. in biochemistry from the California Institute of Technology in 1959, then served in the Laboratory of Cell Biology, National Institute of Allergy and Infectious Diseases, until 1961, when he entered the Albert Einstein College of Medicine. In 1974, he returned to the NIH as head of the Molecular Structure Section in the Laboratory of Molecular Genetics, National Institute of Child Health and Human Development. He has been chief of the Laboratory of Experimental and Computational Biology at the NCI since 1983. On joining the NCI, he created a supercomputer facility known today as the Frederick Biomedical Supercomputer Center.*

Laboratory of Experimental and Computational Biology
Molecular Structures and Their Associations: Applications to Molecular Design

Keywords:
computational biology
computational chemistry
computational methods
protein structure
RNA secondary structure

Research: The basis of diseases and of their treatments resides in knowledge and comprehension of the three-dimensional structures of the proteins, nucleic acids, and other molecules that are involved. The availability of atomic-level structures, advances made in understanding their mechanisms, and highly efficient computational methodology that we have developed enable us to investigate binding sites on molecular surfaces of receptors and to dock a ligand onto a receptor surface, which will be useful in the design of potent inhibitors. We have recently developed and continue to improve highly effective descriptions of molecular surfaces that are successful and efficiently utilized for docking molecules of variable sizes. Using our sequence-order-independent, computer-vision-based methods, we have detected and cataloged interior and surface motifs. Using similar tools, we made a dataset of protein-protein interfaces and utilized it for studies of protein associations and their comparison to protein folding. Comparisons of this dataset to a dataset of protein monomers generated earlier illustrates the similarities and differences between the types of architectures at the interfaces and in single-chain proteins. Theoretical chemical methods are incorporated into the analyses and show that hydrophilic effects are more important than hydrophobic forces in interfaces. An efficient computational algorithm designed for cutting proteins into highly hydrophobic, compact, subdomain modules is used to produce a database of folding units that provides deeper insights into the architecture of proteins and how they are assembled. Analyses of structural information lead to deep understanding of the relationships between protein folding, binding, and function through modeling the view as funnels leading from a large ensemble of less stable conformations into a limited number of optimal states.

RNA structure and function, no less important than that of proteins, is studied using thermodynamics-based algorithms for secondary structure and molecular mechanics and dynamics for three-dimensional predictive modeling. Correlations drawn with experimental data from mutagenesis and

NMR show successful agreement in retroviral ribosomal frameshifting. Internal ribosome entry in 5′ untranslated regions (5′UTR), a hallmark of picornaviruses, correlates with conserved, predicted structural elements. Similar elements are predictions in some 5′UTRs of cellular mRNAs. Many mRNAs have long 3′UTRs that contain unusual structures that may be implicated in stability, expression, localization, and development. Refinements in predictive methods are continually developed.

Recent Publications:

Kumar S, et al. *Biophys J* 2001;80:2439–54.

Tsai CJ, et al. *Proc Natl Acad Sci USA* 2000;97:12038–43.

Chen SL, et al. *Nucleic Acids Res* 2000;28:2375–82.

Maizel JV. *Trends Biochem Sci* 2001;25:590–2.

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Biography: *Dr. Blumenthal obtained his Ph.D. in physical chemistry at the Weizmann Institute. Following postdoctoral work at the Institute Pasteur and at Columbia University, he came to the NIH and was ultimately recruited by the NCI. Dr. Blumenthal has worked in a wide range of areas in membrane biophysics, which includes membrane fusion, membrane transport, cell surface receptors, immune cytotoxic mechanisms, and use of liposomes for delivery of drugs and genes into cells. Dr. Blumenthal's current interest is in the cell biology of virus entry, replication, and pathogenesis.*

Laboratory of Experimental and Computational Biology
Regulation of Membrane Fusion by Viral and Cellular Proteins and Lipids

Keywords:
AIDS
biochemistry
chemokine receptors
dominant-negatives
fluorescence
HIV–1
HIV–1 inhibitor
lymphocytes
membranes
peptides
proteins
receptor functions
SIV
vaccines

Continued on page 499

Research: Our goal is to find out how enveloped animal viruses inject their nucleocapsid into the target cell. Cells are surrounded by cell membranes which impose insurmountable barriers for passage of undesirable molecules and particles into the cell. Viruses are enveloped by similar membranes. The virus has developed strategies to overcome these insurmountable barriers by designing envelope glycoproteins which catalyse the fusion of viral and cellular membranes. We are specifically studying the mode of action of the envelope glycoproteins of human immunodeficiency virus (gp120-gp41), murine leukemia virus, vesicular stomatitis virus, paramyxovirus, and influenza virus.

The viral envelope glycoproteins assemble into a viral fusion machine which forms a molecular scaffold responsible for bringing the viral membrane close to the target cell membrane and creating the architecture that enables lipid bilayers to merge. Triggering the fusion machine results in drastic conformational changes in viral envelope glycoproteins. The fusion reaction then

Keywords (continued):
vaccinia
viral envelope genes
viral receptors
virus-cell interaction

undergoes multiple steps before the final event occurs, which allows delivery of the nucleocapsid into the cell. We have dissected these steps kinetically and analyzed the molecular features of the kinetic intermediates. High-resolution structural studies have led to the proposal that there are native (nonfusogenic) and fusion-active (fusogenic) states of viral membrane fusion proteins. Our conformational exploration has unveiled a number of states en route between the native and fusogenic states, some of which represent inactivation. Photosensitized labeling studies indicate that the conformational transitions are irreversible in the case of influenza HA, and reversible in the case of VSV G, suggesting a velcro-like attachment in the latter case. Using quantitative fluorescence video microscopy, we have continuously measured CD4-induced conformational changes of cell surface-expressed HIV–1 envelope glycoprotein gp120-gp41 in situ using bis-ANS as a fluorescent probe which lights up in hydrophobic pockets. CD4-expressing human T cell lines induced significant and rapid conformational changes in gp120-gp41 from T-tropic but not M-tropic HIV–1 strains. Macrophages induced similar changes in gp120-gp41 from M-tropic but not T-tropic HIV–1 strains. Thus, the conformational changes undergone by gp120-gp41, which lead to membrane fusion, are highly cooperative and require both receptor and coreceptor.

We have also shown that in addition to CD4 and coreceptor, these conformational changes leading to fusion and infection are critically dependent on specific glycosphingolipids in the target membrane. Our data are consistent with the notion that a limited number of specific glycosphingolipids species serves as a crucial element in organizing gp120-gp41, CD4, and an appropriate chemokine receptor into a membrane fusion complex. To study fusion intermediates we have monitored redistribution of membrane-bound and cytosolic dyes following the triggering of fusion events. In HA-mediated fusion, we have dissected out intermediate stages kinetically. In the case of HIV–1 gp120-gp41-mediated fusion, we have revealed intermediates using synthetic peptides corresponding to specific residues of the HIV–1 gp41 sequence which inhibit at various stages of fusion. We have shown that the inhibitory peptide DP178 acts after the CD4 and chemokine receptor-induced conformational changes in gp120-gp41 have occurred. We have assessed the ability of the mutant gp41s to transfer lipid and small solutes from susceptible target cells to the gp120-gp41-expressing cells. We found an interesting phenotype that was not capable of inducing syncytia formation but still mediated dye transfer, indicating that the fusion cascade was blocked beyond the stage of small fusion pore formation. We have analyzed these data in terms of a fusion model which incorporates the recently determined high-resolution structure of the gp41 core.

Collaborators include Eric Hunter, University of Alabama; Stephen Morris, University of Missouri; and Yechiel Shai, Weizmann Institute, Israel.

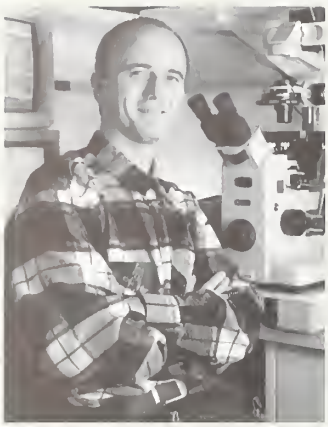
Recent Publications:

Dimitrov D, et al. *J Biol Chem* 2001;276:30335–41.

Kliger Y, et al. *J Biol Chem* 2001;276:1391–7.

Raviv Y, et al. *FASEB J* 2000;14:511–5.

Hug P, et al. *J Virol* 2000;74:6377–85.



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Biography: *Dr. Dimitrov obtained his Ph.D. in chemistry from the University of Sofia in 1976, and his Sc.D. in biology from the Bulgarian Academy of Sciences, where he was a professor of biophysics and chief of the Department of Membrane Interactions. He was also a visiting scientist at the Cancer Research Laboratory of Carnegie-Mellon University and the Department of Cell Biology of the Holland Laboratory for Biomedical Research. He was a member of the editorial boards of* Cell Biophysics, Bioelectrochemistry, Bioenergetic, *and* Pathobiology, *and currently of* Experimental and Molecular Pathology.

Laboratory of Experimental and Computational Biology
HIV Entry, Dynamics, and Pathogenesis; Cell Dynamics in AIDS and Cancer

Keywords:
AIDS
CCR5
HIV

Research: Human immunodeficiency virus (HIV), the etiological agent of acquired immunodeficiency syndrome (AIDS), enters cells by binding its envelope glycoprotein (Env) to receptor molecules and fusing its membrane with the cell membrane. The infection cycle then proceeds through a series of steps which may result in virus transmission and AIDS. Understanding how HIV enters cells and what determines the kinetics of virus infection is important for elucidating the mechanisms of HIV pathogenesis and the development of vaccines and drugs. By using a variety of experimental and mathematical approaches, we discovered important interactions of the receptor molecules with the HIV–1 Env, as well as relationships between parameters of virus infection kinetics and how they affect responses to treatment.

Currently, we are characterizing the interactions in the HIV Env-CD4-coreceptor complex leading to entry. We are producing large amounts of complexes for evaluation of their potential as HIV vaccines. We are also analyzing the dynamics of spreading HIV infections in tissue cultures, monkeys, and humans, as well as turnover of T cells, and how these processes are related to pathogenesis and responses to antiretroviral treatment. An overall conclusion from our work is that Env-CD4-coreceptor complexes play a critical role in HIV entry and may have potential as vaccines, that turnover of T cells is related to HIV pathogenesis, and that efficacy of antiretroviral treatment could be evaluated one week after initiation of therapy by measuring HIV dynamics.

Current collaborators include C.C. Broder, Uniformed Services University of the Health Sciences; H. Golding, FDA; Z. Grossman, Tel Aviv University; and C. Lane, M.A. Martin, W. Paul, M. Polis, and R. Yarchoan, NIH.

Recent Publications:

Xiao X, et al. *Proc Natl Acad Sci USA* 1999;96:7496–501.

Grossman Z, et al. *Nat Med* 1999;5:1099–104.

Polis M, et al. *Lancet* 2001; in press.

Dimitrov DS. *Cell* 2000;101:697–702.

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Biography: *Dr. Guy obtained his Ph.D. in biophysics from the University of Illinois where he used electrophysiological methods to study postsynaptic receptors in neurons. He began to develop models of membrane channel structures during his postdoctoral fellowship at the State University of New York at Albany. His most notable work involves modeling the three-dimensional structure and functional mechanisms of voltage-gated and mechanosensitive ion channels.*

Laboratory of Experimental and Computational Biology
Structural Modeling of Membrane Channel Proteins

Keywords:
ion channels
membrane proteins
molecular models
protein structures

Research: The primary goals of our group are to develop procedures to model the three-dimensional structures of membrane proteins and to use these procedures to develop models of specific proteins. Most of our current work concentrates on superfamilies of ion channel proteins for which a crystal structure of at least one member has been determined. Our most noted work has been on potassium channels and proteins, such as sodium and calcium channels, that evolved from them. The recent determination of a crystal structure of a bacterial potassium channel allows us to better evaluate our methods and results, and lets us move on to a more precise level of molecular modeling. Our early models of the channel protein's secondary structure, its transmembrane topology, and the general location and orientation of the pore forming segments and predictions about which portions of the protein form the ion selective region, voltage sensor for activation gating, the inactivation gate, and binding sites for a variety of drugs and toxins have been confirmed. However, atomic scale models of large proteins that are not constrained by precise structural data always contain errors. The crystal structure allows us to correct these errors for the highly conserved ion selective portion of the potassium channels. Based on this new information, we are currently extending our models to numerous membrane proteins that are homologous to the potassium channel that was crystallized or that may have structures similar to it. These include not only numerous families of potassium channels, but also sodium, calcium, and cyclic nucleotide-gated channels, ionotrophic glutamate receptors, and some types of potassium transport proteins. We are also examining conformational changes in these proteins and have used computer graphic and computational chemistry methods to develop models of the entire transmembrane

portion of the several potassium channels in closed, open, and inactivated conformations. These models are supported by results of many mutagenesis experiments obtained from numerous groups. We have also worked on mscL, which forms mechanosensitive channels in bacterial membranes. This project exemplifies how we use molecular modeling to augment experimental studies of the structure and functional mechanisms of membrane proteins. Beginning with the crystal structure of a closed conformation of mscL from *M. tuberculosis*, we: (1) developed models of the N terminus which was unresolved in the crystal structure; (2) developed homology models of mscL from *Escherichia coli*; (3) developed models of the conformational changes involved in opening the channels; and (4) used mutagenesis experiments on *Escherichia coli* mscL to test these models.

Collaborations include Peter Backx, Toronto University; Evert Bakker, Universität Osnabrück; Stewart Durell, NIH; Saul Goldman, Guelph University; Tatsunosuka Nakamura, Chiba University; and Sergei Sukharev, University of Maryland.

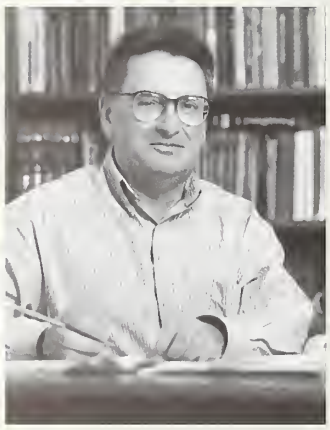
Recent Publications:

Sukharev S, et al. *Nature* 2001;409:720–4.

Durell SR, et al. *Biochem Biophys Res Commun* 2001;281:741–6.

Durell SR, et al. *Biophys J* 2000;78:188–99.

Durell SR, et al. *Biophys J* 1999;77:775–88, 789–807.



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Biography: *Dr. Jernigan received his Ph.D. from Stanford University and studied as a postdoctoral fellow at University of California–San Diego. He has been at the NIH since 1970 and in his present position since 1972. He has served on various NIH committees including the Laboratory of Structural Biology Steering Committee. He serves on the editorial advisory board of the journal* Biochemistry *and the Publications Committee overseeing the* Biophysical Journal.

Laboratory of Experimental and Computational Biology
Proteins and Nucleic Acids: Their Interactions and Folds

Keywords:
bioinformatics
computational methods
conformation
nucleic acid-protein complexes

Research: Protein, nucleic acid, and small molecule structures are investigated with computational methods, focusing on interactions within proteins, how to infer functional mechanisms from structures, and developing molecular models.

Protein Interactions

Simpler ways to view protein structures and their folding patterns are being developed by coarse graining. We have assessed interactions from available

Continued on page 503

Keywords (continued):
nucleic acids
protein stability
protein structure
structure determination
tumor suppression

structures and other experimental data. We developed a standard way to view interaction energies between residues, based on sets of protein structures. This approach led to useful ways to incorporate structural and hydrophobicity information into simulations. Packing of residues has been demonstrated to exhibit a substantial regularity that can be utilized for more efficient computations.

Protein Folding
In applying these interaction potentials, we showed that they are useful for selecting native forms among various folds in one of the earlier demonstrations of threading a sequence through structures; in more recent threading we include insertions and deletions. In recent studies we developed new ways to enumerate protein conformations with extremely high efficiency. This is particularly important for determining native protein conformations, where the problem is akin to searching for a needle in a haystack, and random searches are typically not effective. This new approach opens the way for the computer generation of much larger numbers of protein conformations. Efforts to extract general features of protein structures are continuing.

Inferring Function from Structure
A new approach has been taken to develop mechanisms from structures. This is based on residue contact matrices derived directly from crystal structures. All contacting residues have an identical spring connecting them, and the modes of motion of this system of interconnected springs are developed. The motions are used to calculate the temperature factors and hydrogen exchange factors which compare favorably with experiments. The domain motions can be observed directly with this method, and correlations in fluctuations of different regions can be interpreted mechanistically. For example, motions in reverse transcriptase indicate that the fingers move coherently in a direction perpendicular to the thumb, and both move in a direction opposing that of the ribonuclease H domain, representing a processing motion; i.e., when the "hand" is open, the RnaseH domain pulls the nucleic acid strand away. Other recent applications have been made to the GroEL/GroES system and to tubulin. New methods now permit calculations on extremely large systems.

A New Method for Rapid Protein Structure Determination
X-ray crystallography and NMR are the usual ways to determine protein structures, but they are relatively slow. In addition to the urgent need for a huge number of protein structures, it is easy to envision the need to determine structures of aberrant proteins, even to the extent of providing structures for individual diagnostics. The problem of crystallizing aberrant structures is extremely intimidating, since both flexibility and aggregation can interfere with crystallization. Because of the need for structure determination of many hundreds of thousands of new proteins, *a faster way to determine structures is urgently required*. We are attempting to develop a new faster method for structure determination that could ultimately yield a rapid and efficient rationalization of the functions of large sets of proteins. The basis of this project is a new powerful combination of computational analyses and rapid biochemical/biophysical experiments. The essence of the approach is to determine a limited number of distance measurements, determined rapidly and automatically by mass spectroscopy (or other spectroscopic methods)

followed by computational searches through huge libraries of possible protein structures. We have already generated huge libraries of computed structures. Neither the experimental nor the computational components are robust enough to determine independently the structure. But, by combining an experimental mapping of the protein surface, via mass spectrometry and fluorescence, together with database searches through a structure library, it should be possible to select the correct structure.

Post-Genomic Studies of Nucleoprotein Interactions

In pursuing the ultimate goal of understanding the molecular mechanisms of gene regulation, we have three specific projects:

- **Assembly of prokaryotic repressosomes:** Usually multisubunit protein-DNA complexes involve DNA looping, which brings the protein subunits together. The prokaryotic repressosomes and eukaryotic enhancesomes are among the best characterized complexes of this kind. Despite an ongoing effort to characterize DNA loop structures, our knowledge of any of them remains incomplete largely because of their size and complexity and their dependence on DNA topology. Recently, we suggested an innovative approach for determining such complex structures. First, genetic (sequence variability) data were used to determine the overall orientation of the *gal* repressors subunits in a tetrameric structure. Next, empiracally based computer modeling was employed to determine the trajectory of the DNA loop. According to our data, a similar (antiparallel) DNA loop is formed upon binding of *lac* repressor to DNA. This model immediately suggests experiments to elucidate further details of the 3D organization of the *gal* repressosome, and its modulation by DNA mutations and changes in the environment.

- **Stabilization and regulation of the transcription elongation complex:** Another new method, which permits the visualization of DNA and RNA trajectories in nucleoprotein complexes, is 125-iodine radioprobing, successfully applied to the CRP–DNA complex. This technique is useful when the protein structure is known, but the whole complex is too large and/or flexible to be analyzed by x-ray or NMR. Therefore, the T7 transcription complex is an ideal object for this method. With this approach, we were able to detect for the first time the DNA–RNA heteroduplex outside the T7 polymerase "cleft." Among other applications, this finding suggests a novel mechanism for stabilization and regulation of the elongation complex. We plan to study the eukaryotic Pol II transcription complexes, using radioprobing, time-resolved fluorescence and computer modeling to visualize the behavior of all three DNA/RNA strands during initiation and elongation. We anticipate that this will provide details of those molecular mechanisms, which are responsible for the fidelity of RNA synthesis, and are involved in recognition of DNA mismatches during the course of transcription.

- **The role of mutations on the formation of the p53-DNA complex; modeling the structure of the tetrameric p53 complex and DNA deformations:** Our radioprobing data indicate that, upon binding to p53, DNA is severely bent and overtwisted at CATG tetramers, consistent with gel electrophoresis results. If so, then the apparent role of the sequence-specific DNA deformation may direct p53-p53 interactions in order to form an interface on the surface of the p53 tetramer suitable for binding of other

proteins, such as Mdm2. A next step is to analyze the effects of mutations in p53 and substitutions at the p53 binding sites upon stabilization of the tetrameric p53–DNA assembly.

The expansion of structural databases for nucleic acids, proteins, and nucleoprotein complexes, as well as continued progress in elucidating the sequence-dependent structural properties of DNA and RNA, will improve the accuracy with which large nucleoprotein complexes can be characterized by the methods described above.

Drug Discovery

In other recent studies, we have been developing ways to apply what we have learned about molecular interactions to the selection of new drugs against target proteins. Preliminary screenings based on these approaches are promising.

Collaborators include Sankar Adhya, Ettore Appella, Daniel Camerini-Otero, Angela Gronenborn, Sharlyn Mazur, Ron Neumann, and Igor Panyatin, NIH; Ivet Bahar, University of Pittsburgh; Rodney Harrington, University of Arizona; Tom Jovin, Max Planck Institute, Goettingen; and Wilma Olson, Rutgers University.

Recent Publications:

Bahar I, et al. *J Mol Biol* 1999;285:1023–37.

Nagaich AK, et al. *Proc Natl Acad Sci USA* 1999;96:1875–80.

Keskin O, et al. *Biophys J* 2000;78:2093.

Miyazawa S, et al. *Protein Eng* 2000;13:459.

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Biography: *Dr. Lemkin obtained his Ph.D. in computer science from the University of Maryland where he developed computer methods for image analysis of bone marrow smears. Later research concentrated on computer image and database analysis of cDNA microarrays, 2D electrophoretic PAGE gels with the development of the GELLAB system, and most recently on advanced methods for providing collaborative access to biomedical databases through the World Wide Web. The latter includes cDNA data mining tools, a protein-disease database, 2DE searchable databases, 2D gel comparison over the Internet using Flicker, the 2DWG (2D World Gel) database, and WebGel systems. Recent work includes WebGel analysis of prostate cancer nuclear matrix proteins, and cDNA microarray exploratory data analysis using a system called the MicroArray Explorer (MAExplorer) for a mouse mammary genome.*

Laboratory of Experimental and Computational Biology
Biological Database Software

Keywords:
2DE gels
data mining
databases
Internet
microarray
proteins
Web

Research: The existence of large biological databases requires problem-domain oriented software to better utilize this data. The IPS is working on this in a number of areas: (1) Web-based cDNA microarray data mining tools for the Mouse Mammary Genome Anatomy Project (MGAP); (2) interactive biomedical database image analysis across the Internet using the Flicker system; and (3) Internet groupware for computer aided two-dimensional electrophoretic gel analysis using WebGel.

We have developed a Web-based cDNA microarray database exploratory data analysis tool called the MicroArray Explorer (MAExplorer) for analyzing expression data (HREF=**http://www.lecb.ncifcrf.gov/MAExplorer**). It is a Java program which runs in a user's computer. It allows the exploratory data analysis of quantitative cDNA expression profiles across multiple micro-arrays. Data may be filtered, viewed, and directly manipulated in images, scatter plots, histograms, expression profile plots, cluster analysis, etc. Reports on clones or arrays may be generated with Web access to UniGene, GeneBank, and other Internet databases. Reports may also be exported to Excel. Arrays are used to monitor expression profiles under various physiological conditions. At this point expression profiles have been obtained from several stages of normal mammary gland development and different tumor models. With this program, you may: (1) analyze the expression of individual genes; (2) analyze the expression of gene families and clusters; and (3) compare expression patterns.

Scientists around the world often work on similar data, so the need to share results and compare data arises periodically. We developed a flicker computer method of comparing 2-dimensional (2D) protein gels and other biomedical materials across the Internet (HREF=**http://www.lecb.ncifcrf.gov/flicker**). Comparing similar samples created in different laboratories helps identify or suggest protein spot identification. Now that 2D gels and associated

databases are frequently appearing on the Internet, this opens up the possibility of visually comparing one's own experimental 2D gel image data with data from another gel in a remote Internet database. To aid this effort, we support a meta-database, 2DWG, of 2D gel images in 2DE servers on the Internet (HREF=**http://www.lecb.ncifcrf.gov/2dwgDB**).

Often one wants to do quantitative comparison as well as qualitative comparison of 2DE gels. We developed a collaborative groupware Web-based exploratory analysis system, WebGel, for comparing 2DE gels and sharing results between members of the group. It makes available some of the facilities of the GELLAB–II system but on the Internet. The GELLAB–II software system is an exploratory data analysis system for the analysis of sets of 2D electrophoretic protein gel images. It incorporates image acquisition, processing, database manipulation, graphics, and statistical analysis. It has been applied to a variety of experimental systems which keep track of quantitative and qualitative changes in one or more proteins among hundreds or thousands of unaltered proteins in the basic analytic problem. A composite gel database may be "viewed" under different exploratory data analysis conditions and statistical differences and subtle patterns elucidated from "slices" of an effectively 3D database. Some of the current collaborative projects including using WebGel/GELLAB–II for the identification of nuclear matrix prostate proteins correlated with stages of prostate cancer, which may aid in the screening and staging of men with prostate cancer and determining possible diagnostic marker proteins for studies of Rett Syndrome (CDC).

Collaborators include Ester Asaki, Jai Evans, John Powell, Chris Sailor, CIT; Terry Clark, University of Chicago; L. Hennighausen, NIH; and Bob Stephens and G. Thornwall, SAIC–Frederick.

Recent Publications:

Lemkin PF, et al. *Electrophoresis* 1999;20:3492–507.

Lemkin PF, et al. *Mol Biotechnol* 1999;12:159–72.

Pannek J, et al. *Virology* 1999;54:934–9.

Lemkin PF, et al. *Nucleic Acids Res* 2000;28:4452–9.

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Biography: *Dr. Qasba is the chief of the Structural Glycobiology Section in the Laboratory of Experimental and Computational Biology. After receiving his Ph.D. degree in pharmaceutical synthetic chemistry from Munich University, Germany, he studied the transcription of phages and animal viruses during his postdoctoral work at the Max Planck Institute of Biochemistry, Munich, and later at the Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, NIH. He initiated the molecular biological studies of Golgi glycosyltransferases and α-actalbumin and is currently investigating their structure/function relationships.*

Laboratory of Experimental and Computational Biology
Protein-Carbohydrate Interactions: Structure/Function Relationship of Glycosyltransferases

Keywords:
carbohydrates
conformation
enzymes
protein structure
supercomputing

Research: Complex carbohydrate moieties of glycoproteins and glycolipids are present on the surface of all cells and within the cellular matrix, and they perform specific cellular functions. Although there is no single unifying function assigned to oligosaccharides, they clearly serve as recognition markers. Also, the carbohydrate part of proteoglycans, called glycosaminoglycans, take part in a wide range of biological functions. For example, they are involved in forming the extracellular matrix with collagen to which growth factors bind with a high degree of specificity and thus regulate the growth factor activity. The assembly of complex carbohydrates on glycoproteins and glycolipids requires the concerted action of a superfamily of enzymes that are collectively called glycosyltransferases. These enzymes are grouped into subfamilies based on the type of sugar they transfer from a sugar-nucleotide donor to an acceptor sugar. They reside in different parts of the Golgi apparatus where the catalytic domain of the enzyme faces towards the Golgi lumen where they add sugars to the proteins and lipids. Some glycosyltransferases have also been shown to be present at the cell surface interacting with the cell matrix and have been implicated as signaling molecules for pattern formation during development. The exact processing of oligosaccharide structures depends on the species, tissue developmental stage, and the availability of the repertoire of glycosyltransferases (and glycosidases). Several diseases have been associated with the lack of or alteration in the activity of these enzymes. The three-dimensional structural knowledge of the glycosyltransferases and their complexes with the oligosaccharides is highly desirable for understanding the biosynthesis and functions of glycoproteins, and our laboratory is contributing towards these goals.

For structure/function studies, we are producing by recombinant methods the proteins of β-1,4-galactosyltransferase subfamily (Gal-T) members. We have determined the crystal structure of galactosyltransferase-T1 (Gal-T1)

in complex with α-lactalbumin and bound with various substrates at 2 Å resolution. These structures reveal that upon substrate binding to Gal-T1, a large conformational change occurs in the enzyme in a region that positions the residues in such a way that creates metal, sugar, and α-lactalbumin binding sites on the enzyme. We are now determining the structure of Gal-T1 in complex with various oligosaccharides. By site-directed mutational analysis, crystallographic, and molecular modeling methods we are studying sugar-induced protein-protein interaction between Gal-T1 and α-lactalbumin. An oligosaccharide can exit in several conformations. The protein-carbohydrate interactions that lead to a biological response involve the interactions between a unique conformer of the oligosaccharide and a protein molecule. Thus the precise information about all the conformers that are accessible by the oligosaccharide is essential. Such information is important in the studies on the modulation of protein-protein interactions by carbohydrate moieties or in understanding the biosynthetic pathways of N-linked oligosaccharides. The examples of the involvement of sugars in protein-protein interactions have been assembled in a recent review article from the laboratory. These studies, together with the studies from other laboratories, show that in the oligosaccharide-induced recognition process, sugars orient the molecules in a way that brings about specific protein-protein or protein-carbohydrate interactions.

Recent Publications:

Ramakrishnan B, et al. *J Mol Biol* 2001;310:205–18.

Shah PS, et al. *Biochim Biophys Acta* 2000;1480:222–34.

Qasba PK. *Carbohydr Polym* 2000;41:293–309.

Iyer LK, et al. *Protein Eng* 1999;12:129–39.

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Biography: *After receiving a B.S. in biology at the Massachusetts Institute of Technology, Dr. Schneider obtained his Ph.D. in molecular biology with Larry Gold in the Department of Molecular, Cellular and Developmental Biology at the University of Colorado, Boulder. His thesis and postdoctoral work, also done at Boulder, were on the application of information theory to nucleic-acid binding sites. He was a member of the GenBank Advisory Committee. He helps to organize and run the international news group bionet.info-theory, which is devoted to the application of information theory to biology.*

Laboratory of Experimental and Computational Biology
Information Theory in Molecular Biology

Keywords:
bioinformatics
computational biology
computer analysis
computer graphics
DNA binding protein
DNA sequencing
Escherichia coli
gene regulation
genetic algorithm
G-proteins
information theory
molecular evolution
molecular information theory
molecular machines
nanotechnology
sequence logo
sequence walker
splicing

Research: Shannon's measure of information is useful for characterizing the DNA and RNA patterns that define genetic control systems. Dr. Schneider has shown that binding sites on nucleic acids usually contain just about the amount of information needed for molecules to find the sites in the genome. This is a working hypothesis, and exceptions can either destroy the hypothesis or reveal new phenomena. For this reason, he is studying several interesting anomalies.

The first major anomaly was found at bacteriophage T7 promoters. These sequences conserve twice as much information as the polymerase requires to locate them. The most likely explanation is that a second protein binds to the DNA. In another case, he discovered that the F incD region has a three-fold excess conservation, which implies that three proteins bind there. Both anomalies are being investigated experimentally. Thus, the project has three major components: theory, computer analysis, and molecular biology experiments. The theoretical work can be divided into several levels. Level 0 is the study of genetic sequences bound by proteins or other macromolecules, briefly described above. The success of this theory suggested that other work of Shannon should also apply to molecular biology. Level 1 theory introduces the more general concept of the molecular machine, and the concept of a machine capacity equivalent to Shannon's channel capacity. In Level 2, the Second Law of Thermodynamics is connected to the capacity theorem, and the limits on the functioning of Maxwell's Demon become clear. The practical application of this work for most molecular biologists will be the replacement of consensus sequences with better models. Consensus sequences are being used to characterize the binding sites of macromolecules on DNA and RNA. After aligning a set of binding-site sequences, the most frequent base is chosen. A position that contains 100 percent As will be represented by an A, while a position that is only 75 percent A will also be represented by an A. The consensus is frequently used to search for binding sites, and the number of mismatches to the consensus is counted. A mismatch to a 100 percent A position is much more severe than one to a 75 percent A, but this is not accounted for so the researcher is misled. Mathematically robust graphical

replacements for the consensus sequences called the Sequence Logo and Sequence Walkers won't discard hard-earned data. The Walker, which is patented, has direct medical application because it can be used to distinguish polymorphisms from mutations in human sequences. The Walker method allows one to display many different binding sites simultaneously. This bird's-eye-view is a powerful tool for gene structure analysis. Further information and examples may be found on the Internet at the World Wide Web site **http://www.lecb.ncifcrf.gov/~toms/**.

We collaborated on this research with Peter Rogan, Allegheny University of the Health Sciences.

Recent Publications:

Schneider TD. *Nanotechnology* 1994;5:1–18.

Schneider TD. *Methods Enzymol* 1996;274:445–55.

Schneider TD. *J Theor Biol* 1997;189:427–41.

Schneider TD. *Nucleic Acids Res* 1997;25:4408–15.

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Biography: *Dr. Shapiro has been with the NIH since 1973 and the laboratory since 1983. He received his Ph.D. in computer science from the University of Maryland in 1978, with undergraduate work in mathematics and physics. During his association with the NIH, Dr. Shapiro has done extensive work in image processing and nucleic acid structure prediction and analysis, leading to several algorithms and computer systems in these areas. His latest interests involve the use of massively parallel computing in the realm of computational biology and molecular modeling.*

Laboratory of Experimental and Computational Biology
Parallel/Heterogeneous Computational Biology and RNA Structure

Keywords:

3D molecular motifs
computational biology
computational methods
computer analysis
computer graphics
computer modeling
computer program
conformation
coxsackie B viruses
data mining

Continued on page 512

Research: Revolutionary changes in computational paradigms will be required to maintain the necessary computational power to solve problems in molecular biology over the next several years. Standard methodologies of sequential computer architectures cannot be expected to continually keep pace with the needed computational speeds. In order to accommodate the high speeds that are necessary, heterogeneous and massively parallel computational techniques are going to be required. We have been applying these computational paradigms to the area of RNA structural biology.

Parallel/Heterogeneous Computation and RNA Structure

A complete understanding of the function of RNA molecules requires a knowledge of their higher order structures (2D and 3D) as well as the

Keywords (continued):
databases
dimerization
genetic algorithm
HIV
molecular dynamics
molecular models
molecular structure
motifs
nucleic acids
parallel computing
protein families
protein sequences
retrovirus
RNA folding
RNA secondary structure
sequence analysis
simulation
supercomputing
thymidylate synthase

characteristics of their primary sequence. RNA structure is important for many functions, including regulation of transcription and translation, catalysis, and transport of proteins across membranes. The understandings of these functions are important for basic biology as well as for the development of drugs that can intervene in cases where pathological functionality of these molecules occurs. Methodologies for improving RNA folding techniques help in furthering the understanding of the functional properties of these molecules. We have developed and improved upon an RNA folding technique that uses concepts from genetic algorithms on a massively parallel SIMD supercomputer, a MasPar MP–2 with 16384 processors. We have also adapted the algorithm to massively parallel MIMD supercomputers, a 512 processor CRAY T3E, and a 64 processor ORIGIN 2000. The algorithm can also run on a single processor of an SGI OCTANE. Exceptional scaling characteristics seem to be maintained across these different computational platforms with the ability to run the algorithm with hundreds of thousands of virtual processors. RNA pseudoknot prediction is part of the GA, resulting in its ability to predict tertiary interactions. Other features include a Boltzmann relaxation technique, the ability to incorporate different energy rules, and forced inhibition and embedding of desired helical stems. In addition, STRUCTURELAB, the heterogeneous bioinformatical RNA analysis workbench, has been enhanced in conjunction with RNA_2D3D to produce the predicted atomic coordinates of RNA structures along with the visualization of these structures. Also, a novel interactive visualization methodology has been added to STRUCTURELAB that enables the comparison and analysis of multiple sequence RNA folds from a phylogenetic point of view, thus allowing improvement of predicted structural results across a family of sequences. These systems have been adapted to other environments inside and outside our laboratory and the NIH.

Computational Studies of RNA Folding Pathways

RNA folding pathways are proving to be quite important in the determination of RNA function. Studies indicate that RNA may enter intermediate conformational states that are key to its functionality. These states may have a significant impact on gene expression. It is known that the biologically functional states of RNA molecules may not correspond to their minimum energy state, that kinetic barriers may exist that trap the molecule in a local minimum, that folding often occurs during transcription, and that cases exist in which a molecule will transition between one or more functional conformations before reaching its native state. Thus, methods for simulating the folding pathway of an RNA molecule and locating significant intermediate states are important for the prediction of RNA structure and its associated function. Two representative folding pathway applications, potato spindle tuber viroid and the host-killing mechanism of *Escherichia coli* plasmid R1, have been successfully modeled utilizing the massively parallel genetic algorithm. Computational results are consistent with those derived from biological experiments, and some novel structural interactions are predicted.

Computational Studies of Three-Dimensional RNA Structures

Some structural elements of RNA molecules are being studied using molecular mechanics and molecular dynamics simulations. The structures currently being studied include an RNA tetraloop and a three-way junction that appear in the crystal structure of the central domain of the 30S ribosomal

subunit from *Thermus thermophilus*. It has been experimentally determined that the intermolecular interactions between the three-way junction and the S15 ribosomal protein initiate the process of the assembly of the 30S ribosomal subunit. By using molecular dynamics simulations with the AMBER 6 software package, we have obtained some insights into the conformational transitions of the junction associated with the binding of S15.

In addition, the molecular dynamics trajectories associated with temperature-dependent denaturation of the tetraloop and the subsequent refolding to the original crystal structure have been examined. The stabilizing influence of the S15 ribosomal protein on the tetraloop has been analyzed. In both of these cases, besides revealing new atomic level details, the structural transitions in these regions correspond to results derived from thermodynamic and biochemical experiments.

Experimental/Computational Studies of the HIV Dimer Linkage Site
We have continued a collaborative study of the dimer linkage site of HIV–1 RNA which is important for viral encapsidation. Based on experiments which include ultracentrifugation, gel shifts, RNase mapping studies, and computational approaches, we have been characterizing the RNA structure of this region and the binding affinity of the nucleocapsid protein NCp7.

Experimental/Computational Studies of Thymidylate Synthase RNP
We have recently begun collaborative work on elucidating, using computational methods, the RNA/protein structural interactions between the enzyme thymidylate synthase (TS) and TS mRNA. Experiments have indicated that translation of human TS mRNA is controlled by an a negative autoregulatory mechanism with its own synthesized protein. The existence of these RNA/protein complexes has been found in human colon cancer cells.

Our collaborators include Carmen Allegra and Ettore Appella, NIH; Donald Atha, National Institute of Standards and Technology; Nora Chapman and Steven Tracy, University of Nebraska Medical Center; Kathleen Currey, University of Maryland Medical Center; Hugo Martinez, N. Pattabiraman, Boopathy Ramakrishnan, SAIC–Frederick; Dennis Shasha, New York University; Jason Wang, New Jersey Institute of Technology; and Kaizhong Zhang, University of Western Ontario.

Recent Publications:

Shapiro BA, et al. *J Mol Graph* 1996;14:194–205.

Shapiro BA, et al. *Bioinformatics* 2001;17:137–48.

Kasprzak W, et al. *Bioinformatics* 1999;15:16–31.

Shapiro BA, et al. *Proc Atlantic Symp Computational Biol and Genome Sys Tech* 2001;10–3.

Laboratory of Experimental Carcinogenesis

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The research strategies of the Laboratory of Experimental Carcinogenesis (LEC) are: (1) to use genetic, cellular, and biochemical approaches to elucidate the basic hepatic stem cell biology; (2) to generate transgenic mouse models relevant for human tumor development; (3) to use molecular cytogenetic methods to characterize recurrent chromosomal abnormalities relevant for the pathogenesis of human and experimental cancers; and (4) to characterize the mechanisms of genotoxicity and carcinogenicity of food-derived heterocyclic amines.

Current Research Projects:

- *Hepatic stem cells:* We are studying the stem cell factor/c-kit, flt-3/flt-3 ligand, hepatocyte growth factor/c-met, and TGFβ1/TGFβRI and II. These signal transduction systems are involved in the control of proliferation and survival of early progeny of liver stem cells.
- *Transgenic mouse models:* We have generated several transgenic mouse models to study cellular and genetic factors critical in multistage carcinogenesis. We are analyzing the interaction of c-myc with TGFα, HGF, and TGFβ1 during multistep hepatocarcinogenesis. We are also using targeted gene deletion approaches to extend these studies.
- *Cytogenetic analysis of hepatocarcinogenesis:* We use the transgenic models to characterize the cytogenetic alterations during the neoplastic process and the genetic linkage groups between mouse and human to identify novel tumor susceptibility genes in man.
- *Molecular cytogenetics of human and experimentally induced neoplasia in rodents:* We are undertaking identification and characterization by fluorescence in situ hybridization and polymerase chain reaction of recurrent genomic alterations in human liver, breast, and pancreas cancers to develop specific markers with preventive, diagnostic, or prognostic value, and to isolate genes involved in the neoplastic process. Also, we are mapping cancer related genes on mammalian chromosomes and generating high-density cytogenetic maps by multicolor fluorescence hybridization.
- *Molecular mechanisms of carcinogenesis of food-derived heterocyclic amines (HCAs):* Our research examines metabolic processing, DNA adduct formation, and mutagenesis of the HCAs, carcinogens found in cooked meats. Our focus is on the mammary gland as a target organ for HCA carcinogenesis. Using experimental rodent models, our research addresses the events in cancer initiation and susceptibility to HCAs, early events in HCA-induced mammary carcinogenesis, and the genomic alterations arising in HCA-induced mammary carcinomas.

In addition, LEC has core facilities for DNA sequencing and confocal microscopy.

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Laboratory of Experimental Carcinogenesis
Transgenic Mouse Models for Multistage Carcinogenesis and Hepatic Stem Cell Biology

Keywords:
apoptosis
cell cycle
c-myc
cytokines
growth factors
hepatocarcinogenesis
liver
oncogenes
pancreas
Rb
regeneration
stem cells
TGFα
TGFß
TGFß1
transgenic mice

Research:

NFκB and TGFβ pathways. NFκB/Rel factors have been strongly implicated in the regulation of apoptosis, a key mechanism of normal and malignant cell growth control. In particular, a direct role of NFκB/Rel in the survival of hepatocytes has been demonstrated. We have used the normal and Ras- and Raf-transformed RLE cells to test the hypothesis that the transformed RLE cells have altered NFκB regulation leading to resistance of TGFβ1. The data demonstrate that NFκB is aberrantly activated in Ras- or Raf-transformed RLEs due to increased phosphorylation and degradation of IkBα protein. Furthermore, we demonstrated that inhibition of either IKK–1 or IKK–2 reduced focus-forming activity in Ras-transformed RLEs. Thus we conclude that these studies identified and elucidated a mechanism that contributes to the process of transformation of stem-like liver cells by oncogene Ras and Raf through the IkB kinase complex leading to constitutive activation of NFκB.

Helix-loop-helix proteins and hepatic stem cell differentiation. We have also explored the potential role of helix-loop-helix (HLH) proteins in the regulation of activation and differentiation of hepatic stem cells. The HLH family of transcription regulatory proteins are key regulators in numerous developmental processes. The class I HLH proteins, such as E12, are ubiquitously expressed. Class II HLH proteins, such as MyoD, Atonal, NeuroD/BETA 2 are expressed in a tissue-specific manner. We have identified E-box sequences in promoters/enhancers of a number of genes that both regulate and characterize liver-specific gene expression. These include HNF–1α, HNF–3α and 3γ, HNF–4, and HNF–6, α-fetoprotein (AFP) as well as in the intergenic enhancer between albumin and AFP genes. We have therefore hypothesized that HLH proteins may exist that regulate both developmental pathways and cell lineage commitment in the liver. In an attempt to identify partners for the E12 protein that may exert control during liver development, we performed the yeast two-hybrid screen using the expression complementary DNA library from human fetal liver. A novel dominant inhibitory HLH factor, designated HHM (Human Homolog of

Maid), was isolated and characterized. HHM is structurally related to the Id family and was highly expressed in brain, pituitary gland, lung, heart, placenta, fetal liver, and bone marrow. HHM physically interacted with E12 in mammalian cells in vitro. Although some similarities exist between the expression profiles of HHM and Id2, there are distinct differences best seen in fetal tissues. Recent results show that HHM can interact with E12, Cyclin D1, Grap2, and the HNF–4 promoter, suggesting that HHM may possess multifunctional capacity.

Regulation of NFκB in c-myc/TGFα tumors. We have shown that oncogenic Ras- or Raf-mediated transformation of rat liver epithelial (RLE) cells altered NFκB regulation through IKK complex activation, which rendered these cells more resistant to TGFβ1-induced apoptosis. Based on these findings, we hypothesized that disrupted regulation of NFκB could also be involved in tumor growth of liver cells in vivo. To test this hypothesis, we compared HCCs from the c-myc/TGFα mice to HCCs from c-myc single transgenic mice. The results show that NFκB is activated in HCCs from c-myc/TGFα mice, but not in tumors from the c-myc single transgenic mice, suggesting TGFα mediates induction of NFκB. Furthermore, activation of the IKK complex was observed in the HCCs from c-myc/TGFα mice, implicating this pathway in the NFκB induction.

β-catenin (Wingless/Wnt pathway). Mutations affecting phosphorylation sites in the β-catenin gene have been implicated in the development of human and rodent hepatocellular carcinomas. To further investigate the involvement of this gene in hepatocarcinogenesis, we used several transgenic mouse models of hepatic tumors induced by overexpression of c-myc in the liver either alone or in combination with TGFα or TGFβ1. These studies suggest that nuclear translocation of β-catenin and activation of Wingless/Wnt signaling may represent an early event in liver carcinogenesis, providing a growth advantage in a subset of hepatic tumors with a more differentiated phenotype. This is of considerable interest since mutations in β-catenin are associated with a decreased loss of heterozygosity in human HCC. We have therefore hypothesized that development of liver cancer may advance via two broad pathways. The first pathway would involve β-catenin activation, while the second one would generate genomic instability and produce a mutator phenotype.

Oxidative stress and DNA damage. Our previous work implicated oxidative DNA damage in the etiology of c-myc/TGFα but not c-myc associated hepatocarcinogenesis. It is of considerable interest that mice in which defective DNA repair was generated by targeting the excision repair cross-complementing gene (ERCC-1) have a strikingly similar liver phenotype to our c-myc/TGFα mice. We have therefore hypothesized that attenuation and/or disruption of DNA repair might be required for the accumulation of oxidative DNA lesions. To test this hypothesis, we have sought to determine whether inactivation of DNA repair pathways contributes to an increased chromosomal instability and acceleration of the neoplastic process in c-myc/TGFα mice. Taken together, our results imply that DNA repair responses are dramatically altered in young adult c-myc/TGFα mice.

Collaborators on this research include Marcello Arsura, University of Tennessee, Memphis; Hanne Cathrine Bisgaard, Roskilde University, Denmark; Carla Boccaccio, University Torino, Italy; Nicholas Dean, ISIS Pharmaceuticals, Carlsbad, CA; Joe W. Grisham, James Swenberg, and Ronald G. Thurman, University of North Carolina, Chapel Hill; Michael R. Jensen, European Institute of Oncology, Milan, Italy; Stefan Karlsson, Lund University, Sweden; Jeffrey Kopp, Glenn Merlino, Raji Padmanabhan, Nicholas C. Popescu, Elizabeth G. Snyderwine, and Unnur P. Thorgeirsson, NIH; Michael Muller, Wageningen University, The Netherlands; Peter Nagy, Semmelweis University, Budapest, Hungary; Richard Pestell, Albert Einstein Cancer Center, Bronx, NY; Carol Prives, Columbia University, New York; Alistair Strain, University Birmingham, UK; and Shuji Terai, Yamaguchi University School of Medicine, Yamaguchi, Japan.

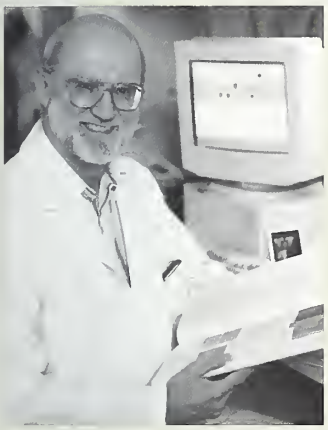
Recent Publications:

Arsura M, et al. *Mol Cell Biol* 2000;20:5381–91.

Terai S, et al. *Hepatology* 2000;32:357–66.

Calvisi DF, et al. *Cancer Res* 2001;61:2085–91.

Nagy P, et al. *Hepatology* 2001;33:339–45.



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Biography: *Dr. Miller is a research chemist in the Laboratory of Experimental Carcinogenesis of NCI. He obtained his Ph.D. from Caltech in 1976 and was a postdoctoral fellow at the University of California–San Diego. He has been at the NIH since 1981, except for a 1-year sabbatical at the Basel Institute for Immunology in Switzerland in 1989. His research interests have focused on computer applications to biological systems. Currently he is responsible for managing the new DNA Sequencing Minicore Facility within the NCI.*

Laboratory of Experimental Carcinogenesis
DNA–Minicore: A Small, Localized DNA Sequencing Core Facility

Keywords:
core facility
DNA sequencing

Research: The DNA Sequencing Minicore Facility (MiniCore) is designed to provide investigators in the Center for Cancer Research (CCR) of the NCI with rapid processing of their DNA sequence samples. Our goal is to generate accurate data as rapidly and efficiently as possible. The facility consists of three ABI 377 Prism automatic DNA sequencers and associated computers, PCR machines, and equipment. These machines are capable of reading up to 750 base pairs with 95 percent accuracy. A professional staff, assigned exclusively to the MiniCore, makes it possible to generate data almost as rapidly as could be expected with a dedicated machine in the investigator's laboratory. Thus we lessen the need for such dedicated machines, which results in significant savings for the division.

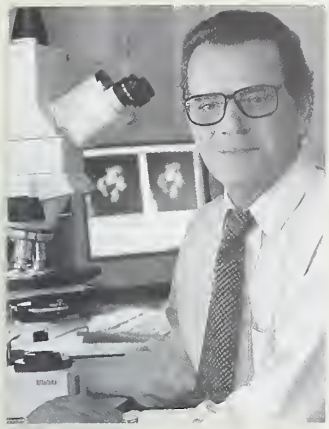
The facility accepts samples from any member of the CCR. We currently list 248 active users from 24 laboratories. Usually the user does the sequencing reaction, but we also accept DNA samples for reactions by the MiniCore. By limiting the range of services we offer, we focus our operation and optimize performance. In the past 12 months we have processed over 24,000 samples, a 10 percent increase over the preceding year. Our average turnover time, however, has actually decreased from 1.41 to 0.62 days (i.e., the weighted average number of business days from sample submission to when the samples are electrophoresed—an extra day should be added to allow the data to be generated and sent to the user).

The division subsidizes the MiniCore's operating expenses. The actual per-sample cost to the division, and the turnover time, are considerably better than can be obtained from external, commercial sources. We charge $8.50 for each sample electrophoresed. An additional $12.00 is charged for each sample submitted for reaction and electrophoresis. We also support several sequence analysis programs for both PC and Macintosh computers. These programs are made available to our user community at no charge. Finally, we also interact with users for troubleshooting, and in the design and interpretation of experiments involving DNA sequencing.

Recent Publications:

Yuan BZ, et al. *Cancer Res* 1998;58:2196–9.

Bliskovski V, et al. *Genomics* 2000;64:106–10.



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Biography: *Dr. Popescu is a cytogeneticist and cell biologist and conducts research in cancer molecular cytogenetics and molecular biology. He received his Ph.D. from the University of Bucharest, Romania. He was chief of the Laboratory of Biology at the Oncological Institute in Bucharest when, in 1971, he joined the Laboratory of Biology, NCI. In 1996 he joined the Laboratory of Experimental Carcinogenesis and became chief of the Molecular Cytogenetics Section. Dr. Popescu has written and cowritten 200 research articles and has received several national and international awards. In 1998 he was the recipient of the NIH's Merit Award for the second time. He serves on the editorial boards of* Cancer Genetics and Cytogenetics, Cytogenetics and Cell Genetics, *the* International Journal of Oncology, *and the* Journal of Cellular and Molecular Medicine.

Laboratory of Experimental Carcinogenesis
Molecular Cytogenetics of Carcinogenesis

Research: The objective of the research program in the Molecular Cytogenetics Section is to identify recurrent genomic alterations critical to the initiation and progression of neoplasia, to identify and isolate genes that contribute to the neoplastic development, and to develop molecular markers useful in the diagnosis and prognosis of the disease and targeted therapy.

Our approach is to integrate molecular cytogenetic and molecular biological methodologies into a coordinated study of cancer cells. Using comparative genomic hybridization and spectral karyotype, the most powerful procedures for the detection of genomic imbalances and structural chromosomal rearrangements across the entire tumor genome, we were able to detect with high accuracy genomic rearrangements in spontaneous human tumors in vitro-transformed human cells and tumors in transgenic animals, and to determine the consequences of DNA reorganization on the structure and expression of genes as well as to identify novel genes with possible roles in cancer development.

We have identified recurrent genomic imbalances, chromosome translocations, and deletions in human liver and breast tumors as well as in liver tumors and acute promyelocitic leukemia (APML) in transgenic mice. At several sites of recurrent genomic change, we have detected alterations of known genes, among which alterations of the fragile histidine triad tumor suppressor gene and the EMS–1 oncogene might be important in hepatocarcinogenesis and may possibly provide markers for early detection and prognosis of disease. In breast cancer, we found that tumor cell lines with an aggressive behavior in nude mice have a higher number of copy alterations than those with a moderate or low degree of tumorigenicity. Aggressive tumors lines also have high-level amplification of erbB–2 and myc genes and deletion of a specific region on chromosome 18. Amplification of the two oncogenes has prognostic value for the recurrence of the disease and deletion of chromosome 18 is prevalent in primary node-positive breast

tumors. In addition, a YAC from chromosome 16 that carries a cell senescence gene for breast cancer was isolated and mapped. The transfer of YAC DNA into immortal human and rat mammary tumor cells restored senescence in both tumors. A similar effect was observed with tumors derived from liver, prostate, and ovarian cancer. We observed that in vitro malignant transformation of normal human mammary epithelial cells by the introduction of mutant alleles of a small number of genes acquired nonrandom chromosome changes involving the MYC gene that mimic those in spontaneously arising breast cancer. Thus it was demonstrated for the first time how a limited, definable number of genes and chromosome changes results in the generation of a tumorigenic phenotype. In the spleen cells from transgenic mice with APML, deletion of the distal region of chromosome 2 was identified by SKY in a majority of the tumors expressing both PML–RARa and RARa–PML. Deletion 2, the first recurrent chromosome alteration identified in APML, appears to predispose to further chromosomal instability, which may lead to the acquisition of additional changes that provide an advantage to the transformed cells. In a human colon carcinoma cell line rendered resistant to ecteinascidine, a potent antitumoral agent, we identified a critical cytogenetic abnormality that leads to the demonstration of a novel mechanism of action for the drug.

We have identified four new genes at the site of recurrent genomic imbalances or translocations in human or mouse hepatocellular carcinoma. They include human DLC–1 (deleted in liver cancer 1), a strong candidate multiple tumor suppressor gene; mouse Gtf2ird1, which encodes a transcription factor that interacts with the retinoblastoma protein (pRb) and is the ortholog of a human gene (GTF2IRD1/WBSCR11) that is hemizygously deleted in Williams-Beuren syndrome; a human gene that encodes a previously unidentified member of the family of zinc finger proteins (KRAB/ZFP), which is highly expressed in hepatocellular carcinoma; and a candidate proto-oncogene.

Several genes isolated in our laboratory and by others were mapped by fluorescence in situ hybridization and include: human MDM2 binding protein and acid ceramidase, which is mutated in Farber disease; mouse DLC–1, mitochondrial aldehyde dehydrogenase; WDR7, a TGFβ resistance-associated gene; and ReLA/p65 gene. These localizations will facilitate the identification of loci in genetic diseases of unknown etiology, mapping at the same chromosomal regions and clarifying their involvement in neoplasia.

Our collaborators are William Hahn and Robert Wiendberg, Massachusetts Institute of Technology; Kay Huebner, Thomas Jefferson University, Philadelphia; Matthias Kraus, European Institute of Oncology, Milan, Italy; Timothy Ley, Washington University School of Medicine, St. Louis; and Anil Mukherjee, Yves Pommier, and Snorri Thorgeirsson, NIH.

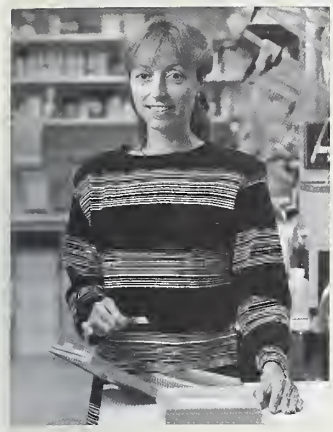
Recent Publications:

Reddy DE, et al. *Oncogene* 2000;19:217–22.

Zimonjic DB, et al. *Proc Natl Acad Sci* 2000;97:13306–11.

Elenbaas B, et al. *Genes Dev* 2001;15:50–65.

Durkin ME, et al. *Genomics* 2001;73:20–7.



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Biography: *Dr. Snyderwine obtained a Ph.D. from East Carolina University School of Medicine in 1984. She was a postdoctoral fellow in environmental toxicology at Johns Hopkins University School of Hygiene and Public Health prior to joining the Laboratory of Experimental Carcinogenesis in 1986 as a guest researcher. She became a senior staff fellow in 1990, and chief of the Chemical Carcinogenesis Section in January 1995.*

Laboratory of Experimental Carcinogenesis
Molecular Mechanisms of Carcinogenesis of Food-Derived Heterocyclic Amines

Keywords:
breast cancer
carcinogen-DNA adducts
carcinogen metabolism
DNA adducts
loss of heterozygosity (LOH)
mammary gland
mammary tumorigenesis
microsatellite
mutagenesis
rat
transgenic mice

Research: Diet is a complex mixture of constituents that may potentially influence the development of neoplasia. From the consumption of cooked meat including beef, chicken, and fish, people are exposed to mutagenic heterocyclic amines (HCAs). Several of these HCAs have been shown to be potent carcinogens in rodent models. Because HCAs are present in dietary staples, there is concern that these compounds may contribute to nutritionally linked cancers common to the western world such as breast, colon, and prostate cancer. Using a rat model, this section recently described the mammary gland carcinogenicity of 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP), one of the most prevalent HCAs in the western diet. The Chemical Carcinogenesis Section investigates the mechanisms involved in the carcinogenicity of the HCAs and focuses on the mammary gland as a target organ. Knowledge gained from studies on the mechanisms of mammary gland carcinogenesis of HCAs is expected to increase our understanding of the factors and processes involved in susceptibility and development of human breast cancer.

Metabolic processing of the HCAs is a critical factor in HCA–DNA adduct formation and susceptibility to HCA carcinogenesis. This section examines pathways of PhIP metabolism and DNA adduct formation in the rat mammary gland model. Our findings indicate that PhIP–DNA adduct formation is a prerequisite for the initiation of mammary gland cancer by this compound. This section is currently examining several additional effects of PhIP that are pertinent to explain its targeting to the mammary gland. During the early stages of carcinogenesis, a carcinogenic dose regimen of PhIP alters epithelial cell proliferation, apoptosis, and differentiation in the mammary gland, and it influences the level of certain serum hormones such as prolactin. Our studies suggest that the formation of PhIP–DNA adducts concomitant with an increase in mammary epithelial cell proliferation is likely to facilitate the carcinogenic targeting of PhIP to the mammary gland.

HCA mutagenesis has been a longstanding research interest in the Chemical Carcinogenesis Section. The role of HCA–DNA adducts in cancer initiation was recently addressed in transgenic mice carrying a mutation reporter gene. Tissue-specific factors modulating mutagenesis are currently being examined. Studies also address whether HCA–DNA adducts induce characteristic "signature" mutations which could be used as biomarkers to assess the role of HCAs in certain human cancers. In the transgenic mouse model, induction of cancer by an HCA was shown to be associated with HCA–DNA adduct-induced base substitution mutations at guanine nucleotides.

Another aspect of research in this section is concerned with the genomic alterations in PhIP-induced rat mammary carcinomas. One recent approach has been to screen carcinomas for allelic imbalance using polymerase chain reaction with primers to known microsatellite regions. These studies have to date revealed specific chromosomal regions showing loss of heterozygosity and microsatellite instability in PhIP-induced mammary carcinomas. These studies are expected to provide insight into the genomic alterations induced by a potential mammary gland carcinogen found in the human diet.

Collaborators on these projects include Roderick Dashwood, Oregon State University; Cindy Davis, USDA; Sarah Roberts-Thomson, University of Queensland; Herman Schut, Medical College of Ohio; Unnur Thorgeirsson, NIH; James Tucker and Kenneth Turteltaub, Lawrence Livermore National Laboratories; and Robert Turesky, Nestec Ltd., Research Center, Lausanne.

Recent Publications:

Yu M, et al. *Mol Carcinog* 2000;27:76–83.

Ryu DY, et al. *Cancer Res* 1999;59:2587–92.

Snyderwine EG, et al. *Nutr Cancer* 1999;31:160–7.

Venugopal M, et al. *Carcinogenesis* 1999;20:1309–14.

Laboratory of Experimental Immunology

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The research staff of the Laboratory of Experimental Immunology (LEI) conducts studies on biological response modification and the application of these studies to cancer therapy. Basic science approaches utilize cellular, biochemical, and molecular techniques to study the regulation of cell-mediated immune effector mechanisms, cytokine gene expression and function, biochemistry of receptor-mediated signaling in leukocytes, and the biology of growth factors. Based on cellular and molecular evaluation of biologicals, this information is then translated into immune modulation and immunotherapy protocols in experimental animals, and hypotheses suitable for testing in cancer patients are formulated.

The LEI is composed of three sections. The Cellular and Molecular Immunology Section studies the molecular mechanism(s) of cytokine-induced gene expression in leukocytes. The Leukocyte Cell Biology Section studies biological response modification at the cellular and molecular levels by better characterizing the key structures, functions, and signaling pathways of receptor leukocytes' responses, in particular T and natural killer cells. The Experimental Therapeutics Section investigates the cellular and molecular mechanisms by which cytokines regulate the immune and hematopoietic systems in vivo, and performs preclinical trials of BRMs in rodent tumor models.

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Xia Zhang	Postdoctoral Fellow

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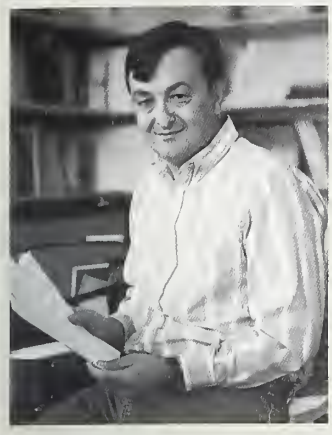

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Biography: *Dr. Ortaldo is chief of the Laboratory of Experimental Immunology (LEI). He obtained his Ph.D. from George Washington University and has studied the cellular and molecular biology of natural killer (NK) cells since 1975.*

Laboratory of Experimental Immunology
The Ly-49 Family: Regulation of Cytotoxicity and Cytokine Production in Murine NK Cells

Research: The goal of the Laboratory of Experimental Immunology is to study the molecular and cellular biology for a novel class of leukocyte regulatory receptors (LRR) that are involved in positive and negative regulation of immune cell function. LRR contain either ITIM- or ITAM-sequences in the cytoplasmic domains and include membrane receptors of the Ig- and C-type lectin superfamily. Their expression is broad since family members are found on a variety of leukocytes (LAIR), including NK and T cells (KIR, Ly-49, CD94, gp49, NKG2), B (PIR) cells, mast cells (gp49, MAFA), and monocyte-macrophage-dendritic cells (ILT, LIR, PIR). This laboratory is focused on the study of mouse and rat LRR and our projects will bring together studies that include expression, structure, signal transduction, and biological functions of LRR, and their interactions with ligands, including tumor cells, and viral and bacterial pathogens.

Recent Publications:

Ortaldo JR, et al. *J Immunol* 2000;166:4994–9.

Ortaldo JR, et al. *J Leukoc Biol* 2000;68:7485–6.

Tomasello E, et al. *Immunity* 2000;13:355–64.

Mason LH, et al. *J Immunol* 2000;164:603–11.

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Biography: *Dr. Stephen Anderson is a senior scientist with SAIC–Frederick working in collaboration with the Laboratory of Experimental Immunology. He obtained his Ph.D. in 1986 from the University of Western Ontario and performed postdoctoral studies on natural killer cells at Mount Sinai Hospital in Toronto. Dr. Anderson was a project leader with the National Research Council of Canada before joining the Laboratory of Experimental Immunology in 1992.*

Laboratory of Experimental Immunology
Cellular and Molecular Studies of Cellular Cytotoxicity

Research: The primary goal of this project is to achieve a more complete understanding of the cellular and molecular mechanisms of natural killer (NK) cell function—in particular, the ability to recognize and lyse tumor cells. A large portion of modern cancer research has focused on the ability of the immune system to destroy cancer cells using tumor-specific antibodies and immunomodulatory agents. A better understanding of the mode of NK cell tumor recognition will allow us to design novel antitumor therapies. We have identified and cloned a unique gene, *Nktr*, that is an essential component of the tumor recognition/killing mechanism of NK cells. This gene codes for a 150-kDa protein (NK–TR) that contains a cyclophilin (cyclosporin A binding protein)-related amino terminal domain while the remainder of the protein contains several repeated domains that are associated with pre-mRNA splicing factors. The possible involvement of NK–TR in the regulation of pre-mRNA splicing has been strengthened by our discovery of NKTR interacting proteins using the yeast two-hybrid system. Two highly related RS-containing pre-mRNA splicing factors were identified, U2AF1–RS1 and –RS2. These data suggest that NKTR may form part of a larger spliceosomal complex, since U2AF1–RS2 was recently shown to interact with U2AF–65, a splicing factor that binds to the 3′ splice acceptor.

The majority of our effort is directed toward the characterization of the Ly49 family of NK cell surface receptors. There are currently 14 *Ly49* genes identified in the C57BL/6 (B6) mouse genome. Ten of these (*Ly49a-j*) have been shown to produce mRNAs with a complete coding region, three represent transcribed pseudogenes (*Ly49k, m*, and *n*), and the remaining potential *Ly49* gene (*Ly49l*) has not been shown to produce any mRNAs in B6 mice. Our laboratory has found that the *Ly49l* gene produces a novel activating Ly49 protein in CBA/J mice, which demonstrates that Ly49 family members may be silenced via germline mutations in certain mouse strains. The observed differences in Ly49 expression between strains indicated the need to survey several strains of mice in order to identify the full *Ly49* gene repertoire. Extensive screening of a 129/J cDNA library by our group led to the discovery of 10 distinct full-length *Ly49*-related coding sequences (*Ly49e, g, i, o, p, r, s, t, u, v*) and seven of these may represent novel genes. Although

129/J mice share the same class I MHC haplotype with B6 mice, only one Ly49 is identical in the two strains (Ly49E). The cDNA sequences of the 129/J class I molecules were found to be identical to the B6 class I sequences, indicating that the changes in the Ly49 repertoire were not related to changes in the class I molecules. Three novel activating Ly49 proteins were discovered, Ly49P, R, and U. The MHC specificity of the total 129/J–Ly49 repertoire was evaluated with soluble class I MHC tetramers. Ly49V bound to many types of class I MHC, including the nonclassical Qa-1^b protein, suggesting that $Ly49V^+$ NK cells may monitor host cells for a global downregulation of MHC molecules. An activating receptor, Ly49R, was shown to bind soluble class I molecules to a moderate degree, a result not previously observed for other activating Ly49 proteins.

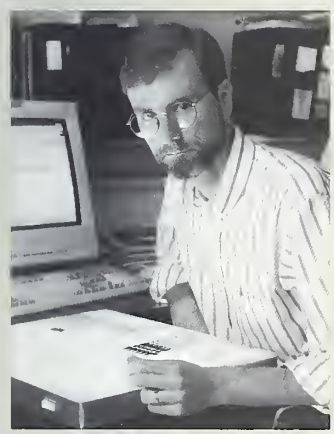
Collaborators on this research include Cynthia Chambers, University of Massachusetts; and John Ortaldo and Brent Passer, NIH.

Recent Publications:

Makrigiannis AP, et al. *J Immunol* 2001;166:5034–43.

Makrigiannis AP, et al. *J Leukoc Biol* 2000;68:765–71.

Makrigiannis AP, et al. *Immunol Res* 2000;21:39–47.



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Biography: *Dr. McVicar obtained his Ph.D. from the Medical College of Virginia, Virginia Commonwealth University, where he studied adoptive immunotherapy of brain tumors. He joined the NCI in 1990, completed a postdoctoral fellowship, and attained his current position within the Laboratory of Experimental Immunology in 1998.*

Laboratory of Experimental Immunology
Protein Tyrosine Phosphorylation in Leukocyte Signal Transduction

Keywords:
monocytes/macrophages
NK cells
protein-tyrosine kinase
signal transduction
tyrosine phosphorylation

Research: The emphasis of our laboratory is to further define the role of protein tyrosine phosphorylation in the regulation of immune cell function. To this end, we study the role(s) of protein tyrosine kinases (PTK) in the signal transduction of immune cell receptors including the T cell receptor (TCR), Fc receptors, and recently described NK cell receptors. In addition, we study the biochemistry and immunobiology of inhibitory receptor systems. As a starting point we use NK cells. These cells are closely related to T cells and share characteristics with monocytes. Unlike T cells, NK cells mediate cytotoxicity without prior activation and produce cytokines easily. Therefore, NK cells represent a constitutively active population that can be used as a source for novel signal transduction pathways that may also be relevant in

monocyte and T cell biology. Using NK cells, we have novel PTK, are characterizing two recently described signal transduction chains, and are studying paired inhibitory and activating receptor systems. Although many of these pathways were first described in NK cells, they are also expressed in T cells, monocytes, and in some cases B cells, validating the use of NK cells for the identification and study of phosphotyrosine-mediated signaling pathways involved in immune cell signal transduction.

Novel Kinases

We searched for PTK expressed in NK cells and found two novel PTK, Jak3 and a Csk homologous kinase, Chk (formerly known as Lsk). Our kinase research is currently focused largely on Chk. This kinase has homology to the carboxyl terminal Src kinase (Csk). Both Chk and Csk phosphorylate the carboxyl terminal tyrosine of Src family kinases, thereby downregulating their activity. Csk is ubiquitously expressed, not transcriptionally regulated, and targeted disruption of the Csk locus is embryonically lethal. In contrast, Chk is constitutively expressed only in neurons, NK cells, and bone marrow, implying that Chk acts as an immune-specific Src family regulator. In T cells, Chk is expressed during T cell activation, suggesting a role in downregulation of TCR function. However, overexpression of Chk did not inhibit Jurkat TCR signaling, indicating that Chk must regulate other T cell functions. Consistent with an effect on receptors other than the TCR, monocytes can also express Chk. Activators of monocytes such as IFN-γ or LPS failed to induce Chk. Conversely, IL–4, IL–13, GMCSF, or IL–3 induced Chk expression. Interestingly, IFN-γ not only failed to induce Chk, but it inhibited induction by other cytokines. The reciprocal effects of IL–4 and IFN-γ on both Chk expression and the Th1 versus Th2 balance of the immune response suggests that Chk may play a role during Th2-like responses. We are now studying the in vivo role of Chk using knock-out mice and studies of protein-protein interaction.

The DAP12 Signal Transduction Chain

We also study the signal transduction of a class of proteins related to the inhibitory MHC class I recognition receptors. These include the Ly49 family in murine NK cells, the killer cell inhibitory (KIR) receptors of human NK cells, the ILT, PIR receptors of monocytes and B cells, and most recently the myeloid receptor, CD33. Members of these families are tyrosine phosphorylated and recruit the phosphatase SHP1, shutting down activation signals. Ly49D, unlike other Ly49s, does not become phosphorylated. In fact, Ly49D delivers a positive signal. A 16 kDa tyrosine phosphoprotein (DAP–12) coimmunoprecipitates with Ly49D and mediates its signal transduction. The expression of DAP12 in monocytes as well as NK cells implies that DAP12 is a common signal transduction component for multiple receptor families. We are testing this hypothesis and dissecting the signal transduction pathways of DAP12-coupled receptors toward an understanding of the role of these receptors in NK cells and monocytes.

Paired Inhibitory and Activating Receptor Systems

The characterization of activating as well as inhibitory members of the Ly49 family has led us to study other paired receptor systems such as the PIR. We have shown that activating PIR can activate monocytes, and that these receptors use Fc ε receptor γ-chain, not DAP12, for their signal transduction.

In addition, we have been studying CD33, a myeloid-specific member of the siglec family of receptors. CD33 uses individual tyrosine motifs within its tail to recruit specific protein phosphatases, suggesting a nonredundant role for these motifs and a basis for the conservation of tyrosine residues within the siglec family. We are currently examining the possibility that CD33 represents a paired family of monocyte receptors.

Recent Publications:

McVicar DW, et al. *J Biol Chem* 1998;273:32934–42.

Taylor LS, et al. *Blood* 1999;94:1790–6.

Paul SP, et al. *Blood* 2000;96:483–90.

Taylor LS, et al. *Rev Immunogenet* 2000;2:204–19.

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Biography: *Dr. Thomas Sayers is a senior scientist with SAIC–Frederick and works in collaboration with the Leukocyte Cell Biology Section of the Laboratory of Experimental Immunology (LEI). Dr. Sayers obtained his Ph.D. in biochemistry from the University of London and performed postdoctoral studies on purification of autoantigens at the Medical Clinic of the University of Tnbingen, Germany. He also worked as a laboratory leader in the Department of Immunotherapy at the Sandoz Research Institute, Vienna, Austria, before coming to the NCI.*

Laboratory of Experimental Immunology
Identification of Factors Involved in Tumor Cell Destruction by the Immune System

Research: Biological therapy of cancer has not as yet provided significant therapeutic benefits for the majority of cancer patients. Nonetheless, some beneficial effects of immunotherapy have been seen in melanoma and renal cancer. These are assumed to be due to the activation of cells of the immune system. However, very little is known about how these cells or their products might mediate tumor cell destruction. Studies in our laboratory using several preclinical murine solid tumor models have determined that natural killer (NK) and T cells are critical for successful immunotherapy. These cells can mediate direct lysis of tumor target cells after cell-cell contact. Cytotoxic lymphocytes produce unique proteins such as the pore-forming protein perforin and a family of serine proteases (granzymes) that are thought to be involved in tumor cell lysis. These proteins are stored in intracellular granules that are released upon contact of effector lymphocytes with tumor targets. We have purified three of these granzymes to homogeneity and determined their enzyme specificities using synthetic substrates. Our current studies are concentrated on identifying the molecular events underlying

tumor cell death, with a focus on the rapidly developing studies related to mechanism(s) of apoptosis. Several specific proteolytic events are required for apoptosis to proceed, and we are investigating whether the granzymes may mediate some of these events. We also are studying a possible role for Fas-ligand, TNFα, and TRAIL in immune-mediated destruction of tumor cells in vivo and their role in controlling the development of tumor metastases. Our overall goal is to better understand the molecular events operating during tumor cell death. This knowledge should be useful in the rational design of agents to trigger these "suicide" pathways in tumor cells.

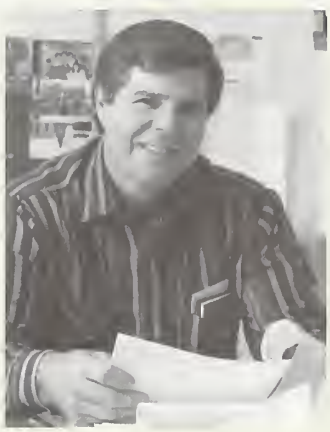
Recent Publications:

Sayers TJ, et al. *J Immunol* 1998;161:3957–65.

Lee JK, et al. *J Immunol* 2000;164:231–9.

Sayers TJ, et al. *J Leukoc Biol* 2000;68:81–6.

Sayers TJ, et al. *J Immunol* 2001;166:765–71.



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Biography: *Dr. Robert Wiltrout obtained his Ph.D. in immunology from Wayne State University in 1979 and performed postdoctoral studies on the regulation of immune responses with Dr. Ronald Herberman at the NCI. He joined the Laboratory of Experimental Immunology in 1986.*

Laboratory of Experimental Immunology
Innate and Adaptive Immune Mechanisms in Cytokine-Mediated Regression of Mouse Kidney and Breast Cancers

Keywords:
angiogenesis
apoptosis
breast cancer
gene therapy
innate immunity
kidney cancer
orthotopic mouse models

Research: The mechanisms by which the immune system can mediate tumor regression in vivo are complex and incompletely understood. Recent studies suggest that successful cytokine-induced tumor regression may depend on a complex interplay between critical elements of the innate immune system (macrophages and NK or NK T cells) and classical elements of the adaptive immune response such as $CD4^+$ or $CD8^+$ T cells. This model predicts that successfully engaged innate mechanisms trigger inflammatory events in the tumor microenvironment that, in turn, provide instructive signals for the development of adaptive responses. Our general hypothesis is that the limited success of antigen-specific therapeutic approaches for patients with kidney cancer (and likely other tumors) derives at least partially from a lack of effective engagement of these early innate mechanisms that initiate and/or support the development of antigen-specific responses. In that regard IL–2, IL–12, and IL–18 all play central regulatory roles for both innate and adaptive

immune responses, reciprocally regulate each other's receptors, and synergize for a variety of immune responses including IFN-γ production, which is central for many of the biological effects of these cytokines.

We have recently demonstrated that the combinations of IL–2 plus IL–12 and IL–2 plus IL–18 exhibit therapeutic synergy against orthotopic mouse kidney and/or breast cancers. In these settings, the mechanisms of early antiangiogenic effects and later tumor regression share a common dependency on IFN-γ and the Fas/FasL pathway. Thus, our present efforts are focused on the molecular and cellular events that are vital for these early phases of cytokine-induced tumor response. In particular, these studies seek to understand the role of the tumor cells versus supporting elements or host leukocytes in the regulation of local gene expression, effector cell recruitment, endothelial and tumor cell apoptosis, and ultimate regression of the tumor. In addition, because both kidney and breast cancers can metastasize to major parenchymal organs in mouse models and patients, studies are under way to delineate organ-specific differences in the regulation of innate immune responses in the lungs versus the liver. The emphasis of these studies is on organ-specific differences in cytokine-induced gene expression in organ-associated innate effector cells (e.g., NK and NK T cells and macrophages) and parenchymal cells unique to those different organ microenvironments, differences in recruitment and maintenance of leukocyte subsets in those organs, and unique cellular and molecular mechanisms for antimetastatic responses. An additional unique aspect of these studies is the use of new gene therapeutic approaches in which naked DNA plasmids encoding cytokine genes can now be delivered so effectively that cytokine protein levels and activities in vivo approach levels following high-dose bolus administration of recombinant cytokines. Overall, these studies seek to identify critical events that regulate the recruitment and antitumor functions of innate immune mechanisms in tumor microenvironments and different anatomical sites, and provide a framework for using innate immunity to amplify antigen-specific adaptive immune antitumor responses.

Collaborators on this project include Bruce Blazar, University of Minnesota; Jeffrey Green, Steve Libutti, Bill Murphy, John Ortaldo, John O'Shea, Tom Sayers, Jon Wigginton, and Howard Young, NIH; Mark Smyth, Peter MacCallum Cancer Institute, Australia; and Stanley Wolf, Genetics Institute, Inc.

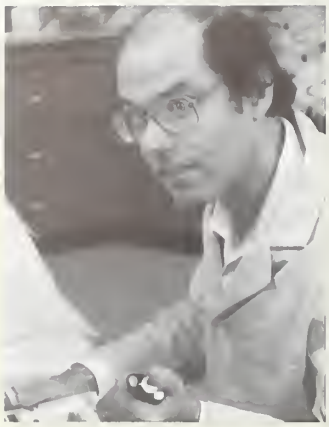
Recent Publications:

Wigginton J, et al. *J Clin Invest* 2001;108:63–72.

Wigginton J, et al. *J Immunol* 2001;166:1156–68.

Park J–W, et al. *J Immunol* 2001;166:3763–70.

Smyth M, et al. *J Exp Med* 2001;193:661–70.



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Biography: *Dr. Howard Young obtained his Ph.D. in microbiology at the University of Washington and carried out postdoctoral research at the NCI under Drs. Edward Scolnick and Wade Parks. He was a member of the Laboratory of Molecular Immunoregulation, NCI, from 1983 to 1989 prior to joining the Laboratory of Experimental Immunology in 1989.*

Laboratory of Experimental Immunology
Regulation of Cytokine Gene Expression

Research: The Cellular and Molecular Immunology Section studies the control of gene expression during the development and maturation of the cellular immune system in mediating antitumor and anti-inflammatory immune responses. The general goals of this section are to use molecular approaches to: (1) investigate in detail the molecular mechanisms by which gene expression is regulated in immune effector cells; and (2) study the mechanism(s) by which tumor cell susceptibility to biological and immunological defense systems can be enhanced through the control of specific gene expression. The specific aim of the Cellular and Molecular Immunology Section is to study human and murine cell-mediated immunity, with emphasis on natural killer (NK) cell- and T cell-specific regulation of gene expression. T cells and NK cells are the two predominant cell types that express interferon-γ (IFN-γ), and IFN-γ transcription occurs after stimulation of these cells with numerous exogenous stimuli, including interleukin 2 (IL–2), IL–4, IL–12, IL–15, and IL–18. Furthermore, the balance between IFN-γ and IL–4 expression in vivo plays an important role in determining host resistance/susceptibility to many disease states. We have been defining the regions of human IFN-γ genomic DNA that regulate transcriptional activation of this gene in order to understand how IFN-γ gene expression is controlled. We have found that IFN-γ gene expression is influenced by DNA methylation, mRNA stability/nuclear localization, and the presence of enhancer and repressor regions in the IFN-γ genomic DNA. It is our continuing goal to determine how specific extracellular signals influence the interactions of DNA-binding proteins with the IFN-γ genomic DNA regulatory elements. In another aspect of this work, we have identified a new type of NK cell that expresses IL–13 and IL–5. Both IL–2 and IL–18 regulate expression of these genes and our current work is focused on the molecular mechanisms involved in the induction of IL–13 gene expression and the biological role of these NK cells in natural immunity. Overall, our studies represent a molecular analysis of the regulation of cytokine gene expression in lymphoid cells and provide a basis for developing a more complete understanding of the role of IFN-γ, IL–13, and other cytokines during the pathogenesis of cancer and infection.

Our collaborators include Thomas Aune, Vanderbilt University School of Medicine; Tom Geibert and Lisa Hensley, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD; and Rivkah Gonsky, Cedars-Sinai Medical Center, Los Angeles, CA.

Recent Publications:

Ortaldo JR, et al. *J Immunol* 2000;166:4994–9.

Zhang X, et al. *J Immunol* 2001;166:7104–11.

Hoshino T, et al. *J Immunol* 2000;30:1998–2006.

Hwu P, et al. *J Immunol* 2000;164:3596–9.

Gene Regulation and Chromosome Biology Laboratory

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The Gene Regulation and Chromosome Biology Laboratory includes research in a variety of organisms, notably bacteriophage λ, *Escherichia coli*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Caenorhabditis elegans*. This research is unified by interests in chromosome dynamics (recombination, chromosome segregation, and transposable elements) and regulation (transcription, silencing, and cell cycle control).

Dr. Strathern's laboratory is focused on the mechanisms and fidelity of recombination. The investigators have identified mutations that elevate the level of aberrant genome rearrangements. Their studies also revealed that the DNA synthesis associated with recombination is of relatively low fidelity, and they have identified genes that control this fidelity. They also study the fidelity of retrotransposition.

Dr. Klar's laboratory studies the positional silencing and the site-specific recombination mechanisms that are components of cell type switching in *S. pombe*. Their studies have revealed several novel genes involved in establishing or maintaining epigenetic states of gene expression. They are also characterizing a strand-specific genome marking system that programs cell type switching. In addition, Dr. Klar's laboratory studies the genetic basis of human handedness.

Using plasmid segregation in bacteria as a model for how chromosomes are properly partitioned to daughter cells at each division, Dr. Austin's laboratory identified specific protein/DNA contacts required for accurate plasmid segregation and continues to focus on mechanisms of chromosome partitioning.

Dr. Court's laboratory has demonstrated that the *N* gene of bacteriophage λ exhibits two different levels of regulation on λ genes: N binds to its own mRNA and inhibits the synthesis of more N protein, and it modifies RNA polymerase so that transcription continues through termination sites into downstream genes. The laboratory staff is investigating how these two mechanisms are related and what other cellular functions contribute to these processes. Recently, they developed improved methods for gene targeting in *Escherichia coli*.

Dr. Garfinkel's laboratory is elucidating the life cycle of Ty, a retrovirus-like element that provides an excellent model for the interaction between a retrotransposon and its host. Recently, their studies identified many yeast genes involved in limiting the transposition level of Ty1. The Garfinkel and

Strathern laboratories have made hybrid Ty/HIV elements in which Ty replication is dependent on the reverse transcriptase (RT) of HIV–1. Such hybrids have been used to identify new drugs that inhibit HIV–1 RT.

Dr. Kashlev's laboratory, established in August 1996, is elucidating the role of RNA polymerase in transcription elongation and termination in *Escherichia coli*. The lab is also carrying out an investigation of transcription elongation in yeast.

Gene Regulation and Chromosome Biology Laboratory Staff

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Movable Genetic Elements Section

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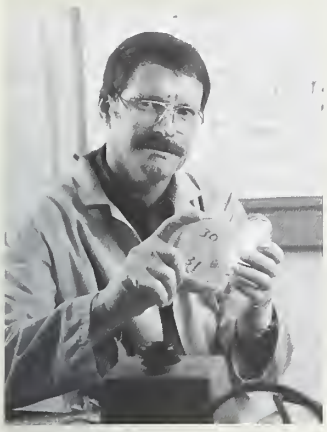
Katherine Nyswaner	Technician
Karen Stefanisko	Technician
Anuradha Sundararajan	Postdoctoral Fellow



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Biography: *Dr. Strathern obtained his Ph.D. from the Molecular Biology Institute at the University of Oregon in 1977, then moved to Cold Spring Harbor Laboratory where he became a senior staff member with the Yeast Genetics Laboratory. In 1984, he joined the ABL–Basic Research Program at the NCI's Frederick Cancer Research Development Center (now NCI–Frederick). His research remains centered on aspects of gene regulation and genetic recombination as revealed by studies in yeast. In 1999, Dr. Strathern joined the Division of Basic Sciences, NCI. In March 2001, the Division of Basic Sciences merged with the Division of Clinical Sciences to form the NCI Center for Cancer Research.*

Gene Regulation and Chromosome Biology Laboratory
Regulation, Recombination, and Retrotransposition in Yeast

Keywords:
recombination
regulation
retrotransposon
yeast genetics

Research: Two general areas of research are pursued in our section: mechanisms of genetic recombination and mechanisms of gene regulation. These areas come together in the control of mating type in yeast, where cell type is changed by a programmed genetic rearrangement that allows expression of alternative genes encoding trans-acting regulatory proteins. The programmed recombination event is initiated by a site-specific DNA cleavage. Our studies of this process led us into the area of genetic recombination in general and double-strand-break repair in particular. Currently, we are screening for strains defective in recombination to define additional functions involved in this process. We expect that such genes could add to the list of recombination and DNA damage repair defects known to be related to neoplastic disease. We recently demonstrated that the DNA synthesis associated with genetic recombination has substantially lower fidelity than that found in general cell duplication. Our recent results suggest at least two different DNA polymerases have roles in this elevated mutation rate: Base substitutions reverting a non-sense allele are dependent on the translesion polymerase, Pol ζ, encoded by *REV3*, whereas reversion of frameshift alleles is *REV3* independent. We continue to investigate the mechanism of double-strand-break repair and the proteins involved in controlling the fidelity of DNA synthesis during this process. We are currently determining the rate-limiting steps in meiotic recombination between ectopic sites.

We demonstrated that reverse transcription of cellular mRNAs can generate substrates for recombination, resulting in processed pseudogenes and RNA-mediated gene conversion. We continue to explore the role of reverse transcripts in genome evolution. In a collaboration with David Garfinkel, we developed an in vivo assay for HIV–1 reverse transcriptase (RT), based on hybrid Ty1/HIV–1 elements and a homologous recombination assay. This assay is sensitive to some known nonnucleoside inhibitors of HIV–1 RT.

In collaboration with Christopher Michejda, we have also identified several new drugs that inhibit HIV–1 RT. This year, we initiated an analysis of the fidelity of retrotransposition using tools similar to those used in our studies of the fidelity of recombination. This topic provides us with an opportunity to investigate not only the features of RT that govern its fidelity, but also an opportunity to investigate the properties that govern the fidelity of RNA polymerase.

Our interests in gene regulation are currently centered on the mechanism of gene silencing. This mechanism was first discovered as the means of inhibiting the transcription of donor loci used in the recombination event associated with mating-type switching in yeast. The source copies of the genes activated in this process are kept silent by the *SIR* genes (silent *i*nformation *r*egulators) and several other genes. A demonstrated role of histones, and an altered accessibility of the DNA in the silenced region, implicates chromatin modification within a defined domain as the means by which silencing occurs. The *SIR2* gene has also been shown to have a role in silencing near the telomeres and in controlling recombination in the rDNA repeats. We showed that yeast has five genes closely related to *SIR2* and that *SIR2* homologs can be found in organisms ranging from prokaryotes to humans. We are continuing to define the roles of this gene family in genome regulation.

Our collaborators are Stephen Hughes and Christopher Michejda, NIH.

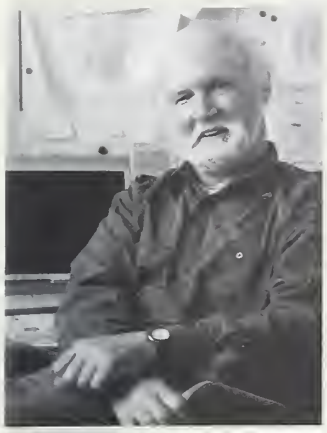
Recent Publications:

Holbeck SL, et al. *Genetics* 1997;147:1017–24.

Nissley DV, et al. *Proc Natl Acad Sci USA* 1998;95:13905–10.

Holbeck SL, et al. *Ann NY Acad Sci* 1999;18:375–7.

Davis ES, et al. *Genetics* 2000;155:1019–32.



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Biography: *Dr. Austin received his Ph.D. from the University of Edinburgh in 1971 and was a postdoctoral fellow at Harvard Medical School and the University of Paris before joining the NCI–Frederick in 1976. He has been head of the Cell Cycle Regulation Section since 1984. In 1999, Dr. Austin joined the Center for Cancer Research, NCI.*

Gene Regulation and Chromosome Biology Laboratory
The Control of the Replication and Segregation of Model Chromosomes

Keywords:
chromosome
Escherichia coli
P1 plasmid
replication
segregation

Research:

The Control of the Replication and Segregation of Bacterial Chromosomes
A fundamental property of cells is their ability to produce daughter cells that carry a faithful complement of genes. The mechanisms by which chromosome replication is controlled and the daughter chromosomes are equitably distributed to daughter cells are not understood in any detail. We seek to understand these processes at the molecular level. Because the problems are complex, we choose to tackle them by using the simplest possible model system. The bacterium *Escherichia coli* provides a powerful genetic system and, in addition to the bacterial chromosome itself, the organism provides a series of unique genetic elements that act as model chromosomes for some of our experiments. Extrachromosomal elements called unit-copy plasmids act as analogs of the chromosome and yet, unlike the host chromosome, are dispensable for cell survival. They are maintained at a 1:1 ratio with the host chromosome and are distributed with great precision to daughter cells. Our earlier studies focused on one of these elements, the plasmid prophage of bacteriophage P1. We continue these studies, but are now applying the tools we have developed and the information we have gained to try to understand how the host chromosome itself is organized and segregated.

The Role of Adenine Methylation in DNA Replication and Segregation
P1 DNA is controlled by a DNA marking system that distinguishes newly replicated DNA from unreplicated DNA and prevents a second round from occurring in a single cell cycle. Similar systems probably exist in all organisms, but the nature of the DNA marking and the replication block are generally not understood. Newly replicated P1 DNA is marked by hemimethylation of DNA sites within the origin of replication. The replication block is imposed by the recognition of the hemimethylated DNA by the SeqA protein, a protein whose selective DNA-binding activity was discovered in our laboratory. SeqA also regulates host chromosome replication in a similar fashion. However, we found that SeqA interacts with many other sequences than just the P1 and host chromosome origins of DNA replication. SeqA appears to interact with multiple hemimethylated GATC-binding sites to

form a filament with the intervening DNA looped out. There are many potential sites distributed around the chromosome so that each replication fork should produce a transient wave of hemimethylation, and thus SeqA binding. Microscopy of cells with two replication forks showed pairs of SeqA–GFP foci located at the cell center. The number of foci increase sharply with increasing growth rate, corresponding approximately to the number of forks present in the cells. SeqA appears to organize newly replicated DNA into a structure that may aid DNA segregation away from replication forks that are tethered to the cell center.

The Roles of the Par and Muk Proteins in DNA Segregation

P1 has a DNA site, *parS*, that appears to be an analog of the centromere of eukaryotic chromosomes; *parS* promotes the accurate distribution (partition) of daughter plasmids to daughter cells. We have shown that the site is acted upon by two P1-encoded proteins that have been purified and characterized in our laboratory. The hypothesis that this par system is responsible for an activity akin to mitosis has recently received dramatic support. Homologs of the P1 ParA and ParB proteins have recently been shown to be responsible for the proper segregation of the chromosomes of two bacterial species. Moreover, studies of the location of these Par homologs within the cell and their likely DNA-binding sites provide important clues as to how partition works. Partition appears to involve attachment of chromosomes (or plasmids) to unique membrane-binding sites present in each half of the dividing cell. Condensation of the DNA then gathers each chromosome toward its point of attachment to allow cell division.

The *Escherichia coli* MukB protein resembles eukaryotic dyneins, proteins that are involved in the energy-dependent movement of cell components. Mutants in the *muk* genes produce frequent anucleate cells. We discovered that mutations in topoisomerase I are general suppressors of muk mutants. These mutations eliminate anucleate cell production by increasing the negative supercoiling of the chromosomal DNA. This observation is consistent with a role for the Muk proteins in chromosome folding or DNA condensation. This result, along with our observations on the SeqA protein, support models in which chromosome replication is coreplicational. The newly replicated DNA emerges from the replication forks at the cell center and is directed toward each cell pole by membrane attachment and DNA condensation to form two separate chromosome masses.

Collaborators on this study have included Michael Davis and Anthony Maurelli, Uniformed Services University of the Health Sciences; Lyndsay Radnedge, Lawrence Livermore National Laboratory; Paul Tucker, European Molecular Biology Laboratory; and Andrew Wright, Tufts University.

Recent Publications:

Brendler T, et al. *EMBO J* 1999;18:2304–10.

Youngren B, et al. *J Bacteriol* 2000;182:3924–8.

Sayeed S, et al. *J Bacteriol* 2000;182:2416–21.

Sawitzke J, et al. *Proc Natl Acad Sci USA* 2000;97:1671–6.

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Biography: *Dr. Court received his Ph.D. from the University of Rochester, Department of Biology. He was a research associate at Stanford University and the University of California–Berkeley in the laboratories of Drs. Alan Campbell and Harrison Echols, respectively, before joining the NCI in 1972. Dr. Court joined the ABL–Basic Research Program as head of the Molecular Control and Genetics Section in 1981, and in 1999, he joined the Center for Cancer Research.*

Gene Regulation and Chromosome Biology Laboratory
Control of Gene Expression and the Cell Cycle

Keywords:
cell cycle control
in vivo cloning
RNA processing
transcriptional control
translational control

Research: Developmental systems are controlled by modulating gene expression in response to internally programmed signals as well as to external signals. Our laboratory is interested in studying the molecular interactions and the signaling that occur to regulate gene expression. We exploit the genetic systems available in *Escherichia coli*, its plasmids, and its viruses (e.g., bacteriophage λ) to help us understand (1) gene regulation at the levels of transcription initiation and elongation, and translation initiation, and (2) cell growth and cell cycle control signals.

The *N* gene of λ is the first gene expressed following viral infection. The function of the N protein is necessary for expression of most other λ genes by its actions as a positive regulator. Positive activation of other genes occurs by N binding to specific RNA sites called NUT, modifying the RNA polymerase transcription complex. This modified polymerase complex reads through transcription terminators to distal λ genes. Thus, the expression and action of N are central to the control of λ development.

We have recently determined that N is subject to novel posttranscriptional regulatory circuits. Expression of the *N* gene is autoregulated by N binding to the NUT RNA site 150 bases upstream of the *N* gene, thereby repressing the translation of N more than 100-fold over this long distance. The N-modified RNA polymerase complex is required for translational repression. Thus, antitermination and translation repression by N are coupled. This may be caused by a specific folding of the RNA structure that brings the NUT RNA into close juxtaposition with the *N* ribosome-binding site. RNaseIII, an endonuclease, recognizes the stem structure and cleaves it, separating NUT from the *N* RNA. This cleavage prevents N translational repression but actually enhances antitermination, presumably by releasing the antitermination complex from its interaction with the NUT RNA.

Additionally, we have found that RNaseIII is expressed from an operon in which an essential low-molecular-weight GTP-binding protein, Era, is also encoded. From this operon, RNaseIII and Era expression is coordinately regulated and increases in relation to growth rate. This growth rate regulation

of RNaseIII and Era occurs at the posttranscriptional level, but the mechanism remains unknown. The accumulation of adequate levels of Era is essential for cytokinesis to be completed and cell growth to continue. We speculate that a threshold level of Era must accumulate before Era-GTPase is activated by a cellular signal to cause cell division and to allow cell growth to continue. We believe RNaseIII and Era are key components that couple regulation of growth and the cell cycle.

We also wish to understand how RNaseIII levels in the cell affect N expression and whether growth conditions affect N repression by modulating RNaseIII levels. To accomplish this, we will be examining how Era signals cytokinesis and what other cell components are involved in Era signaling.

Recently, we have developed an in vivo cloning and gene modification system using λ-mediated homologous recombination with short (<50 bp) homologies. This system eliminates the need to cut DNA with restriction enzymes or join DNA fragments with DNA ligase. This extremely efficient recombination system could, we believe, revolutionize the way in which recombinant DNA work is done.

Our collaborators are Robert Britton, Massachusetts Institute of Technology; Santanu Dasgupta, University of Upsalla; David Friedman, University of Michigan; and James Lupski, Baylor College of Medicine.

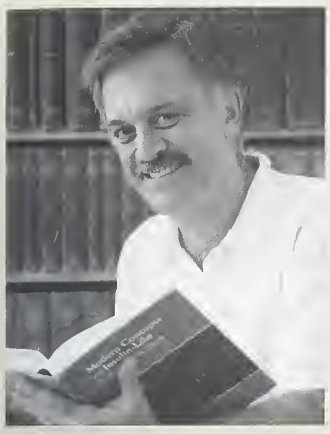
Recent Publications:

Powell B, et al. *J Bacteriol* 1999;181:5111–3.

Altieri AS, et al. *Nat Struct Biol* 2000;7:470–4.

Yu D, et al. *Proc Natl Acad Sci USA* 2000;97:5978–83.

Britton RA, et al. *Genomics* 2000;67:78–82.



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Biography: *Dr. Garfinkel received his Ph.D. in microbiology at the University of Washington, where he studied crown gall tumorigenesis in plants with Dr. Eugene W. Nester. He then studied yeast genetics and Ty element transposition as a post-doctoral fellow with Dr. Gerald R. Fink at the Whitehead Institute. In 1985, Dr. Garfinkel joined the ABL–Basic Research Program at the NCI–Frederick campus, and in 1999, he joined the Center for Cancer Research.*

Gene Regulation and Chromosome Biology Laboratory
Ty Element Retrotransposition in *Saccharomyces cerevisiae*

Keywords:
DNA repair
retrotransposition
Saccharomyces cerevisiae

Research: Our research efforts are directed toward understanding the mechanism and consequences of Ty element retrotransposition. Retrotransposons are a class of transposable elements that resemble retroviruses, such as HIV–1, in their structure and mode of replication. Ty element relatives also comprise a significant fraction of mammalian and plant genomes. Ty elements are a paradigm for studying many aspects of retrotransposition because these elements are found in *Saccharomyces cerevisiae*, a highly developed eukaryotic model system. Here, we report on our recent work addressing the mechanism of Ty1 integration, the response of hybrid Ty1/HIV–1 hybrid elements to reverse transcriptase (RT) inhibitors, and new lines of host defense used by yeast to minimize the level of Ty1 retrotransposition. We also present a technique utilizing microhomologous recombination to generate targeting constructs for mammalian genes.

Correct Integration of Model Substrates by Ty1 Integrase
The Ty1 retrovirus-like mobile genetic element transposes to new genomic locations via the element-encoded integrase (IN) protein. We have reported that purified recombinant IN catalyzes correct integration of a linear DNA into a supercoiled target plasmid. Ty1 virus-like particles (VLPs) integrate donor DNA more efficiently than IN. VLP and IN-mediated insertions occur at random sites in the target. Mg^{+2} is preferred over Mn^{+2} for correct integration, and neither cation enhances nonspecific nuclease activity of IN. Products consistent with correct integration events have also been obtained by Southern analysis. Recombinant IN and VLPs utilize many, but not all, linear donor fragments containing non-Ty1 ends, including a U3 mutation which has been shown to be defective for transposition in vivo. Together, our results suggest that IN is sufficient for Ty1 integration in vitro and IN interacts with exogenous donors less stringently than with endogenous elements.

Hybrid Ty1/HIV–1 Elements Used to Detect Inhibitors and Monitor the Activity of HIV–1 RT

We have previously demonstrated that hybrid retrotransposons comprised of Ty1 and the RT of HIV–1 are active in yeast. The RT activity of these hybrid (HART) elements can be monitored using a simple genetic assay. This year, we have shown that the HART assay is sensitive to nonnucleoside inhibitors of HIV–1 RT. We also show that drug-resistant variants of HIV–1 RT can be used to identify compounds active against drug-resistant viruses.

The *Saccharomyces cerevisiae* DNA Recombination and Repair Functions of the *RAD52* Epistasis Group Inhibit Ty1 Transposition

RNA transcribed from Ty1 accumulates to a high level in mitotically growing haploid cells, yet transposition occurs at very low frequency. The product of Ty1 reverse transcription is a linear double-stranded DNA molecule that reenters the genome by either Ty1–IN-mediated insertion or homologous recombination with one of the preexisting genomic Ty1 elements. We have examined the role that the major cellular homologous recombination functions have on Ty1 transposition. We find that transposition is elevated in cells mutated for genes in the *RAD52* recombinational repair pathway, such as *RAD50, RAD51, RAD52, RAD54*, or *RAD57*, or in the DNA ligase I gene *CDC9*, but is not elevated in cells mutated in the DNA repair functions encoded by the *RAD1, RAD2*, or *MSH2* genes. The increase in Ty1 transposition observed when genes in the *RAD52* recombinational pathway are mutated is not associated with a significant increase in Ty1 RNA or proteins. However, unincorporated Ty1 cDNA levels are markedly elevated. These results suggest that members of the *RAD52* recombinational repair pathway inhibit Ty1 posttranslationally by influencing the fate of Ty1 cDNA.

Generation of Targeting Constructs for Mammalian Genes by Microhomologous Recombination in Yeast

We have developed a new procedure utilizing microhomologous recombination in yeast to generate targeting constructs for producing targeted mutations in mice. This procedure is rapid and efficient and should be directly applicable to all mammalian genes. Furthermore, only minimal information about the locus being targeted is required. The feasibility of this approach has been demonstrated by producing another allele of the mouse polycystic kidney gene, *Tg737*.

We have collaborated on this research with Adam Bailis, City of Hope; M. Joan Curcio, Wadsworth Center and School of Public Health; Stephen Hughes, NIH; Conrad Lichtenstein, Queen Mary and Westfields College; and Richard Woychik, Oak Ridge National Laboratory.

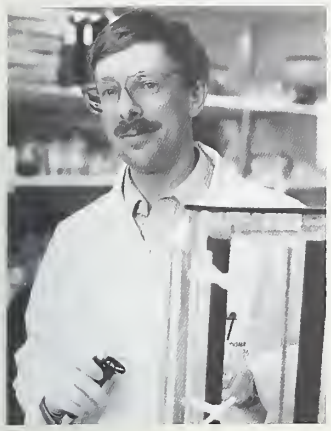
Recent Publications:

Curcio MJ, et al. *Trends Genet* 1999;15:43–5.

Rattray AJ, et al. *Genetics* 2000;154:543–56.

Lee B–S, et al. *Mol Cell Biol* 2000;20:2436–45.

Moore SP, et al. *J Virol* 2000;74:11522–30.



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Biography: *Dr. Kashlev received his Ph.D. in molecular biology from Moscow State University in 1990. He was a postdoctoral fellow in the Department of Microbiology at Columbia University from 1991 to 1992 and a research associate at the Public Health Research Institute from 1993 to 1996. In 1996, Dr. Kashlev joined the ABL–Basic Research Program and established the Molecular Mechanisms of Transcription Group. In 1999, he joined the Center for Cancer Research, NCI–Frederick.*

Gene Regulation and Chromosome Biology Laboratory

The Mechanism of Transcription Elongation by RNA Polymerase from *Escherichia coli* and RNA Polymerase II (POL II) from Yeast *Saccharomyces cerevisiae*

Keywords:
RNA polymerase
transcription elongation and termination
transcription through nucleosomes

Research: We conduct the structure/function studies of transcription elongation and investigate the mechanism of high processivity of the two closely related enzymes *Escherichia coli* RNA polymerase (RNAP) and RNA polymerase II (Pol II) from the yeast *Saccharomyces cerevisiae*. For both polymerases, we have developed a new experimental approach using synthetic DNA and RNA oligonucleotides and the solid-phase-immobilized enzymes to build in vitro authentic ternary elongation complexes (TECs) that bypass promoter-specific initiation and enzymatic synthesis of the transcript. Recently, we extended our interest toward unraveling the details of transcription through chromatin by the yeast Pol I, Pol II, and Pol III.

In the process of intrinsic transcription termination elongation complex dissociates as it passes across a DNA sequence that encodes a GC-rich hairpin in the RNA followed by a series of U residues. We investigate the mechanism of termination in *Escherichia coli* in the highly purified immobilized in vitro transcription system. We address the mechanism of destabilizing action of the hairpin, the input of oligo-U sequence to the destabilization, and the integration of both signals in the termination process.

We address the structural basis for high processivity of transcription by studying the role of RNA and DNA in the stability of TECs. We found that formation of a stable TEC by *Escherichia coli* RNAP depends on two distinct nucleic acid components: an 8-9 nt transcript-template hybrid, and a DNA duplex immediately downstream from the hybrid. For the Pol II, only the 8-9 nt RNA:DNA hybrid is required. To analyze the long-distance interactions between RNAP and DNA, which may affect the stability of TECs, we modified a specific site in the RNAP with Fe^{2+}-BABE molecule. This agent is capable of generating hydroxyl radicals that locally break the DNA strands. This approach allows us: (1) to address the global DNA folding in TEC and its role in the complex stability and catalytic activity, and (2) to probe the DNA arrangement in the TEC interior, which is not accessible with other

chemical probes. We also explore the highly sensitive method of iodine-125 radioprobing to study the geometry of nucleic acids in the TEC interior and to detect rearrangements of that geometry throughout the catalytic cycle of RNAP.

The project aims crystallization of TEC of *Escherichia coli* RNAP alone, and in combination with the transcription elongation factors NusA and NusG. In the future, this project will include cocrystallization of the TEC with a variety of modified substrate NTPs in the active center of the enzyme to address the catalytic mechanism of RNAP.

This project addresses the intriguing question of how eukaryotic RNA polymerases transcribe through chromatin. The project involves development of the minimal in vitro system for transcription through nucleosome by Pol I, Pol II, and Pol III from *Saccharomyces cerevisiae*. RNAP from *Escherichia coli*, which behaves very similarly to the eukaryotic enzyme in nucleosomal transcription, is used in this project as an easy-handled prototype of Pol II.

In this joint venture project, we take advantage of the floating visiting scientist position, which greatly facilitates collaboration between the sections in our laboratory. Dr. Kashlev's part in the project involves development of an in vitro system for purification and analysis of the Pol II enzymes having decreased transcription fidelity in vivo. These mutations are being isolated in Dr. Strathern's laboratory by the genetic screen exploring the Ty1 element retrotransposition assay.

Our collaborators include Herman Heumann and Evgeny Zaychikov, Max Planck Institut fur Biochemie, Germany; Xinhua Ji, Valery Karamichev, and Jeffrey Strathern, NIH; and Vasily Studitsky, Wayne State University, Detroit, MI.

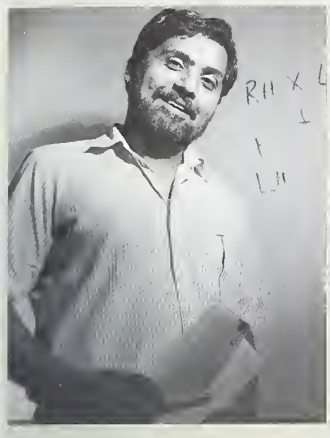
Recent Publications:

Komissarova N, et al. *Proc Natl Acad Sci USA* 1998;95:14699–704.

Sidorenkov I, et al. *Mol Cell* 1998;2:55–64.

Kireeva N, et al. *J Biol Chem* 2000;275:6530–6.

Kireeva N, et al. *J Mol Biol* 2000;299:325–35.



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Biography: *Dr. Klar received his Ph.D. in bacteriology at the University of Wisconsin in 1975 under Dr. Harlyn O. Halverson. He then studied genetics as a postdoctoral fellow at the University of California with Dr. Seymour Fogel. In 1978, Dr. Klar joined the staff at Cold Spring Harbor Laboratory, where he served as director of the Delbruck laboratory from 1985 to 1988. In 1988, he joined the ABL–Basic Research Program as head of the Developmental Genetics Section. In 1999, Dr. Klar joined the Center for Cancer Research, NCI–Frederick.*

Gene Regulation and Chromosome Biology Laboratory
Molecular Genetics of Development

Keywords:
cellular differentiation
genetics mechanisms
Schizosaccharomyces pombe

Research: The Developmental Genetics Section is mainly interested in the genetics and molecular biology of gene silencing and mating-type switching in *Schizosaccharomyces pombe*. In this yeast, the mating-type region consists of three loci called *mat1*, *mat2*, and *mat3*. The *mat2* and *mat3* loci are always silent and only act as donors of genetic information required for switching the transcriptionally active *mat1* locus. We found that the silencing mechanism involves a chromosomally heritable epigenetic alteration, presumably of chromatin associated with the *mat* region. We showed that the epigenetic event is maintained and inherited through meiosis as a conventional Mendelian marker. We have identified cis-acting sequences and trans-acting genes whose products participate in silencing. Molecular analysis of two such genes, designated *clr3* and *clr6* (for *c*ryptic *l*oci *r*egulator), showed that they encode homologs of histone deacetylase. We showed that another gene, *clr4*, encodes a chromodomain protein, a motif thought to be essential for silencing; furthermore, we showed that this gene is essential to the directionality of switching. This work strongly supports our model that gene silencing in the mating-type region is mediated by organization of the repressive chromatin structure. Future work will address the molecular biology of *clr* gene products as well as the mechanism of inheritance and duplication of the epigenetic mark during DNA replication.

Another aspect of our research has explained the mechanism of asymmetric cell division such that only one among four related granddaughter cells undergoes a mating-type switch. We showed that this pattern results from another site- and DNA strand-specific alkali-labile modification at *mat1* that efficiently initiates the recombination required for *mat1* interconversion. We have shown that the orientation of DNA replication with respect to leading- and lagging-strand synthesis establishes the mating-type switching pattern.

In another project, we have evidence that a single locus, *RGHT*, specifies preference for hand utilization in humans and that individuals with the recessive allele have a 50:50 chance of being either right-handed (RH) or left-handed (LH). We proposed a model for a single gene (*RGHT* = *Right-* and

r = *r*andom-handedness) controlling human hand preference and obtained data satisfying two key genetic predictions of the model. First, by calculating the gene frequency from RH x LH families, the model explains the percentage of LH children observed in RH x RH families. Second, the RH children of LH x LH families carry the *r/r* genotype, because they produce a higher percentage of LH children as predicted, similar to that of LH individuals in the general population. This model also explains discordance of handedness in monozygotic twins due to randomicity in hand preference of *r/r* individuals. We speculate that the *RGHT* locus functions primarily to specify cerebral laterality such that language is processed in the left-brain hemisphere and secondarily to coordinate left-brain dominance with development of the right-hand preference. Encouraged by these results, we plan to map the gene, with the ultimate goal of cloning it for molecular analysis. For this purpose, we are searching for families in which one or both parents are RH with two LH children (over 16 years old) and all additional children, if any, should be RH. Curiously, schizophrenic and bipolar disorder patients are three times more often LH compared to those in the general population. We speculate that genetics controlling handedness predisposes individuals for the psychosis. However, this partial association of psychosis with handedness needs to be experimentally verified.

Recent Publications:

Dalgaard JZ, Klar AJS. *Nature* 1999;400:181–4.

Klar AJS. *Schizophr Res* 1999;39:207–18.

Dalgaard JZ, Klar AJS. *Cell* 2000;102:745–51.

Nakayama J, et al. *EMBO J* 2001;20:1–10.

Laboratory of Genetics

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Since its inception in 1982, the Laboratory of Genetics has focused on the tumor biology and genetics of hematopoietic tumors with special emphasis on mouse plasmacytomagenesis. The laboratory is composed of five principal investigators whose interests are focused on genetic mechanisms in neoplastic development. Dr. Janz's B Cell Mutagenesis Group is studying the occurrence, fine structure, and mechanisms of the chromosomal trans-locations that lead to the deregulation of c-*myc* transcription. His group has developed new model systems for inducing "spontaneous" plasmacytomas in mice by "knocking" *c-myc* into new sites in the Ig heavy-chain gene complex. His group also studies the role of mutagenesis in various plasmacytoma models using transgenic reporter genes. Dr. Mock's Cancer Genetics Group identifies clones and characterizes tumor susceptibility/resistance genes in mouse plasmacytomagenesis. Dr. Mushinski's Oncogenesis Group within the Molecular Genetics Section is focused on the neoplastic effects of the cMyc proto-oncogene and the protein kinase C family of protein kinases and their effects on cell growth and programmed cell death. Dr. Potter's Plasmacytomagenesis Section is studying the role of inflammation and antigens in pristane-induced plasmacytomagenesis and the effects of various anti-inflammatory agents and immunization on this process. A major goal is to identify the nature of the B cell precursors of plasmacytomas. Dr. Law, formerly chief of the Laboratory of Cell Biology, is Scientist Emeritus, and his current studies have focused on tumor antigens.

Laboratory of Genetics Staff

Office of the Chief

Michael Potter	Chief of the Laboratory
Lloyd Law	Scientist Emeritus
Mary Millison	Support Staff
Victoria Rogers	Administrative Laboratory Manager

Laboratory of Genetics Staff (continued)

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Klaus Felix	Adjunct Investigator
Lynne Rockwood	Postdoctoral Fellow

Cancer Genetics Group

Beverly Mock	Tenure Track Investigator
Valery Bliskovsky	Postdoctoral Fellow
Edward Ramsay	Technician
Shuling Zhang	Postdoctoral Fellow
Wei Shi	Postdoctoral Fellow

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Mouse Facility

Wendy DuBois	Technician
Vaishali Jarral	Technician
Tina Wellington	Technician

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Biography: *Dr. Potter obtained his M.D. from the University of Virginia and joined the Leukemia Studies Section at the NCI under Dr. Lloyd Law in 1954. In 1982, he was appointed chief of the Laboratory of Genetics.*

Laboratory of Genetics
Pathogenesis of Plasma Cell Tumors in Mice

Keywords:
B cell
chromosomal translocations
c-myc
inflammation
susceptibility/resistance genes

Research: Our basic research interest is to understand the underlying process of neoplastic development—specifically, utilizing the model system of plasmacytomagenesis (PCTgenesis) in mice. Plasma cell tumor development offers special advantages for relating B cell clonal history—i.e., the punctuated developmental stages—associated with immunoglobulin (Ig) gene rearrangements V–D–J joining and heavy-chain isotype switching to the process of PCTgenesis. In fact, 80 to 90 percent of pristane-induced plasmacytomas (PCTs) have developed a chromosomal translocation in which the c-myc oncogene is illegitimately joined to an Ig switch region sequence. This activates and deregulates c-myc, which may be the initiating mutation in PCT formation. Recently, the evidence that BALB/c.CBA/N carrying a defective btk allele are resistant to PCT induction suggests the B–1 cells may be the target cells in plasmacytomagenesis. Our conventional mouse colony was moved to new quarters and the incidence of PCTs induced by pristane dropped from 60 to 70 percent in 300 days to 20 percent or less. In the last year, however, the incidence of PCTs following pristane induction has returned to the expected levels of 60 to 70 percent in 300 days. The sensitivity of pristane plasmacytomagenesis to environmental changes opens up new approaches to understanding mechanisms that determine neoplastic development. A longstanding focus of our work is to understand the role of inflammation and antigenic stimulation in plasmacytomagenesis. When added to the drinking water, the nonsteroidal anti-inflammatory (NSAID) agent indomethacin (IND) dramatically inhibits plasmacytomagenesis, but does not depress either the formation of the granulomatous tissue (OG) in which the preneoplastic proliferations (foci) take place or the formation of chromosomal translocation. Other NSAIDs including COX–2-specific agents and various antioxidants are being tested. We continue to study the antigen-binding activities of myeloma proteins to find clues concerning the origin of the neoplastic clones and we are currently studying the role of immunization with high molecular weight antigens and small peptides on plasmacytoma development. We continue to define new plasmacytoma resistance genes from DBA/2 and C57βL/6 mice in plasmacytoma genesis, and we carry out collaborative projects on the role of TGFβ/TGFβRII alterations in plasmacytoma formation.

Recent Publications:

Potter M, et al. *Blood* 1997;90:260–9.

Amoroso SR, et al. *Proc Natl Acad Sci USA* 1998;95:189–94.

Potter M, et al. *Int Immunol* 1999;11:1059–64.

Siegfried Janz, M.D.

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Biography: *Dr. Janz obtained his M.D. from the University of Leipzig, Germany, where he also took postgraduate training in immunology and received board certification in immunology.*

Laboratory of Genetics
Myc-Induced B Cell Neoplasia in Mice

Keywords:

Myc-activating chromosomal translocations
Myc-induced genomic instability
Myc transgenes

Research: We continue to evaluate the role of the proto-oncogene, Myc, in the development of malignant B cell tumors in mice. In fiscal year 2000, we made significant advances in this research. Three key results shall be briefly described here. First, we observed previously that Myc-activating 12;15 translocations, the hallmark mutations of inflammation-induced peritoneal BALB/c plasmacytomas (PCTs), can originate by illegitimate genetic exchange between Myc and the most upstream gene of the immunoglobulin heavy-chain (IgH) gene cluster, Cμ, and later progress to an exchange of Myc with the most downstream IgH gene, Cα. The Cμ/Myc to Cα/Myc transition appears to take place in PCT precursors where it is effected by a process that resembles isotype switching in normal B cells. The finding inspired us to mimic the two states of 12;15 translocation by gene targeting experiments, in which a histidine-tagged Myc gene was inserted on chromosome 12, either 5′ of Cμ or 5′ of Cα. The first mutation recreates the PCT precursor-typical Cμ/Myc recombination, while the latter reproduces the Myc recombination that is most frequently observed (~80 percent) in mature plasmacytomas (Cα/Myc recombination). We recently demonstrated that insertion of Myc 5′ of Cμ causes PCT development in mice. The tumors appeared to originate in Peyer's patches or mesenteric lymph nodes, produced copious amounts of monoclonal Ig, and performed in many cases abnormal isotype switching in the vicinity of the knocked-in Myc gene. Mice that harbor the inserted Myc gene upstream of Cα are currently being monitored for incipient B cell neoplasia, yet the first tumors have already been observed. In a related second study, we were able to show that mice bearing a properly engineered Myc transgene develop B cell lymphomas with histologic, cytologic, phenotypic, and molecular features that resemble human Burkitt's lymphoma (BL). The Myc transgene was controlled in this situation by reconstructed Ig light-chain loci (λ or κ) that encompass all regulatory elements required to

establish locus control in vitro. We have designated these tumors "mouse BL." In a third study, we exploited BALB/c mice congenic for a human IL–6 transgene driven by the histocompatibility H–2L^d promoter to demonstrate for the first time that transplantable plasmacytomas harboring Myc-activating 12;15 translocations can develop spontaneously in lymphoid tissues of untreated mice. This finding suggested that the cooperation of three pathogenetic factors suffices to trigger PCT development in mice: constitutive IL–6 signaling (transgenic expression of IL–6), activation of Myc (12;15 translocation), and PCT susceptibility alleles (BALB/c genotype). Our studies have provided important new information on Myc-induced neoplastic development in B cells. In addition, they have resulted in the design of exciting new mouse models for human disease.

Our collaborators are G.–W. Bornkamm, Institute for Clinical Molecular Biology and Tumor Genetics, GSF, Munich, Germany; Lionel Feigenbaum, SAIC–Frederick; and T. Ried and L. Tessarollo, NIH.

Recent Publications:

Kovalchuk AL, et al. *Leukemia* 2000;14:1127–35.

Coleman AE, et al. *Genes Chromo Cancer* 2000;29:70–4.

Kovalchuk AL, et al. *J Exp Med* 2000;192:1183–90.

Kovalchuk AL, et al. *Genes Chromo Cancer* 2001;30:282–91.

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Biography: *Dr. Mock obtained her Ph.D. in zoology from the University of Maryland and continued her studies on the genetics of susceptibility to parasitic diseases in the Department of Immunology at the Walter Reed Army Institute of Research. Since coming to the NIH, she has focused her research on complex genetic traits of cancer, and she serves as the committee chair in the International Mouse Genome Society for compiling linkage maps of mouse chromosome 4.*

Laboratory of Genetics
The Genetics of Susceptibility to Mouse Plasmacytomagenesis

Keywords:
B cell
backcross
bacterial artificial chromosomes
Burkitt's lymphoma
cancer
cancer genetics
candidate genes
cell cycle control
cyclin dependent kinase
DNA methylation
gene mapping
gene mutation
genetic linkage analysis
genetic susceptibility
germline mutations
hereditary cancer
modifier loci
multiple myeloma
plasma cell tumors
susceptibility/resistance genes

Research: Our research is directed at combining classical and molecular genetic studies to fine-map, isolate, and characterize disease-trait loci associated with multistep models of tumor initiation, promotion, and progression found in mouse plasmacytomas and carcinogen-induced skin tumors. To this end, our lab is involved in mapping the chromosomal locations of genes associated with the susceptibility of BALB/c mice to the induction of mouse plasmacytomas. These B cell tumors are induced by a variety of agents including pristane, mineral oil, plastic discs or shavings, silicones, and retroviral constructs containing cooperating pairs of oncogenes. At least three distinct regions of the genome have been linked by RFLP/SSLP analyses to pristane-induced plasmacytomagenesis in a series of intercross and backcross progeny derived from susceptible and resistant strains. Two of these genes (*Pctr1* and *2*) reside in noncontiguous, nonoverlapping segments of the distal half of mouse Chr 4. A third gene, *Pctr3*, resides in the interval of mouse Chr 4 between *Pctr1* and *2*. A fourth gene, *Pctm*, is linked to mouse Chr 1; tumor latency is also linked to mouse Chr 1. These genes reside in regions of the mouse genome that share linkage homology with human Chrs 1 and 9; a number of human malignancies, including multiple myeloma, have shown chromosomal abnormalities involving the human 1p region.

To map the positions of these genes more precisely, panels of congenic strains of mice (C.D2) have been constructed to contain regions of DBA/2 chromatin harboring the *Pctr1* and *2* resistance genes on a BALB/c background. As these strains are being advanced, mice which harbor recombinations across the 4 to 5 cM intervals surrounding these resistance genes are being partitioned into 1 to 2 cM intervals. To obtain markers surrounding *Pctr1* and *2*, we have used our resistant and susceptible congeners in PCR-based DNA subtraction protocols to isolate three clones linked to the resistance phenotype. In the *Pctr1* region, single base pair substitutions in a candidate gene, *Cdkn2a*, have been shown to have functional differences at the protein level in in vitro kinase assays with the

RB protein as substrate. Current studies have focused on evaluating tumor phenotypes in knock-out mice. Tumor incidence is higher and tumor latency is greatly reduced in Cdkn2a knock-out mice, when compared with the tumor incidences and latencies seen in C57BL/6 mice. Cell cycle profiles in plasma cell lines transfected with wild-type (DBA) versus variant (BALB/c) p16 differed dramatically; DBA p16 induced cell cycle growth arrest. In contrast, BALB/c p16 did not. Physical mapping studies in the *Pctr2* region have been initiated, and several new genes identified in this region are under evaluation as candidates. Any candidate genes isolated by one or more of the above methods will be examined for mutations in patients with B cell disorders.

Alternative models of PCT induction are being used to determine the genetic basis of susceptibility to mouse myelomas which are induced by a combination of pristane and retroviruses harboring cooperating pairs of oncogenes (myc and abl). These tumors do not harbor the T(12;15) or T(6;15) translocations which occur in more than 95 percent of the tumors induced by pristane alone, but the majority are trisomy for Chr 11. This system may identify genes involved in tumor progression, rather than initiation. A salient feature of the retrovirally induced tumors, in contrast to tumors induced by pristane alone, is that susceptibility is dominant in F1 hybrids. Tumors induced in F1 hybrids may be examined for evidence of loss of heterozygosity, an event characteristic of tumor suppressor genes or microsatellite instability frequently associated with mismatch repair defects.

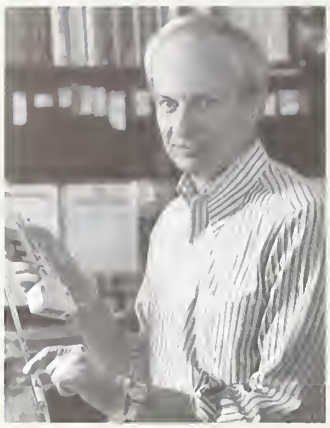
Recent Publications:

Mock BA, et al. *Blood* 1997;90:4092–8.

Mock BA, et al. *Proc Natl Acad Sci USA* 1998;95:2429–34.

Mock BA, et al. *Carcinogenesis* 1998;19:1109–15.

Mock BA, et al. *Curr Top Microbiol Immunol* 1999;246:363–8.



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Biography: *Dr. Mushinski is also a research professor in the Molecular and Cell Biology Program of the University of Maryland. He obtained his B.A. from Yale and his M.D. from Harvard University. He completed postgraduate training in internal medicine at Duke University and in the Department of Molecular Biology at the Max Planck Institute for Experimental Medicine in Göttingen, Germany.*

Laboratory of Genetics
Oncogene Expression and Signal Transduction in Hematopoietic Transformation and Differentiation

Keywords:
animal model
antibodies (monoclonal)
antioncogene
apoptosis
B lymphocytes
cell cycle control
cell transformation
chromosomal translocations
c-myc
cyclin D
cytoskeleton
differentiation
extrachromosomal elements
genomic instability
lymphoma
macrophages
microarrays
monoclonal antibodies
mouse
mRNA expression
multiple myeloma
myeloma
oncogenes
phorbol esters
PKC
plasma cell tumors
plasmacytoma
prostate cancer
protein kinase C
proto-oncogene
retroviruses
tumor suppressor genes
tumorigenicity

Research: Our research objective is to understand the molecular and genetic mechanisms responsible for cell growth, differentiation, and neoplastic transformation. We study the oncogenes, tumor-suppressor genes, and signal transducing proteins involved in BALB/c mouse plasmacytomas, B cell lymphomas, and other mouse and human experimental tumor systems. These are valuable experimental models, because they have many biological and molecular genetic features in common with human multiple myeloma, non-Hodgkin's lymphomas, and other human malignancies that are in need of mechanistic understanding in order to devise more specific therapy and preventive measures. BALB/c plasmacytomas, like rat immunocytomas and human Burkitt's lymphomas, are characterized by constitutive expression of messenger RNA and protein from the master oncogene, c-Myc. Most commonly, c-Myc expression in plasmacytomas is dysregulated secondary to a chromosomal translocation in the vicinity of the c-Myc gene.

It is still not clear why the c-Myc oncogene is universally involved in plasma cell tumors nor how overexpression of this gene leads to many different forms of tumors in human and mouse cells. We think we have found a clue to this mechanism in that we have found that the gene encoding an important protein that drives the cell cycle, cyclin D2, is amplified and overexpressed in human and mouse tumor cells that overexpress c-Myc. In addition, we have found that 3 or 4 days of overexpression of c-Myc is sufficient to destabilize the genome and to cause the generation of intranuclear fragments of chromatin, called extrachromosomal elements. These can be detected with the fluorescent microscope. Hybridization techniques show that a number of genes can be found on these nonchromosomal nuclear DNAs, some of which result in elevated expression of important growth-stimulatory proteins, including cyclin D2. We are actively engaged in learning how many such genes can be amplified by this mechanism.

In the study of signal transduction, we are investigating protein kinase C (PKC), a multigene family of at least 12 structurally related isoenzymes that are important mediators of many forms of signal transduction. Using a variety of expression vectors, we have overexpressed many of the PKCs in fibroblasts, lymphocytic, and myeloid cell lines. This has made possible the identification of specific functions and intracellular targets for the individual PKC isoenzymes. We have been focusing on the delta and epsilon isoenzymes, which seem to have opposite effects on cell growth. We have shown that PKCδ is responsible for myeloid differentiation and growth inhibition, while overexpressed PKCε stimulates cell growth and transforms fibroblasts into tumor cells. We are dissecting the structure of these isoenzymes to determine which protein domains control these functions. We have shown that most of the isoenzyme-specific determinants are located in the catalytic half (the carboxyl-terminal domain) of these PKCs by creating chimeric molecules that are half PKCδ and half PKCε. Chimeric molecules that have carboxyl-terminal PKCδ sequences are able to cause macrophage differentiation much like the parent all-PKCδ protein. Similarly, a PKC chimera with a PKCε carboxyl terminus retains the neoplastic transformation potential of the all-PKCε protein.

We are also studying the nature of PKC's involvement in prostate tumor induction, smooth muscle apoptosis, cytoskeletal changes in cell shape, and its relationship to metastasis of these and other types of tumors. Recently we have shown that phorbol ester activation of overexpressed PKCδ disrupts the actin cytoskeleton in human and mouse lymphocytes, leading to the loss of membrane ruffling, a surface alteration needed for cell movement, and the loss of the typical elongated shape of these cells. This is the first of our studies into the important interrelationship between PKC, the cytoskeleton, and signal transduction. We are also using microarray hybridization to study changes in gene expression during cell signaling.

Collaborators on this research include Peter Blumberg and Jane Trepel, NIH; Sabine Mai, University of Manitoba, Winnipeg, Canada; and Michael Kracht and Harald Mischak, Medizinische Hochschule Hannover, Germany.

Recent Publications:

Wang QJ, et al. *Oncogene* 1998;16:53–60.

Mai S, et al. *Neoplasia* 1999;1:214–52.

Romanova LY, et al. *J Cell Physiol* 1999;179:157–69.

Laboratory of Genomic Diversity

The Laboratory of Genomic Diversity (LGD) has five primary purposes: (1) to develop research on identification and characterization of genes that influence human cancers and human infectious diseases, principally AIDS, HTLV–1 disease, hepatitis, and other viral agents; (2) to contribute to the international human genome project by mapping human genetic markers and functional gene loci; (3) to develop comparative genome mapping by assembly of a gene map in non-human mammalian species that serves as a model for cancer and infectious diseases; (4) to conduct investigations on animal models of infectious disease and cancer; and (5) to apply evolutionary and phylogenetic methodology to genome analysis related to health imperatives.

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The strength of the LGD is in the cooperative interplay of diverse skills and interests of its scientists, who are encouraged to pursue different lines of investigation ranging from fundamental genetic mechanisms to approaches that we hope will be directly applicable to understanding and treating human cancer.

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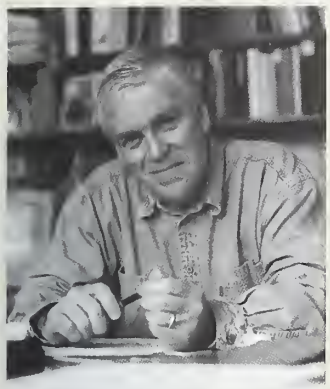

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Biography: *Dr. Stephen O'Brien is chief of the Laboratory of Genomic Diversity and head of the Genetics Section. He studied* Drosophila *genetics at Cornell University where he received a Ph.D. in 1971. He came to the National Institutes of Health as a postdoctoral fellow and built a program based on mammalian somatic cell genetics. He is cochairman of the International Committee on Comparative Gene Mapping and editor of* Genetic Maps *and* Journal of Heredity.

Laboratory of Genomic Diversity
Comparative Genetics, Emerging Viruses, and Host Responses in Natural Populations

Keywords:
Felidae
FIV
genetics
HIV

Research: The principal focus of our investigation concerns the collaborative interaction of mammalian cellular genes operative in concordant evolutionary descent of the immune system, retroviruses, and cancer onset in pursuit of comparative mammalian genetic principles which participate in these processes. Three sharply focused research projects are currently in progress.

The Development of the Domestic Cat, *Felis catus*, as a Model for Genetic Analysis

To facilitate the understanding of mammalian development, infectious disease, and neoplasia, and also to provide a balance to possible biological exceptions that may occur in rodent modeling of human genetics, we are developing a comparative genetic map of the domestic cat (*Felis catus*). The cat was an attractive candidate for laboratory genetics for several reasons, including abundant polymorphic morphological loci, heritable defects homologous to human genetic diseases, and epidemics of two viruses, feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV), that cause neoplasias and immunodeficiencies, respectively. Recent advances include (1) a radiation hybrid map of the domestic cat containing 424 Type I (coding gene) marker loci and 176 hypervariable short tandem repeat polymorphisms (STRPs or microsatellites); (2) a genetic map containing over 100 Type I marker anchor loci distributed over 17 of the 19 feline chromosomes, a map built using somatic cell hybrid panels, high-resolution G-banding karyology, fluorescence in situ hybridization (FISH), and pedigree analysis, which revealed extensive syntenic conservation between feline and human genomes in several cases spanning entire chromosomes; (3) identification of 321 Type I marker anchor loci selected to provide a 5- to 10-centiMorgan density of gene markers for use in comparative mapping of all vertebrate species, termed "comparative anchor tagged sequences" (CATS); (4) isolation, sequencing, and polymerase chain reaction primer design, and genetic mapping of 235 STRPs; and (5) development of interspecies sexual crosses and backcrosses between the domestic cat and Asian leopard cat suitable for rapid mapping of Type I and STRP loci.

Emerging Viruses and Host Responses in Natural Populations
Throughout evolution, animal species have been continually afflicted with devastating viral disease outbreaks that have driven the coevolution of both host and pathogen genomes, which today are punctuated with the molecular footprints of these outbreaks. The specific objectives of this project are to identify new viruses and disease agents which influence the health of individuals and the demographic stability of the population, to monitor the dynamics of emerging viruses and associated epidemics in situ, and to track the coevolution and coadaptation of parasite and host genomes driven by natural epidemics, using genetic and phylogenetic inference. Current work is focused on the natural history and acute epidemic emergence of four viruses: human T cell lymphotropic virus (HTLV–1), feline infectious peritonitis virus (FIPV), feline immunodeficiency virus (FIV), and canine distemper morbillivirus. Recent results include (1) the demonstration that FIV exposure is a widespread occurrence in felid species, and (2) a phylogenetic analysis of lion FIV *pol* gene sequences from four geographically isolated African populations revealed remarkably high intra- and interindividual genetic diversity at the sequence level resolvable into three phylogenetic clusters. The latter study suggests that the ancestors of lion FIV evolved in allopatric (geographically isolated) populations that converged recently and, since there is no clear evidence of lion FIV-associated pathology, raises the possibility of a historic genetic accommodation between the lion lentivirus and its host.

The Identification of Human Genetic Loci Which Influence Susceptibility to HIV Infection, Disease Progression, and Host Immune Response
The HIV–AIDS epidemic is characterized by considerable epidemiologic heterogeneity in infection, rate of progression of HIV-infected patients to AIDS, and disease sequelae. Epidemiologic explanations for heterogeneity generally involve either virus or host genetic differences, another pathogen, or an environmental component. The specific objective of this project is to discover and characterize human genetic loci operative in differential host responses to two pathological viruses, HIV and hepatitis B virus, using the combined methods of human molecular genetics, population genetic theory, and epidemiology. Towards this goal, we have developed a new approach to mapping disease loci in populations when family studies are not feasible (as in the case of infectious disease susceptibility). The method, termed "mapping by admixture linkage disequilibrium" (MALD), takes advantage of linkage disequilibrium that occurs temporarily in admixed racial groups, such as African Americans and Hispanics. We have recently discovered mutations in the HIV macrophage coreceptors and one of the coreceptor ligands that provides genetic resistance to disease progression among HIV-infected patients.

Recent Publications:

O'Brien SJ, et al. *Annu Rev Genet* 2000;34:563–91.

O'Brien SJ, et al. *Science* 1999;286:458–81.

O'Brien SJ, et al. *Immunol Rev* 1999;167:133–44.

O'Brien SJ, et al. *Sci Am* 1997;277:44–51.

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Biography: *Dr. Carrington obtained her Ph.D. at Iowa State University in the Immunobiology Department. She performed her postdoctoral studies in the departments of immunology and microbiology at Duke University and the University of North Carolina, respectively. Prior to her current appointment, she was a faculty member in the Immunology Department at Duke University.*

Laboratory of Genomic Diversity
The Human Major Histocompatibility Complex and Its Association with Disease

Keywords:
hepatitis viruses
HIV–1
HLA
HPV
killer inhibitory receptors
linkage disequilibrium
major histocompatibility complex
microsatellites
natural killer cells
recombination
single sperm typing

Research: The main goal of the HLA Genetics Group is to understand the interactions between HLA and disease using a genetic approach. A confounding factor in determining the precise disease locus (loci) among the many candidates within the major histocompatibility complex (MHC) is the nonrandom association between pairs of loci within the complex. Accounting for molecular genetic characteristics of this region, such as meiotic recombination rates and levels of linkage disequilibrium between pairs of loci, may help us to identify more accurately which locus is truly involved in the disease process. Since all diseases associated with the HLA loci are multigenic, it is helpful to identify effects of other genes on a particular disease when characterizing the relationship between that disease and HLA. Thus, in order to determine the effects of HLA on a given disease, we have taken into account the following: (1) direct analysis of allelic and haplotypic associations between HLA and selected infectious diseases; (2) characterization of patterns of recombination and linkage disequilibrium between loci of the MHC; and (3) identification of loci located outside the MHC that have an effect on disease outcome, some of which may act in a epistatic manner with HLA.

Our previous studies have convinced us that genes within the MHC play a role in controlling diseases caused by HIV–1, hepatitis B virus, and human papilloma virus. For these diseases, we now hope to dissect the genetic mechanism by which the HLA loci are exerting their influence. This is being investigated by detailed analysis of allelic HLA typing data using allelic, haplotypic, heterozygosity, and nucleotide site-specific analysis.

Natural killer (NK) cells destroy target cells that lack expression of HLA class I and are inhibited by recognition of class I on the target surface through receptors called killer inhibitory receptors (KIR). NK cells play an important role in defense against viruses that inhibit class I molecule expression and thereby avoid recognition by cytotoxic T lymphocytes. The KIR haplotypes have undergone contraction and expansion, resulting in great complexity and diversity of KIR haplotypes. We have developed a molecular typing technique for the KIR genes and are defining KIR haplotypes using the CEPH

families. We are also studying the effects of this locus, in combination with HLA, on a number of autoimmune and infectious diseases.

Microsatellites are markers which contain repetitive elements of varying sizes within a species and they are dispersed frequently throughout the genome. We have characterized a number of these repeat markers which map within the MHC, which we are using as a rapid means to identify regions of disease association and to identify HLA matched bone marrow recipients and donors. Recently the entire sequence of the human MHC has become available and we are now characterizing all di-, tri-, and tetranucleotide repeats throughout the MHC.

The high level of polymorphism within the MHC provides the means for studying the mechanism of meiotic recombination. Further, identification of regions within the MHC where recombination occurs and determination of its frequency of occurrence may reflect selection for certain fixed combinations of alleles at two or more loci. Single sperm typing has been established in the laboratory and recombination frequencies of individual donors are being determined. These data, along with linkage disequilibrium studies being generated in the laboratory, should provide useful information regarding associations among genes in the MHC.

Collaborators are William Klitz, University of California–Berkeley; and John Trowsdale, Cambridge University.

Recent Publications:

Carrington M. *Immunol Rev* 1999;167:245–56.

Carrington M, et al. *Science* 1999;283:1748–52.

Carrington M, et al. *Hum Mol Genet* 1999;8:1939–45.

Thio CL, et al. *Hepatology* 2000;31:819–27.

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Biography: *Dr. Dean obtained his Ph.D. from the Biochemistry Department at the Boston University School of Medicine. He performed his postdoctoral studies at the National Cancer Institute on the MET oncogene and cystic fibrosis gene. He is a member of the Centre Etude du Polymorphisme Humaine (CEPH), the Human Genome Organization (HUGO), and an adjunct faculty member at Hood College.*

Laboratory of Genomic Diversity
Genetic Analysis of Complex Diseases

Keywords:
ATP-binding cassette genes
cancer genetics
chemotherapy
drug screening
human genetics
multidrug resistance
tumor suppressor genes

Research: Our laboratory's objective is to develop methods for analyzing complex diseases and to apply them to human genetic conditions, cancer, HIV infection, and disease. Our laboratory is part of the Genetic Annotation Initiative (GAI), a component of the Cancer Genome Anatomy Project. The GAI aims to identify frequent sequence variants in approximately 1000 cancer-related genes. The goal of this project is to identify genetic markers that will be useful in cancer family studies as well as in population-based approaches. Resistance of tumors to several drugs (multidrug resistance, or MDR) is a major limitation of cancer chemotherapy. In some tumors, MDR is due to the overexpression of the P-glycoprotein (PGP)/MDR gene, or the multidrug resistance-related protein (MRP). PGP and MRP are members of the ATP-binding cassette (ABC) family of transporters. To identify new genes involved in multidrug resistance, we have characterized at least 25 new human ABC genes. The genetic location of each of these genes as well as their expression pattern has been determined. One of these genes, *ABCR*, is expressed exclusively in the retina, in rod photoreceptor cells. The gene is responsible for the recessive retinal degeneration syndrome Stargardt disease, and is also mutated in some patients with retinitis pigmentosa and cone-rod dystrophy. Some parents of Stargardt disease patients have age-related macular degeneration (AMD). AMD is the most common cause of vision loss in the elderly, and we have found that a significant portion of AMD patients have mutations in one allele of the *ABCR* gene, suggesting that this gene can predispose individuals to AMD.

In all well-characterized epidemics there are individuals in the population who respond differently to the infectious agent. The HIV–1 epidemic presents a critical challenge to apply current genetic techniques to the study of host genetic variation for infection and susceptibility to infection. The *CCR5* gene serves as a secondary receptor on CD4+ T lymphocytes for certain strains of human immunodeficiency virus. We previously identified a 32-base pair deletion allele (*CCR5Δ32*) that is present at a frequency of approximately 0.10 in the Caucasian population. Homozygotes for the deletion occur almost exclusively among exposed HIV–1-antibody negative individuals, indicating that individuals lacking a functional copy of the gene are highly resistant to HIV infection. In addition, *CCR5* deletion heterozygotes (+/*Δ32*) are

significantly elevated among patients who survive HIV–1 infection for more than 10 years. In addition, we have identified 15 other alterations in the *CCR5* gene, many in conserved residues, and an amino acid deletion. Polymorphisms in the promoter and regulatory regions of the *CCR5* gene are also associated with either faster or slower progression to AIDS, suggesting that differential expression of this gene may play an important role in the early stages of the disease process.

Collaborators on our research are Rando Allikmets and Bernard Gerrard, SAIC–Frederick; Mark Leppert, University of Utah; and James Lupski, Baylor University.

Recent Publications:

Hahn H, et al. *Cell* 1996;80:841–51.

Dean M, et al. *Science* 1996;273:1856–62.

Allikmets R, et al. *Science* 1997;277:1805–7.

Martin MP, et al. *Science* 1998;282:1907–11.

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Biography: *Dr. Smith obtained his Ph.D. from The Johns Hopkins University working in the field of population genetics, and he completed postdoctoral training in molecular evolution at the University of California–San Diego. Subsequently, he was the assistant director of the Human Genome Center at the Salk Institute for Biological Studies. He is now a principal investigator at the National Cancer Institute with expertise in the areas of HIV–1/AIDS and human genetic analysis.*

Laboratory of Genomic Diversity
HIV–1/AIDS and Prostate Cancer: Genetic Epidemiology Using Candidate Genes and Genome Scans

Keywords:
genetic epidemiology
genetic polymorphism
genetic susceptibility
genotyping technology
HIV–1
phylogenetic analysis
population genetics

Research: A novel method for disease gene identification in patient cohorts is being developed. African-American HIV–1/AIDS and prostate cancer patients are being genotyped for the identification of genes involved in the development and progression of these diseases. A complementary approach of examining candidate gene polymorphisms in patient cohorts for testing hypotheses of susceptibility to HIV–1 and progression to AIDS is also in progress. High-throughput genotyping technology is continuing to be developed and incorporated into the laboratory to accomplish these objectives.

HIV–1 and AIDS Candidate Genes

The approach of screening polymorphisms in or near a gene depends on associations between functional genetic lesions and flanking polymorphisms that exist in most human populations on the scale of up to one or two centiMorgans. Candidate gene searches exploit that fine-scale linkage disequilibrium by screening any available polymorphisms flanking or within candidate genes against patient populations. We have identified five cohorts that have several thousand HIV–1-infected and -exposed patients: AIDS Link to the Intravenous Experience (ALIVE); DC Gay (DCG); Multicenter AIDS Cohort Study (MACS); Multicenter Hemophilia Cohort Study (MHCS); and San Francisco City Cohort (SFCC). Screening is under way with polymorphisms at over 100 selected candidate genes, and any additional newly identified genes, to identify human loci that influence viral susceptibility, disease progression and sequelae in patients. Such loci would be useful as targets for drug development and gene therapy in HIV-infected patients. The research uses available candidate gene polymorphisms and develops new ones for screening the patient cohorts as follows: (1) screening with the candidate genes that currently have known polymorphisms and additional loci that become available either from our own development or from the literature; (2) identifying polymorphisms for the remaining loci by denaturing HPLC and direct sequencing; (3) converting these polymorphisms to high-throughput single nucleotide polymorphism (SNP) assays; (4) identifying flanking polymorphic microsatellites, insertion/deletions, and SNPs as these candidate loci are placed onto physical and genetic maps by radiation hybrid mapping and/or sequencing at the genome centers; and (5) performing survival and categorical analysis of the clinical data to determine relationships between genotypes and phenotypes.

Development of Polymorphic Admixture Typing Map

We are implementing a new genetic method termed "mapping by admixture linkage disequilibrium" (MALD) which provides a population- and patient cohort-based approach for disease gene identification. The method uses genetic markers which have significant allele frequency differences between racial groups and a patient population with a recent admixture history like African Americans and Hispanics. Microsatellites are a class of markers with larger differences between racial groups than SNPs, making them more suitable for MALD mapping. Like traditional linkage studies, MALD can use additional markers to confirm a positive result and pinpoint the exact disease gene location. We have recently demonstrated the presence of admixture linkage disequilibrium across a core 8 cM flanking the FY locus which extends to a region of up to 30 cM.

High-Throughput Genotyping Laboratory for Gene Discovery

A semiautomated laboratory has been set up which types highly informative microsatellite markers by multiplex analysis with fluorescent labels, and types SNPs using 96-well format assays and gels. Efforts currently under way are moving those laboratory experiments over to the new 384-well platform for genetic analysis. We have developed dinucleotide microsatellite markers with a systematic spacing of 10 centiMorgans for multiplex fluorescent detection on an Applied Biosystems sequencer. About 450 markers are available, and ongoing experiments will identify the remainder needed for MALD mapping.

Prostate Cancer and AIDS Gene Mapping and Identification
Many diseases occur sporadically in patients without a family history of the disease and, in others, exposure to an environmental or viral agent is a causative factor. In the case of sporadic prostate cancer and AIDS, family members are not available for study, precluding traditional linkage analysis and making MALD gene mapping one of the few genetic means for identifying the previously unknown genes. The complementary candidate gene approach has already identified a deletion in the *CCR5* gene, an amino acid change in *CCR2(64I)*, a 3′ untranslated region variant in *SDF1*, and promoter variants of *CCR5* (+.P1.+ homozygosity) and *IL–10* (5′A) as important factors in HIV–1 infection and disease progression. Screening of the 100 candidate genes identified in the literature by the AIDS research community will likely determine other important host gene polymorphisms.

Our collaborators are Susan Buchbinder and Eric Vittinghoff, San Francisco City Clinic; Kyu Woan Cho, James Goedert, Alisa Goldstein, William Modi, and Hyoung Doo Shin, NIH; Joseph Coresh, Homayoon Farzadeghan, Michael Klag, Alvaro Munoz, and David Thomas, Johns Hopkins University; Roger Detels, University of California–Los Angeles; Sharyne Donfield, Rho, Inc.; Brian Henderson, University of Southern California; Richard Kaslow, University of Alabama at Birmingham; Charles Rinaldo, University of Pittsburgh; Steffanie Strathdee and David Vlahov, New York Academy of Sciences.

Recent Publications:

Smith MW, et al. *Science* 1997;277:959–65.

Bream JH, et al. *Immunogenetics* 2000;51:50–8.

Lautenberger JA, et al. *Am J Hum Genet* 2000;66:969–78.

Shrestha S, et al. *AIDS* 2000;14:1507–13.

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Biography: *Dr. Winkler received her graduate degree in immunogenetics from the University of Maryland under the direction of Dr. Stephen J. O'Brien at the NIH. During graduate school, Dr. Winkler's work on the major histocompatibility complex in cats led to the notable finding that the endangered cheetah was highly inbred and more susceptible to infectious diseases as a consequence. Dr. Winkler now investigates the interaction of host genetic variation and complex diseases in humans.*

Laboratory of Genomic Diversity
Identification of Human Genetic Loci That Influence Susceptibility to Viral Infection

Keywords:
gene discovery
genetic epidemiology
hepatitis viruses
HIV–1

Research: A hallmark of viral infections in natural populations is the range of host response to exposure and infection. Differences in infection rates, rate of progression, and specific outcomes are likely due to both strain differences and variation in host genes that either directly or indirectly interact with the infectious agent. We are utilizing a candidate gene approach to identify polymorphic sites in genes that may have a role in viral infection and disease progression. The discovery of genetic variants provides a valuable tool for the identification of host cellular components that are operative in viral infection, replication, viral assembly, and immune regulation of the infection. Defining the mechanisms by which host factors restrict viral disease processes will advance our understanding of viral pathogenesis and may lead to possible therapeutic interventions. Differences in mutation frequencies between ethnic/racial groups may also explain at least in part the geographical variation observed for both HIV and the hepatitis viruses.

Identification of Human Genetic Loci That Influence Resistance to HIV and the Hepatitis Viruses, HCV and HBV

Our group is utilizing a candidate gene approach to identify genes that: (1) restrict infection following viral exposure; (2) modulate or control the immune response; (3) influence outcome to infection; (4) regulate the rate of pathogenesis; and (5) contribute to antiviral therapy resistance or intolerance. In collaboration with five large multicenter cohort studies, we have established B cell lines from more than 4000 HIV-infected or HIV-exposed uninfected study participants as a renewable source of DNA for genetic analysis and high throughput genotyping. Using either known polymorphisms or novel polymorphisms discovered by our group in regulatory and coding regions of candidate genes, patient DNA is genotyped and analyzed for association with specific disease phenotypes. This approach to deciphering the genetic influence on complex infectious diseases has proven remarkably successful. These resources have been used by investigators within our lab to identify a series of polymorphisms in genes involved in immune regulation (*IL10* and *HLA*) and cell entry (*CCR2*, *CCR5*, and *SDF1*), and in transcriptional factors that affect infection, rate of progression to AIDS,

and specific outcomes from HIV–1. Although the individual effects of some of these variants are small, together they account for approximately 30 to 50 percent of long-term survivors. Because viral pathogens depend on specific molecular interactions with host factors for cell entry, replication, and virion assembly, it is not surprising that viral pathogenesis may be modulated by host genetic variation.

Using the genetic analysis of HIV–1 as a model for association analysis of complex diseases that have both genetic and environmental components, we are employing a similar strategy to investigate the host genetic contributions to outcomes following infection with the hepatitis viruses, HCV and HBV. These important human viral infections have global distributions and extremely high prevalence rate in some regions of the world and among certain risk groups and cause considerable morbidity and mortality from chronic liver disease leading to cirrhosis and hepatocellular carcinoma. The pathogenic effects of the hepatitis viruses are highly variable and not fully explained by strain differences or subtypes. We are using a candidate gene approach to elucidate the genetic basis for the extensive variation observed within risk groups in viral clearance, liver disease progression, and resistance to infection for these important pathogens.

Genetics of Renal Failure in African Americans

African Americans have an increased incidence of renal disease, probably due to the combined impact of several disease processes including hypertension, diabetes, and glomerulonephritis. The incidence of nondiabetic glomerulonephritis is approximately two-fold elevated in African Americans compared to other racial groups. The most common form of nondiabetic glomerular disease among African Americans is focal segmental glomerulosclerosis (FSGS). The worldwide epidemiologic pattern argues strongly that genetic differences rather than sociocultural differences are responsible for the increased risk. In collaboration with Dr. Jeffrey Kopp, we have initiated a population-based approach to identifying genetic loci that contribute to the pathogenesis of FSGS, an aggressive disease that progresses to end stage renal disease in approximately 50 percent of patients. At least two polymorphic genes encoding proteins in the pathway regulating blood pressure have been found to be associated with an increased risk for FSGS. The development of more rational and effective therapy likely awaits a better understanding of pathogenesis, and an understanding of the genetic basis for FSGS might provide a key insight into pathogenesis.

Collaborators on our research include Eric Daar and Roger Detels, University of California; Sharyne Donfield, Rho Federal Systems; James Goedert, Jeffrey Kopp, and Thomas O'Brien, NIH; Lisa Jacobson, Alvaro Munoz, and David Vlahov, Johns Hopkins School of Public Health; and Charles Rinaldo, University of Pittsburgh.

Recent Publications:

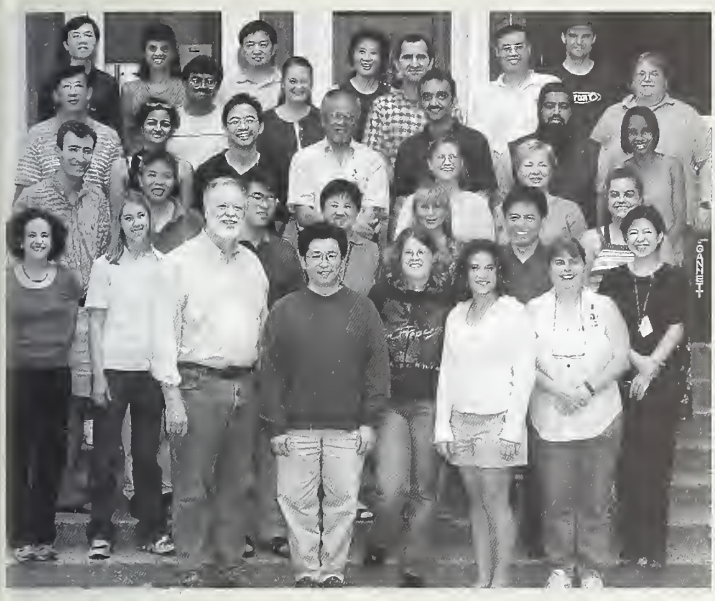
Smith MW, et al. *Science* 1997;277:959–65.

Winkler C, et al. *Science* 1998;279:389–93.

Martin MP, et al. *Science* 1998;282:1907–11.

An P, et al. *AIDS* 2000;14:2117–22.

Laboratory of Human Carcinogenesis



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The Laboratory of Human Carcinogenesis conducts investigations to assess: (1) mechanisms of carcinogenesis including the cellular functions of tumor suppressor genes and oncogenes; (2) experimental approaches in biological systems for the extrapolation of carcinogenesis data and mechanisms from experimental animals to humans; and (3) molecular epidemiology of human cancer risk.

The scientific strategy of the laboratory is reflected in its organization into three sections, the In Vitro Carcinogenesis Section, the Molecular Genetics and Carcinogenesis Section, and the Molecular Epidemiology Section. Scientifically, the emphasis is on the role of inherited or acquired host factors as important determinants of an individual's cancer susceptibility. Our investigations of host factors involve interspecies studies among laboratory animals and humans, and are multidisciplinary, to include molecular and cellular biology, pathology, epidemiology, and clinical investigation. The In Vitro Carcinogenesis and the Molecular Genetics and Carcinogenesis Sections devote their major efforts to more fundamental and mechanistic studies. The Liver Carcinogenesis Group within the Molecular Carcinogenesis Section focuses on the interactive effects of hepatitis viruses and chemical carcinogens in the molecular pathogenesis of hepatocellular carcinogenesis. The Molecular Epidemiology Section utilizes the scientific findings, techniques, and concepts developed by our two other sections and by the scientific community at large in selected and more applied studies of molecular genetics, carcinogenesis, and cancer prevention.

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Biography: *Dr. Harris received his M.D. from Kansas University School of Medicine. He completed his clinical training in internal medicine at the University of California–Los Angeles, and at the NCI. He has held positions of increasing responsibility at the NCI, and is also a clinical professor of medicine and oncology at Georgetown University School of Medicine. Dr. Harris has a wide range of scientific interests and accomplishments spanning molecular genetics of human cancer to molecular epidemiology of human cancer risk.*

Laboratory of Human Carcinogenesis
Tumor Suppressor Genes in Human Carcinogenesis

Keywords:
apoptosis
cell cycle
hepatitis viruses
molecular epidemiology
tumor suppressor gene

Research: Carcinogenesis is a multistage process involving activation of proto-oncogenes, e.g., K-ras, and inactivation of tumor suppressor genes, e.g., p53 and $p16^{INK4}$. Analysis of the p53 mutational spectrum is providing clues to the etiology of cancer and the function of specific regions of p53. Mutational hot spots at CpG dinucleotides in codons 175, 245, 248, 273, and 282 may reflect endogenous mutagenic mechanisms, e.g., deamination of 5-methylcytosine to thymidine. Oxyradicals, including nitric oxide, may enhance the rate of deamination and also produce DNA strand breaks that lead to the accumulation of p53, which then transrepresses nitric oxide synthase-2 (NOS2) in a negative feedback loop. Increased expression of NOS2 is associated with C to T transitions at CpG sites in the p53 gene during colon carcinogenesis. G to T transversions are frequently observed in lung, breast, esophagus, and liver cancers, and are more likely to be due to bulky carcinogen-DNA adducts. For example, G to T transversions are more common in lung cancers from smokers when compared with those who never smoked, are more common in lung cancers from women smokers then men, and are frequently observed in codon 249 in liver cancers associated with aflatoxin B_1 exposure. Other associations between p53 mutational spectra and carcinogen exposure include: skin carcinoma caused by ultraviolet light and the occurrence of mutations at dipyrimidine sites, including CC to TT transitions; and hepatic angiosarcoma induced by occupational exposure to vinyl chloride and a high frequency of A:T to T:A mutations. These differences in mutational frequency and spectrum among human cancer types indicate: (1) the etiological contributions of both exogenous and endogenous factors to human carcinogenesis; (2) specific proliferative effects conferred by different mutant p53 genes in different human cell types; and (3) hypotheses for investigation.

P53 has multiple functions including cell cycle control in response to DNA damage, apoptosis, neurological development, and DNA repair. The high frequency of p53 mutations in its nontranscribed DNA strand is a reflection of strand-specific repair in that the transcribed strand is more rapidly repaired than the nontranscribed (DNA coding) strand. P53 can also modulate DNA repair by protein-protein interactions with DNA helicases,

XPB, XPD, and in the transcription-repair complex, TFIIH. P53 reduces the helicase activities of the TFIIH in vitro without affecting its transcription or ATPase activities. Human cells with p53 mutations—e.g., cells from donors with the Li-Fraumeni syndrome—have a deficiency in the repair of the nontranscribed DNA strand of active genes. GADD45, which is regulated by p53, has also been demonstrated to be a G2–M cell cycle checkpoint gene in human and murine cells. A dose-dependent relationship between dietary aflatoxin B_1 intake and codon 249^{ser} p53 mutations is observed in hepatocellular carcinoma. Inactivation of p53 tumor suppressor gene functions including DNA repair and apoptosis also may be a consequence of cellular protein-hepatitis B virus (HBV) oncoprotein complex formation. The HBVX protein binds to p53 and inhibits its sequence-specific DNA binding as well as its transcriptional and apoptotic activities. p53 may induce apoptosis by two interactive transcriptional transactivator-independent and -dependent pathways. Because many of the effective anticancer therapies utilize p53-dependent apoptosis, our recent finding that p53 transmits an apoptotic signal via XPB and XPD DNA helicases in the TFIIH complex suggests new molecular targets of cancer therapy. Recent studies indicate that p53 also modulates the function of DNA helicases involved in other cancer-prone diseases, i.e., Werner and Bloom syndromes.

Recent Publications:

Hussain SP, et al. *Proc Natl Acad Sci USA* 2000;97:12770–5.

Bennett WP, et al. *J Natl Cancer Inst* 2000;92:760–1.

Spillare EA, et al. *Genes Dev* 1999;13:1355–60.

Wang XW, et al. *J Biol Chem* 2001; in press.

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Biography: *Dr. Huang obtained his medical degree from Shanghai Medical University School of Medicine and his Ph.D. in cell and developmental biology from Rutgers University in 1994. He received his postdoctoral training at the Brigham and Women's Hospital–Harvard Medical School with Dr. H. Franklin Bunn, where he began his studies on the molecular mechanisms of hypoxic response. He was an instructor in medicine at Harvard Medical School before joining the NCI.*

Laboratory of Human Carcinogenesis
Molecular Mechanisms of Hypoxic Response

Keywords:
angiogenesis
carcinogenesis
hypoxia
tumor suppressor gene
ubiquitination
wound repair

Research: Hypoxic adaptation is of fundamental importance in developmental, physiological, and pathological processes including neural and cardiac development, hematopoiesis, angiogenesis, and tumorigenesis. Organisms respond to hypoxia in part by transcriptional upregulation of a group of physiologically relevant genes such as those encoding erythropoietin, vascular endothelial growth factor, and glycolytic isoenzymes. Remarkably, induction of most of the hypoxia responsive genes appears to rely on the activation of a crucial transcription factor, hypoxia-inducible factor 1 (HIF–1). HIF–1 was identified as a result of specific binding to a 3′ enhancer of human erythropoietin gene. Although the erythropoietin gene is tissue-specifically expressed, HIF–1 activity is detected in a wide variety of cells, suggesting a general role for HIF–1 in transcriptional induction of hypoxic responsive genes. HIF–1 is composed of α and β/ARNT subunits, both of which belong to the basic helix-loop-helix PAS family of transcription factors. Either Hif1α or Arnt null mutant mouse embryos exhibited developmental arrest and lethality, resulting essentially from impaired vascularization. Moreover, HIF–1α is also involved in cell cycle control as indicated by its stabilization of p53 protein under hypoxia and by its pro-apoptotic effects in tumorigenesis.

HIF–1 activity is determined primarily by its alpha subunit (HIF–1α), resulting from hypoxia-induced posttranslational stabilization, nuclear translocation, and transcriptional activation. HIF–1α is oxygen sensitive and rapidly degraded via the ubiquitin-proteasomal pathway. The proteolytic pathway appears to be controlled by an oxygen-dependent degradation (ODD) domain within HIF–1α and involves tumor suppressor genes such as the von Hippel-Lindau protein and p53 as E3 ubiquitin ligases.

The transcription coactivator p300/CBP is required for HIF–1-mediated transcription, at least in part, by binding of its C/H1 domain to the C terminal activation domain (CAD) of HIF–1α. We have recently developed a new approach named "RAMSY" (random mutagenesis screen in yeast) to efficiently identify and unravel the molecular basis of the protein-protein interaction.

Our research focuses on the molecular mechanism underlying HIF–1 activation, which in turn sheds light on the fundamental processes involved in angiogenesis and tumorigenesis.

Recent Publications:

Huang LE, et al. *J Biol Chem* 1996;271:32253–9.

Huang LE, et al. *Proc Natl Acad Sci USA* 1998;95:7987–92.

Ohh M, et al. *Nat Cell Biol* 2000;2:423–7.

Gu J, et al. *J Biol Chem* 2001;276:3550–4.

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Biography: *Dr. Wang obtained his undergraduate degree from Shanghai Medical University in 1982, his M.S. in cancer pharmacology from the Chinese Academia of Science in 1985, and his Ph.D. in environmental oncology from New York University in 1991. He completed postdoctoral training at the Roche Institute of Molecular Biology and at the NCI.*

Laboratory of Human Carcinogenesis
Human Liver Carcinogenesis

Keywords:
cell cycle checkpoints
Crm1
Gadd 45
genomic instability
hepatitis viruses
hepatocarcinogenesis
nucleocytoplasmic transport
oncogenes
Ran GTPase
tumor suppressor genes

Research: Hepatocellular carcinoma (HCC) is one of the most prevalent malignant diseases worldwide with an extremely high mortality rate. Despite the efforts of epidemiological and basic research studies, little is known about the mechanism(s) of liver carcinogenesis. Little progress has been made toward the discovery of efficient therapies, largely due to the lack of early diagnostic methods and the lack of information on the phenotypic changes as well as the changes in gene expression during the development of HCC. The major causative factors are hepatitis B (HBV) and C (HCV) viruses, that account for more than 80 percent of HCC cases worldwide. In the last year, our group has focused our interest in liver carcinogenesis by initiating new studies emphasizing the mechanism of hepatitis virus-mediated liver carcinogenesis and on the gene expression profile in HCC. We have identified a new function associated both with the X protein (HBx) of HBV and core protein (HC-core) of HCV. We found that both the HBx and the HC-core proteins contain functional nuclear export signals (NES) that utilize cellular Crm1-mediated nuclear export pathways, a feature shared by many viral and cellular oncoproteins. Our working hypothesis is that HBx or HC-core induce neoplastic transformation by a persistent disruption of the Crm1-mediated nuclear export process. We are currently examining their physical and functional interactions with Crm1 and its cofactors including RanGTPase, RanGAP1, and SUMO–1. We also

are examining if HBx- or HC-core-mediated activation of the NF–B and NF–AT signaling cascades, a common pleiotropic effect associated with these viral proteins, is the result of dysregulation of nuclear export. The major cellular activities associated with HBx and HC-core are their abilities to transactivate cellular genes. Currently, we are examining gene expression profiles in human primary hepatocytes expressing either HBx or HC-core by the method of Serial Analysis of Gene Expression (SAGE), as well as the NCI Human OncoChip Genes microarray. The plan is to identify a potential group of genes whose expressions are specifically and abnormally regulated by either HBx or HC-core in primary hepatocytes derived from healthy donors. The revealed expression changes in the genes of interest are used to compare with the gene expression profile from either HBx-positive or HCV-positive HCC.

Recent Publications:

Yang Q, et al. *J Biol Chem* 2000;275:36892–8.

Liu J, et al. *Cell* 2001;104:353–63.

Forgues M, et al. *J Biol Chem* 2001;276:22797–803.

Wu CG, et al. *Oncogene* 2001; in press.

Laboratory of Immune Cell Biology

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The Laboratory of Immune Cell Biology is composed of independent laboratories with a common interest in the biology of the immune system at the molecular and cellular levels.

Dr. Ashwell's laboratory focuses on the mechanisms and consequences of apoptosis (regulated cell death). Among the major approaches being taken to understand this important biological phenomenon are (1) characterization of the ubiquitin protein ligase (E3) activity of the IAP (Inhibitor of **AP**optosis) family of molecules, (2) determination of the role of IAPs in signaling via TNF receptors, and (3) studies of the mechanisms of glucocorticoid-induced killing and immunosuppression. Other studies are aimed at understanding the molecular mechanisms underlying antigen-specific thymocyte selection.

Dr. Bosselut's group investigates how T cell receptor (TCR) engagement promotes survival and differentiation of T lymphocyte precursors during their intrathymic development (a process known as positive selection). Previous studies have examined how signaling by CD4 and CD8 molecules impacts TCR-induced differentiation events during positive selection. Current projects aim at identifying intracellular pathways, and their target genes, that transduce survival and differentiation signals during positive selection. Ongoing approaches make use of models generated in the laboratory, in which premature interruption of TCR signals blocks thymocyte development at intermediate steps of the selection process.

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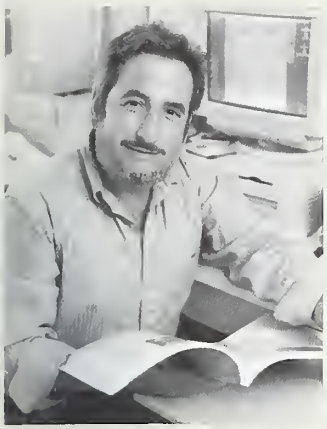

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Biography: *Dr. Ashwell received his M.D. from Columbia University College of Physicians and Surgeons. He trained in internal medicine at Presbyterian Hospital in New York City. Following a postdoctoral fellowship in immunology in the laboratory of Dr. Ronald Schwartz (National Institute of Allergy and Infectious Diseases/NIH), Dr. Ashwell joined the NCI as a principal investigator. He was named chief of the Laboratory of Immune Cell Biology in 1992.*

Laboratory of Immune Cell Biology
Physiologic and Pathologic Regulation of Apoptosis

Keywords:
apoptosis
immunology
signal transduction
T cell

Research: Apoptosis, or physiologic programmed cell death, is a means of eliminating unwanted cells. Many cells, including immature and mature lymphocytes, undergo apoptosis under the appropriate conditions. Inappropriate apoptosis has clearly been shown to result in both tumor formation and autoimmunity (lack of appropriate cell death) and has been implicated in the loss of CD4+ cells in AIDS (death of functionally useful cells). We are examining the mechanisms and biological relevance of apoptosis in a number of settings.

Glucocorticoids and Antigen-Specific Thymocyte Selection
We found that glucocorticoids and T cell receptor (TCR)-mediated activation, each of which induces apoptosis of T cell hybridomas, are mutually antagonistic, and proposed that crosstalk between these independent receptor/ligand systems may determine the outcome of thymocyte selection. Consistent with this model, we have found that steroids are synthesized in the thymus itself. Pharmacologic inhibition of corticosterone production synergistically enhances the ability of a relatively weak deletional stimulus to kill thymocytes, and inhibition of local corticosterone production results in the antigen-specific deletion of thymocytes that are normally positively selected. Using MHC-congenic mice, we have found that the effects of inhibiting local steroid production are absolutely dependent upon the number of different MHC-encoded molecules. We have also created transgenic mice in which glucocorticoid receptor (GR) expression is specifically decreased in immature thymocytes. Thymic development is severely impaired in these animals, and there is evidence for a steroid requirement at two different points of development: the CD4–CD8– to CD4+CD8+ transition, and the progression of CD4+CD8+ cells to mature T cells (positive selection). Mice bearing this transgene are specifically unresponsive to a subset of antigens because the T cells that normally respond were deleted in the thymus (i.e., positive selection was converted to negative selection). Thus, locally-produced steroids are critical for normal T cell development and, more importantly, the generation of the antigen-specific T cell repertoire.

Molecules Involved in Apoptosis

T cell hybridomas and activated peripheral T cells undergo apoptosis when stimulated via the TCR. We have shown that activation induces expression of both Fas, a transmembrane molecule whose cross linking induces apoptosis, and its ligand, FasL, and that cell death is actually caused by the interaction of these two proteins. Furthermore, both retinoic acid and glucocorticoids, which prevent activation-induced T cell death, do so by preventing the upregulation of FasL. We have identified a critical regulatory region in the 5′ FasL enhancer termed the "FLRE." This site specifically binds members of the Egr family of transcription factors. Interestingly, although all bind with roughly equal affinity, Egr-2 and Egr-3 but not Egr-1 drive FasL expression. These factors are elevated in lpr and gld T cells, accounting for the high levels of FasL expressed in these cells. We are also studying the role of the IPA antiapoptotic molecules in physiologic cell death. We have recently found that these proteins undergo proteasome-mediated degradation prior to the death of glucocorticoid- or etoposide-treated thymocytes. The regulation of this event is currently under study.

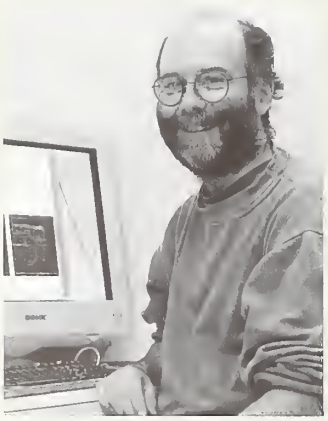
Recent Publications:

D'oro U, et al. *J Immunol* 1999;162:1879–83.

Vacchio MS, et al. *J Immunol* 1999;163:1327–33.

Lu FWM, et al. *Immunity* 2000;12:183–92.

Yang Y, et al. *Science* 2000;288:874–7.



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Biography: *Dr. Rémy Bosselut trained at the Institut Curie in Paris, France. He earned his M.D. degree in 1992 from the Xavier Bichat School of Medicine and his Ph.D. degree in 1993 from the University Denis Diderot, both in Paris. Dr. Bosselut obtained postdoctoral training at the NCI Experimental Immunology Branch and joined the Laboratory of Immune Cell Biology in February 2000.*

Laboratory of Immune Cell Biology
Signal Transduction During T Lymphocyte Differentiation

Keywords:
CD4+ and CD8+ T lymphocytes
lymphocyte
T cell activation
T cell development
T cell receptor

Research: The main research interests of this group reside in elucidating signals that mediate T lymphocyte (T cell) differentiation and survival. A variety of studies have demonstrated that T cell survival and activation are controlled through engagement of the clonotypic T cell receptor (TCR) by peptide antigens bound to major histocompatibility complex (MHC) molecules. On most T cells, TCR recognition of MHC/peptide complexes is promoted by CD4 or CD8 coreceptors, two surface glycoproteins that bind to constant regions of MHC molecules. While studies with recombinant

mice have clearly established that CD4 and CD8 are important for T cell differentiation and function, the actual signaling function(s) of the coreceptors remain poorly understood; we have addressed this issue using a combination of biochemical and genetic approaches. We first reinvestigated the role of CD4 and CD8 in TCR signal transduction. We made the unexpected observation that CD4 and CD8 coreceptors associate with LAT, an adapter protein whose tyrosine phosphorylation by TCR-associated Zap70 kinase molecules is critical for TCR signal transduction. We showed that coengagement of TCR with CD4/CD8 coreceptors induces LAT phosphorylation and results in the specific recruitment of downstream signaling intermediates to coreceptor-associated LAT molecules. These observations point to a new function for CD4 and CD8 in TCR signal transduction—namely, that TCR/coreceptor coengagement by MHC/peptide complexes would juxtapose coreceptor-associated LAT with TCR complexes, thereby promoting LAT phosphorylation by TCR-associated Zap70 molecules and enhancing TCR signal transduction. In addition to these studies, we are currently examining the role of signals transduced by CD8 and CD4 during T cell differentiation in the thymus.

We also addressed in more detail the function of CD8 coreceptor molecules. CD8 molecules are made of two polypeptides expressed from two distinct genes, CD8α and CD8β. While gene knock-out experiments have demonstrated that CD8β is required for the generation of most CD8+ T cells, the molecular bases of CD8βα function were unknown. We have addressed this issue and demonstrated that the extracellular domain of CD8β promotes CD8 binding to MHC–I molecules and that the intracellular domain of CD8β promotes CD8 binding to the tyrosine kinase Lck and to LAT. By generating recombinant mice expressing CD8 complexes selectively lacking the extracellular or the intracellular domain of CD8β, we have shown that each CD8β domain independently contributes to CD8 coreceptor function during T cell differentiation, and that both domains are required for the generation of normal numbers of CD8+ T cells.

Ongoing studies are directed at understanding how signals resulting from TCR engagement can either promote T cell survival or proliferation or, to the contrary, induce T cell death. Selection events during intrathymic T cell development provide a dramatic illustration of this dichotomy, as TCR engagement on immature thymocytes can lead to diametrically opposed outcomes. Thymocytes whose TCR binds with high avidity to MHC/peptide complexes are actively deleted by TCR-induced cell death. In contrast, thymocytes whose TCR binds with intermediate avidity to MHC/peptide complexes survive and differentiate into mature T cells (the vast majority of thymocytes, whose TCR avidity for MHC/peptide complexes is too low to result in efficient TCR engagement, die by "neglect"). Our goal is to understand the differences between the signaling cascades that mediate these opposite outcomes of TCR engagement, and to analyze the gene expression patterns they induce. An important obstacle faced by previous studies addressing this issue has been to obtain in vivo signaled homogenous cell populations large enough to perform signal transduction or gene expression analyses. To overcome such difficulties, we are planning to address these questions by generating recombinant mouse lines in which TCR signaling is altered at defined stages of T cell differentiation or activation.

Recent Publications:

Singer A, et al. *Semin Immunol* 1999;11:273–81.

Bosselut R, et al. *J Exp Med* 1999;190:1517–26.

Bosselut R, et al. *Immunity* 2000;12:409–18.

Laboratory of Immunobiology

The Laboratory of Immunobiology contains three sections. In order to identify genes involved in the pathogenesis of renal cancers, the Cellular Immunity Section studies families with inherited predispositions to develop these carcinomas. Some 600 members of kidney cancer families have been brought to the Clinical Center at the NIH for clinical and molecular genetic evaluation. Neoplastic manifestations of the disease are correlated with the results of genetic analysis performed in the laboratory. The work includes identification of families with kidney cancer, cloning tumor suppressor genes, mutation analysis, and biochemical studies of the proteins encoded by the tumor suppressor genes.

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The Cancer Causing Genes Section identifies, clones, and characterizes tumor suppressor genes located on chromosomes 3p and 8p that are involved in the origin or development of carcinomas of the lung, breast, and prostate.

The Immunopathology Section studies proteins that mediate the host response to infection or injury. A major interest is in protein chemoattractants that recruit specific types of leukocytes to inflammatory foci. This has led to isolation and cloning of interleukin 8 (IL–8), which attracts neutrophils, and monocyte chemoattractant protein-1 (MCP–1), which attracts monocytes. An additional discovery is macrophage stimulating protein (MSP), which acts not only on macrophages but also on keratinocytes and cells of the hematopoietic system. Current studies of these proteins focus on stimuli for their production or activation, and downstream events following ligand-receptor interaction.

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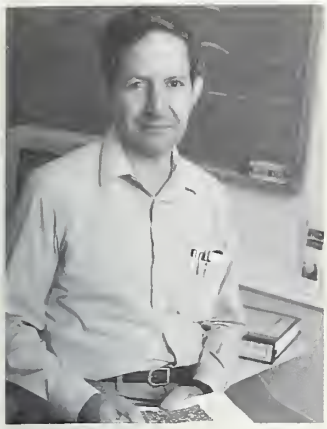

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Biography: *Dr. Zbar was trained in medicine at the Downstate Medical Center, State University of New York. He received postgraduate training in internal medicine and oncology at the Utah School of Medicine. He came to the National Cancer Institute in 1965.*

Laboratory of Immunobiology
Genetic Studies of Inherited Renal Cancers: VHL Disease and Hereditary Papillary Renal Carcinoma

Keywords:
Birt-Hogg-Dube syndrome
genodermatoses
hereditary kidney cancer
hereditary papillary renal carcinoma
MET proto-oncogene
renal carcinoma
von Hippel-Lindau disease

Research: Renal carcinoma affects some 27,000 individuals in the United States each year. Until recently, relatively little attention was paid to the genetics and histology of renal carcinomas. Improved understanding of the genetic basis of human renal carcinoma has come from studies of families with an inherited predisposition to develop this disease. We have studied the genetic basis of three of its inherited forms: von Hippel-Lindau disease (VHL), hereditary papillary renal carcinoma (HPRC), and renal carcinoma associated with the chromosome 3;8 translocation. VHL is an inherited multisystem neoplastic disorder characterized by a predisposition to develop tumors in the eye, brain, spinal cord, pancreas, adrenal gland, and kidney. Kidney cancer in VHL is the clear-cell type. HPRC is characterized by a predisposition to develop multiple tumors in both kidneys; this type of renal carcinoma is characterized by a papillary growth pattern. In the family with the 3;8 translocation, individuals who inherit a balanced translocation between chromosomes 3 and 8 have a greatly increased risk of developing clear-cell renal carcinoma. We used positional cloning strategies to isolate the VHL gene. The gene was shown to be mutated in the germline of patients affected with VHL, in sporadic clear-cell renal carcinomas, and in renal carcinomas from patients with the 3;8 translocation. Currently, VHL research in our laboratory is concentrated on biochemical studies to the VHL protein, which has been shown to regulate the rate of transcription mediated by RNA polymerase II. The VHL protein regulates transcription by binding to elongins B and C, subunits of a protein that regulate the rate of elongation. Two binding domains of the VHL protein have been defined, and one binding domain has been shown to be compromised by germline missense mutations. We have demonstrated that the MET proto-oncogene is the HPRC gene. Missense mutations in the tyrosine kinase domain of MET were found in affected members of HPRC families. In vitro experiments demonstrated that the missense mutations produced constitutive activation of the MET kinase. Current studies aim to identify the signaling pathways triggered by mutant MET genes. We have identified five families with multiple members affected with renal oncocytomas. These families provide a foundation for studies aimed at defining genes involved in the pathogenesis of renal oncocytoma.

Recent Publications:

Schmidt L, et al. *Cancer Res* 1998;58:1719–22.

Schmidt L, et al. *Nat Genet* 1997;16:68–73.

Jeffers M, et al. *Proc Natl Acad Sci USA* 1997;94:11445–50.

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Biography: *Dr. Steven Hou received his Ph.D. from the University of Chicago in 1994. He completed postdoctoral training with Dr. Norbert Perrimon at Harvard Medical School, and was offered a tenure track fellowship in the Laboratory of Immunobiology in September 1997.*

Laboratory of Immunobiology
Signal Transduction in *Drosophila* Embryogenesis and Tumor Development

Keywords:

Drosophila genetics
epithelial cell sheet movement
JAK
JNK
Jun
melanotic tumor
oncogene
segmentation
sex determination
signal transduction
STAT
TGFß
tracheal formation

Research: Cells in a developing embryo and in normal adult tissues send signals to each other to coordinate the differentiation, proliferation, and movement of cells. Not only do the signals have to be correct; they also must be perfectly coordinated. Otherwise, disasters like cancer can result. To explore the mechanisms of cell communication in a multicellular organism, we have turned to the fruit fly, *Drosophila melanogaster*. As a model system, *Drosophila* offers both complex patterns of the mammals and powerful methods for genetic analysis. More importantly, the signal transduction mechanisms between the fly and the mammals are remarkably similar.

Our group is currently focusing on two key signal transduction pathways: the *Drosophila* JNK/JUN and JAK/STAT signal transduction pathways. The *Drosophila* JNK/JUN pathway regulates epithelial cells sheet movement during embryogenesis and imaginal disc formation. Studies on the JNK/JUN-regulated cell movement may shed light on cell migration and tumor metastasis in mammals. The *Drosophila* JAK/STAT pathway regulates cell differentiation and cell fate determination in the developing fly. Hyperactivation of this pathway leads to melanotic tumor formation in larvae and the adult fly, due to cell fate misdetermination in the imaginal discs. The JAK/STAT system provides an excellent genetic model to study cell fate determination in development and tumor formation.

Critical to our understanding the signal transduction mechanisms is the identification of all of the components that are involved in signal transmission. To achieve that goal, we have performed a P element-mediated gene disruption screen, examining over 50,000 mutant lines to identify genes that

are essential to fly life. We recovered more than 2,500 such mutants. Using a PCR-based sequencing protocol, we have sequenced the genomic DNA of all P insertion points. Taking advantage of the recently completed *Drosophila* genome sequence, we have identified the disrupted genes for most of the mutants. We are now doing a germline clone assay of the interesting genes to identify genes that function in the JNK/JUN and JAK/STAT signal transduction pathways. So far, we have identified several new genes in the two signal transduction pathways. In addition, we also identified a number of genes that function in other signal transduction pathways and biological processes; they will be distributed to interested laboratories for in-depth studies.

In the JNK/JUN pathway, we have characterized a multidomain protein in detail. It functions as a scaffold or hub molecule that brings kinases and transcription factors together for efficient signaling. A manuscript on this work has been submitted.

In the JAK/STAT pathway, we are focusing on three most interesting genes. Two of them are cell surface molecules that may identify the missing receptors of the signal transduction pathway. The third one is a molecule that connects the pathway to cell cycle regulation. These results will be published soon.

In the coming year, we will continue to examine in detail the functions of several of these genes. Meanwhile, in our P element screen we isolated several genes that are the fly homologs of human disease genes. We will carefully examine the disease-related genes' functions in the *Drosophila* model system.

Recent Publications:

Hou XS, et al. *Cell* 1996;84:411–9.

Hou XS, et al. *Trends Genet* 1997;13:105–10.

Hou XS, et al. *Genes Dev* 1997;11:1728–37.

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Biography: *Dr. Lerman obtained his M.D. from the First Moscow Medical School, U.S.S.R., and his Ph.D. and D.Sci. from the Academy of Medical Sciences, U.S.S.R. At the NIH, he studied repetitive elements with Maxine Singer and cloned the ß-amyloid gene with D. Carleton Gajdusek.*

Laboratory of Immunobiology
Cloning Tumor Suppressor Genes (TSG) from Human Chromosome 3p

Keywords:
carcinogenesis
hereditary cancer
lung cancer
tumor suppressor

Research: This research takes stock in cloning and analysis of TSG located on chromosome 3p. Cytogenetic, allelotyping, and genetic linkage studies have implicated defined regions of chromosome 3p as harboring TSG involved in the causation or development of several common and hereditary cancers, notably carcinomas of the lung, breast, ovary, cervix, testes, head and neck, colon (NPCC), and kidney (VHL). More recently, the discovery of homozygous deletions on 3p21.3, and 3p12 in lung, cervix, and breast cancers, marked more precisely the physical location of the respective genes and provided an access and means to identify them. In 1993, we reported the identification of the VHL TSG and demonstrated its causative role in sporadic renal carcinoma (RCC) of the clear-cell type. Our current efforts are directed towards: (1) further characterization of the VHL gene and its product, pVHL, and (2) the molecular cloning and analysis of TSGs involved in the causation and/or development of lung cancer at 3p21.3 and 3p12.

VHL TSG (3p25)

To analyze the function(s) of the VHL gene and its carcinogenic pathway(s), we obtained the entire genomic sequence of the gene including the promoter, introns, and flanks, and constructed a set of VHL minigenes (wild-type and mutant) and a complete intronless VHL gene driven by the VHL promoter. We set out to discover target genes controlled by pVHL. The differential display technology was employed to discover these genes using the UMRC6 and 786–0 cells stably transfected with wt and mutant VHL minigenes. As of June 2001, six downregulated genes were identified—namely, NOTCH2 and DEC1—that specify cell fate determination and may have oncogenic potential, two transmembrane type carbonic anhydrases, CA9 and CA12, and two new unknown genes. The CA9 and CA12 genes are overexpressed in many tumor types and could control the extracellular pH of the milieu surrounding the cells and thus create a microenvironment conducive to tumor growth and spread. Analysis of the methylation of the VHL promoter in renal carcinoma cells carrying a methylated VHL endogene by monochromosome gene transfer, cell fusion, and VHL gene transfections showed that the meth+ phenotype is dominant in the UOK 21 cells, probably resulting from changes in cis-acting elements of the VHL locus. The future

work will be focused on (1) the role of carbonic anhydrases (CAs) in the regulation of tumor pH and its impact on cancer growth, (2) the effect of pVHL and DEC1 on the cell cycle, (3) the nature of the cis-acting elements in the VHL locus involved in de novo aberrant methylation, and (4) development of new treatment modalities for cancer (CA9/CA12 cDNA-based vaccines and new inhibitors).

Lung Cancer TSGs

Previously, we identified and defined by overlapping homozygous deletions two small regions on chromosome 3p, located in 3p21.3 and 3p12, to contain TSGs that cause all major forms of lung cancer. By allelotyping these genes, we found that they were also implicated in the origin or development of breast, cervix, ovary, kidney, prostate, and head and neck cancers.

The 3p21.3 TSG

We used overlapping homozygous deletions, contig building, genomic sequencing, and physical and transcript mapping to define a ~600 kb tumor suppressor region harboring a multiple tumor suppressor gene(s) on chromosome 3p21.3. This location was identified through somatic genetic mapping in tumors, cancer cell lines, and premalignant lesions of the lung and breast. The combination of molecular manual methods and computational predictions permitted us to detect, isolate, characterize, and annotate a set of 23 genes which likely constitute the complete set of protein-coding genes residing in this ~600 kb sequence. A subset of 19 genes found in the deletions' overlap of ~370 kb were subdivided by a nesting deletion into 2 gene sets: 8 genes lying in the proximal ~120 kb segment and 11 genes lying in the distal ~250 kb segment. Both gene sets were analyzed extensively by manual and computational methods. Five of the 19 genes showed loss-of-expression or reduced mRNA levels in small cell (SEMA V and RASSF1) or nonsmall cell (a2d-2) or both (BLU RASSF1 and LUCA1) cancer cell lines. None of the eight genes showed a frequent (>10 percent) mutation rate in lung cancer samples, leading us to conclude that, with the exception of the three genes with reduced expression in this set, they should be excluded as classical tumor suppressors in sporadic lung cancer. The mutation analysis of the 11-gene set is not yet completed and may reveal a classical TSG with homozygotic inactivation in tumors at this location. Further mutational analysis in breast tumors and functional testing of the eight critical genes by gene transfer and gene disruption strategies is ongoing.

The 3p12 TSG

We covered the ~8 MB U2020 homozygous deletion which we mapped to 3p12 by a gapped overlapping YAC contig and narrowed the gene region by overlapping and nesting deletions. We discovered and characterized a new candidate gene that is a member of the cell adhesion molecule superfamily (CAMs) and showed homology to the TSG DCC. Two homozygous deletions (~230 kb) removing several exons of this gene were discovered. A sequenced BAC clone covering the deletions was constructed to identify the residing gene(s). Future work will focus on the molecular and functional analyses of the emerging putative lung and breast cancer gene(s).

Collaborators on this research are Bruce Johnson, Harvard Medical School; George Klein and Eugene Zabarovsky, Karolinska Institute, Stockholm, Sweden; A.D. Miller, Fred Hutchinson Cancer Research Center, Seattle; John Minna, University of Texas; Svetlana Pack, NIH; Pamela Rabbitts, Medical Research Council, Cambridge, UK; and Eric Stanbridge, University of California–Irvine.

Recent Publications:

Barbee DG, et al. *J Natl Cancer Inst* 2001;93:691–9.

Dreijerink K, et al. *Proc Natl Acad Sci USA* 2001;98:7504–9.

Lerman MI, et al. *Cancer Res* 2000;60:6116–33.

Rai SK, et al. *Proc Natl Acad Sci USA* 2001;98:4443–8.

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The interests of the Macromolecular Crystallography Laboratory (MCL) cover a wide range of systems and techniques relevant to macromolecular crystallography and its applications. Under the direction of Dr. Alexander Wlodawer, the Protein Structure Section has been investigating retroviral protease and integrase; proteins used as anticancer drugs, such as asparaginase and Onconase; a variety of other proteases and ribonucleases; and a number of cytokines and cytokine-receptor complexes. The principal interest of Dr. Xinhua Ji's Biomolecular Structure Section is the structure and function of biomolecular systems with anticancer and antimicrobial significance and the feasibility of structure-based drug design targeting these biomolecules. Systems under study include detoxification enzymes, retinoic acid-binding proteins, mono- and pyrophosphoryl transferases, and cell cycle regulatory proteins. Dr. Jacek Lubkowski's Macromolecular Assembly Structure and Cell Signaling Section is investigating the structural basis of antiviral and antibacterial activity of various proteins involved in intercellular signaling. Current studies involving a wide range of chemokines, defensins, and their analogs will soon be extended to chemokine receptors. The focus of the Protein Engineering Section, headed by Dr. David Waugh, is the expression and purification of proteins studied in the MCL, original studies on protein expression, and structural genomics of the virulence factors of *Yersinia pestis*. The Synchrotron Radiation Research Section, based at the National Synchrotron Light Source at Brookhaven National Laboratory and headed by Dr. Zbigniew Dauter, is involved in developing new methods for phasing macromolecular structures by anomalous scattering, particularly with signals from comparatively light atoms such as bromine. This section has also been involved in collaborative efforts with a number of groups inside and outside the MCL in extending the resolution of crystal structures to atomic levels.

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Biography: *Dr. Wlodawer received his Ph.D. from the University of California–Los Angeles in 1974. Having completed his postdoctoral training at Stanford University, he joined the National Bureau of Standards in 1976, then moved to the ABL–Basic Research Program at the NCI–Frederick in 1987 as director of the newly formed Crystallography. From October 1998 to March 1999, he was on sabbatical as an elected visiting fellow of Sidney Sussex College, University of Cambridge. In 1999, Dr. Wlodawer became chief of the Macromolecular Crystallography Laboratory, CCR, NCI. He is a member of the American Crystallographic Association and the Protein Society and has been an elected officer in both organizations.*

Macromolecular Crystallography Laboratory
Protein Structure

Research: We are studying the relationship between protein structure and function, using the technique of high-resolution x-ray diffraction. In the past year, our work has been concentrated in four distinct areas.

Enzymes with Anticancer Properties

We have been investigating crystal structures of several members of the family of L-asparaginases, some of which are used clinically as drugs directed against childhood lymphoblastic leukemia. While the mechanism of anti-cancer activity of these enzymes is not yet clear, we have concentrated on the studies of their enzymatic properties. We investigated a number of mutants of *Escherichia coli* L-asparaginase and many complexes of the *Erwinia chrysanthemi* enzyme with substrates and products, leading to the elucidation of the enzymatic mechanism. The structure of the latter enzyme, solved at 1 Å resolution, represents the largest protein investigated at atomic level. Another enzyme with potential therapeutic properties is Onconase, a cytotoxic ribonuclease isolated from frog eggs. We have been involved in reengineering this enzyme in order to make it applicable to human cancer therapy and to restore its activity in the absence of posttranslational modifications. The structure of the eosinophil-derived neurotoxin (EDN), another related antitumor ribonuclease, has also been recently solved by us at atomic resolution.

Cytokines and Cytokine Receptors

Our section has been investigating the crystal structures of several cytokines and has made progress in preparing their receptor complexes. We have established that a helical cytokine, interleukin 10 (IL–10), is a domain-swapped dimer in which each compact half is composed of fragments of two identical molecules. The structure of a related cytokine encoded in the genome of Epstein-Barr virus has now been determined, providing the first glimpse of the molecular architecture of an agent used by the virus to control the host's immune system. We have purified and crystallized complexes of

IL–10 with its specific receptor and are studying complexes of several other cytokines related to IL–10.

Retroviral Enzymes

Enzymes encoded by retroviruses such as HIV are prime targets for designing effective drug therapies. We have been studying the structure of native and drug-resistant HIV–1 protease (PR) complexed with inhibitors with the aim of tracing the molecular basis of the resistance phenomenon. We have also determined the structures of related enzymes from feline immunodeficiency virus (FIV) and equine infectious anemia virus (EIAV). The latter PRs are poorly inhibited by most inhibitors of HIV–1 PR, including those in clinical use, although they are capable of cleaving HIV–1-derived sequences. To study the mechanism of drug resistance, we solved the structures of HIV–1, FIV, and EIAV PRs complexed with an identical inhibitor, while the studies of an inactive mutant of FIV PR with a substrate helped in delineating the catalytic mechanism. Another retroviral enzyme under investigation in our laboratory is integrase. We have solved the structure of the catalytic domain of avian sarcoma virus integrase in the presence and absence of divalent cations to atomic resolution, and are attempting cocrystallization of complexes with different substrates.

Proteases and RNA-Processing Enzymes

The structures of a number of different proteases are under investigation in our section. In particular, we have discovered that the *Pseudomonas* serine-carboxyl protease (PSCP) is a novel serine protease with a unique catalytic triad. The structures of a number of complexes of this enzyme, many of them solved at atomic resolution, have been helpful in the analysis of the mechanism of action of this family. We have also solved crystal structures of the plant aspartic protease phytepsin, as well as of inhibitor complexes of yeast proteinase A. Among the RNA-processing enzymes, we have solved the structure of RNA cyclase, as well as of native and semireduced cyclic nucleotide phosphodiesterase from *Arabidopsis thaliana*.

Our collaborators include Ben Dunn, University of Florida; John Elder, The Scripps Research Institute; Witold Filipowicz, Friedrich Miescher-Institut, Basel, Switzerland; John Kay, University of Cardiff; Jonathan Leis, Case Western Reserve University; Klaus Röhm, Philips University; and Anna Marie Skalka, Fox Chase Cancer Center.

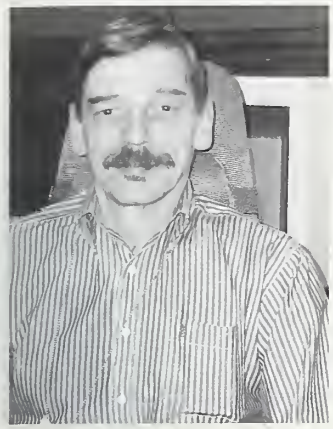
Recent Publications:

Palm GJ, et al. *Structure* 2000;8:13–23.

Li M, et al. *Nat Struct Biol* 2000;7:113–7.

Hofmann A, et al. *EMBO J* 2000;19:6207–17.

Wlodawer A, et al. *Nat Struct Biol* 2001;8:442–6.



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Biography: *Dr. Dauter earned his Ph.D. in crystallography from the Technical University of Gdansk in 1975 under the direction of Professor Z. Kosturkiewicz. From 1975 to 1997, he conducted research in structural crystallography at the University of York, European Molecular Biology Laboratory, and Technical University of Gdansk. He established the Synchrotron Radiation Research Section at Brookhaven National Laboratory (BNL) in late 1997, and joined the Macromolecular Crystallography Laboratory as a section chief in 2000. Based at the National Synchrotron Light Source, BNL, Dr. Dauter is in charge of the NIH operations of beamline X9B, which is currently being used by about a dozen crystallography groups from five institutes of the NIH.*

Macromolecular Crystallography Laboratory
Macromolecular Crystallography Activity at Beamline X9B of the National Synchrotron Light Source

Keywords:
anomalous scattering signal
crystal structure
halide cryosoaking
synchrotron radiation
x-ray crystallography

Research: The aim of the research within this section is to utilize the unique properties of the x-ray radiation generated at synchrotron storage rings—in particular, at beamline X9B of the National Synchrotron Light Source, Brookhaven National Laboratory. The two most important characteristics of synchrotron radiation are the high intensity of the x-ray beam and the tunability of the wavelength; both of these properties are utilized in the research investigations within the section.

In addition to conducting research investigations, the section personnel provides technological and scientific support for NIH researchers collecting diffraction data at beamline X9B of the National Synchrotron Light Source. The NIH Intramural Synchrotron Consortium of macromolecular crystallographers is a part of the Participating Research Team and uses 37.5 percent of the total available time at beamline X9B, which is equivalent to about 25 days per four-month scheduling period. Collecting diffraction data at the synchrotron beamline involves a very high level of technology—but it is a scientific process, not a technicality. Therefore, a proper selection of all the necessary parameters can be done only if based on scientific considerations. The Synchrotron Radiation Research Section supports the users of beamline X9B in all aspects of diffraction data collection.

Recent Publications:

Dauter Z, et al. *Acta Cryst* 2000;D56:232–7.

Dauter Z, et al. *J Mol Biol* 1999;289:83–92.

Li J, et al. *Nat Struct Biol* 2000;7:555–9.

Lubkowski J, et al. *Biochemistry* 1999;38:13512–22.

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Biography: *Dr. Ji received his Ph.D. in chemistry from the University of Oklahoma in 1990. He was a postdoctoral fellow and then a research assistant professor at the Center for Advanced Research in Biotechnology of the University of Maryland Biotechnology Institute and the National Institute of Standards and Technology. He joined the NCI–Frederick in 1995. The primary goals of his section are to address the structure and function of biomolecular systems with anticancer and antimicrobial significance and to explore the feasibility of drug design targeting such biomolecules.*

Macromolecular Crystallography Laboratory
Structural Chemistry of Biomolecular Systems by X-Ray Diffraction

Keywords:

molecular target
structural biology
structure and function
structure-based drug design
x-ray crystallography

Research:

Glutathione S-Transferase: Structure-Based Design of Electrophilic Diazeniumdiolates for Pharmacologic Delivery of Nitric Oxide

Many tumors become drug resistant by overexpressing the detoxification enzyme glutathione S-transferase (GST). Of the three major isoforms, alpha, mu, and pi, pi is the predominant form in cancer cells. We are attempting to design agents that will overcome this drug resistance by generating nitric oxide (NO) selectively in the active site of GST-pi, which could increase the effectiveness of anticancer therapies. Comparison of the active sites and transition state analogs of the three isozymes revealed a potential strategy for achieving isozyme selectivity. Application of this strategy has resulted in a pi-selective NO donor. If planned cytotoxicity studies show that this or subsequent NO donors improve the potency of electrophilic anticancer agents toward GST-pi-overexpressing cells, means of overcoming drug resistance in some clinically important tumor types may be forthcoming.

6-Hydroxymethyl-7,8-Dihydropterin Pyrophosphokinase: Mechanism of Pyrophosphoryl Transfer and Structure-Based Design of Novel Antimicrobial Agents

6-Hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK) is the first enzyme in the folate biosynthetic pathway, catalyzing the transfer of pyrophosphate from ATP to 6-hydroxymethyl-7,8-dihydropterin (HP). Folate cofactors are essential for life. Mammals derive folates from their diets. In contrast, most microorganisms must synthesize folate de novo. Therefore, HPPK is an ideal target for the development of novel antimicrobial agents, which are urgently needed to fight the worldwide crisis of antibiotic resistance. HPPK contains 158 amino acid residues and is thermostable, which makes it an excellent model system for the study of the pyrophosphoryl transfer mechanism, of which little is known. At atomic resolutions (up to 0.89 Å), we have determined the crystal structures of ligand-free enzyme as well as various well-chosen complexes. Our analysis of these

structures has provided essential information on the reaction mechanism of pyrophosphoryl transfer and critical knowledge for the design of novel antimicrobial molecules. Of particular importance is the structure of HPPK•HP•$2Mg^{2+}$•AMPCPP at 1.25-Å resolution, which mimics most closely the ternary complex of the enzyme and reveals the atomic details of the catalytic assembly, and therefore has been the basis of our structure-based inhibitor design effort. We have carried out the design, synthesis, biochemical, and crystallographic studies of three bisubstrate-mimicking analogs, each of which consists of a pterin, an adenosine moiety, and a linker composed of 2–4 phosphoryl groups.

Era Protein: GTPase-Dependent Cell Cycle Regulator

Era is an essential GTPase found in every bacterium sequenced to date. Highly conserved Era homologs are also found in eukaryotes, such as mouse and human. The Era homolog may be a candidate for a tumor suppressor, because it is located in a chromosomal region where loss of heterozygosity is often associated with various types of cancer. In bacteria, Era has a regulatory role in cell cycle control by coupling cell growth rate with cytokinesis. Cell division is signaled when a threshold of Era activity is reached. Artificially reducing the expression or impairing the activity of Era results in bacterial cell cycle arrest at a predivisional two-cell stage. The arrest lasts until Era activity accumulates to the threshold level, allowing another cell cycle to start. Because the synthesis of Era itself is positively correlated with growth rate, the cell division rate is thus coordinately maintained. We have determined the crystal structure of Era from *Escherichia coli* at 2.4 Å, which reveals a two-domain arrangement: an N terminal domain that resembles p21 Ras and a unique C terminal domain that contains an RNA-binding motif. The crystal structure determination of Era in complex with GDP and with a GTP analog is in progress. Our analysis of these structures will provide insight into the conformational changes of the protein during GTP hydrolysis, which may be part of the signaling pathway of this cell cycle regulator.

Our collaborators are Yogesh Awasthi, University of Texas Medical Branch; Donald Court, Larry Keefer, and Christopher Michejda, NIH; Shivendra Singh, University of Pittsburgh Cancer Institute; Honggao Yan, Michigan State University; and Piotr Zimniak, University of Arkansas for Medical Sciences.

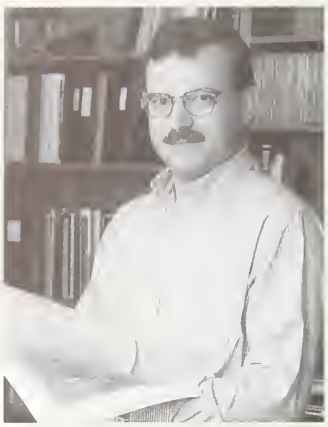
Recent Publications:

Chen X, et al. *Proc Natl Acad Sci USA* 1999;96:8396–401.

Ji X, et al. *Biochemistry* 1999;38:10231–8.

Blaszczyk J, et al. *Structure* 2000;8:1049–58.

Shi G, et al. *J Med Chem* 2001;44:1364–71.



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Biography: *Dr. Lubkowski received his Ph.D. from the University of Gdansk, Poland, in 1991, specializing in physical and theoretical chemistry. Following the defense of his dissertation, he spent 6 months as a visiting scientist in the laboratory of Professor Z. Kosturkiewicz (Adam Mickiewicz University in Poznan, Poland), extending his expertise in small molecule x-ray crystallography. In 1992, as a postdoctoral fellow, Dr. Lubkowski joined the Macromolecular Crystallography Laboratory (MCL) headed by Dr. Alexander Wlodawer. In 1999, he was promoted to staff scientist. In 2001, Dr. Lubkowski became chief of the Macromolecular Assembly and Cell Signaling Section, MCL, CCR, NCI. He is a member of the American Crystallographic Association.*

Macromolecular Crystallography Laboratory
Protein Structure

Keywords:
chemokine receptors
chemokines
defensins
L-asparaginases
x-ray crystallography

Research: Our laboratory is investigating the structural basis of intercellular signaling via chemotaxis and the role of chemokines in the process of viral infection, as well as correlating the structures of defensins with their antimicrobial and chemotactic properties. We are also studying the enzymatic mechanism and specificity of L-asparaginases. Although we primarily use x-ray crystallography in our studies, our research techniques also extend into biochemistry and molecular biology. Our recent efforts have been concentrated in three distinct areas: (1) chemokines and chemokine receptors, (2) defensins, and (3) L-asparaginases.

Chemokines and Chemokine Receptors

Chemokines have been recognized for many years as proteins associated with inflammation, making them interesting targets for the treatment of immune disorders. Recent discoveries link chemokines and chemokine receptors to processes such as viral infection, hematopoiesis, and cancer metastasis, thus significantly broadening the biological role of this system and creating the potential for their use as therapeutic targets. We are particularly interested in understanding the structural factors that determine the interactions between chemokines and their receptors. Despite the extensive overall structural similarity among all chemokines, individual proteins display a significant degree of specificity toward their receptors. We believe this specificity can be understood by analyzing the structural features of specific chemokines. The x-ray structures of MCP–1, fractalkine, Met-, and AOP–RANTES, studied previously by our laboratory, have allowed us to correlate some important properties of these proteins with the topological features of their molecules. However, more general correlations will require a significantly larger amount of structural data; we are currently acquiring this information through our studies of novel members of the chemokine family and their mutants and/or analogs. Another, more challenging, component of this project will aim at understanding the structural features of chemokine receptors.

Defensins

Defensins are small basic proteins that, until recently, were mainly known for their potent antimicrobial properties. We now know that human β-defensins are also potent chemoattractants, and that they specifically interact with CCR6—the receptor for the chemokine MIP–3α. The mechanisms underlying the antimicrobial and chemotactic activity of defensins is not well understood, although both of these properties are of practical interest. The crystal structures of human β-defensin-2, solved by our laboratory, were the first published for human β-defensins. These structures revealed a topology and an oligomerization mode for defensins that had not been reported previously. Our findings shed new light on a possible mechanism for the antimicrobial properties of these proteins, and we recently extended our research by solving the x-ray structure of another human β-defensin, hBD1. One of our primary goals in studying defensins is to establish the molecular basis of their chemotactic properties, which greatly complements our interest in chemokines. Additionally, we will focus on determining the mechanism of the antimicrobial activity of these proteins. Future studies will involve numerous mutant proteins and will be complemented by extensive biological assays.

L-Asparaginases

L-asparaginases, in particular two bacterial enzymes from *Escherichia coli* and *Erwinia chrysanthemi*, have been used for nearly 30 years to treat certain leukemias and lymphomas. However, the moderately low specificity and immunological incompatibility of bacterial L-asparaginases results in the severe side effects observed during therapeutic applications involving these enzymes. Our longstanding interest in bacterial L-asparaginases focuses on understanding their enzymatic activity and substrate specificity. Thus far, we have determined the structures of L-asparaginases from five bacterial sources, including their mutants, as well as the complexes they form with substrates and inhibitors. Our results have allowed us to correlate, in detail, the structural characteristics of specific enzymes with their unique substrate specificities. We have formulated a model for the mechanism of catalysis by L-asparaginases that agrees with all of the structural and kinetic information available. At present, our efforts are directed toward the cloning, purification, and crystallization of human L-asparaginase, as the genomic sequence for this enzyme is now available.

Our collaborators include Dimiter Dimitrov, Joost Oppenheim, and Alexander Wlodawer, NIH; Stephen Kent, Gryphon Sciences, San Francisco, CA; Wuyuan Lu, University of Maryland; Amanda Proudfoot, Serono Pharmaceutical Research Institute, Geneva, Switzerland, and Klaus H. Röhm, Phillips University.

Recent Publications:

Lubkowski J, et al. *Nat Struct Biol* 1997;4:64–9.

Wilken J, et al. *Chem Biol* 1999;6:43–51.

Hoover DM, et al. *J Biol Chem* 2000;275:32911–8.

Aghaiypour K, et al. *Biochemistry* 2001;40:5655–64.

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Macromolecular Crystallography Laboratory
High-Throughput Protein Expression and Purification; Structural Genomics

Keywords:
affinity tags
bioterrorism
protein expression
protein purification
structural genomics
x-ray crystallography
Yersinia pestis

Research: Our goal is to create a unified technological infrastructure for high-throughput protein expression and purification that will be suitable for large-scale structural biology initiatives. Central to our approach is the use of multiple genetically engineered affinity tags. We are currently trying to establish which combination of affinity tags is most appropriate for our purposes and how they can be utilized with maximal efficiency. At the same time, we are striving to develop more reliable methods for removing affinity tags, which is another critical element of our strategy. The feasibility of this approach is being evaluated in the context of two structural genomics projects.

One of the greatest technical obstacles that we face is "the inclusion body problem"—i.e., the tendency of proteins to accumulate in an insoluble, inactive form. Because refolding of proteins seems incompatible with high-throughput applications, some means of circumventing the formation of inclusion bodies is needed. Sometimes this can be accomplished by fusing an aggregation-prone polypeptide to a highly soluble partner. To study this phenomenon in greater detail, we compared the ability of three soluble fusion partners—maltose-binding protein (MBP), glutathione S-transferase, and thioredoxin—to inhibit the aggregation of six diverse proteins that normally accumulate in an insoluble form. Remarkably, we found that MBP is a much more effective solubilizing agent than the other two fusion partners. Moreover, in some cases we were able to demonstrate that fusion to MBP can promote the proper folding of the attached protein into its biologically active conformation. This chaperone-like quality distinguishes MBP from other affinity domains and greatly enhances its value as a fusion partner. Accordingly, MBP fusion proteins have become a cornerstone of our strategy for protein expression. We are using a variety of experimental approaches to try to understand how MBP influences the folding of its fusion partners. At the same time, to improve its utility as a fusion partner, we are attempting to endow MBP with an engineered affinity for additional ligands.

Affinity tags would probably be used more often if it were not so difficult to remove them. This is usually accomplished by endoproteolysis of a fusion protein at a designed site. The main difficulty with this approach stems from the intrinsically promiscuous activity of proteolytic reagents that are commonly used to cleave fusion proteins. This problem is compounded by the fact that it is very expensive to purchase enough of any of these reagents to cleave fusion proteins on a scale amenable for structural studies. To overcome these problems, we are producing our own supply of TEV protease, the catalytic domain of the nuclear inclusion protease from tobacco etch virus. TEV protease cleaves the amino acid sequence ENLYFQG between Q and G with high specificity; in contrast to factor Xa, enteropeptidase, and thrombin, there have never been any reports of cleavage at noncanonical sites by TEV protease. The production of TEV protease in *Escherichia coli* has been hampered in the past by low yield and poor solubility, but we have been able to solve both problems by making synonymous codon replacements and producing the protease in the form of an MBP fusion protein. The most troublesome shortcoming of TEV protease is that it cleaves itself at a specific site, yielding a truncated protease with greatly diminished activity. We have been able to rectify this problem as well by introducing amino acid substitutions that prevent autoinactivation without impeding the ability of the protease to cleave canonical target sequences. Further improvements (e.g., modifications that increase stability or alter specificity) may be possible once the structure of TEV protease has been solved or a genetic assay for protease activity in vivo has been developed.

The technological framework that we are assembling for high-throughput protein expression and purification is being field tested in the context of a structural genomics project involving *Yersinia pestis*, the causative agent of plague. Our principal objective is to determine the three-dimensional structures of the effector proteins, a subset of the virulence factors (*Yersinia* outer proteins, or Yops) that are injected into the cytosol of mammalian cells and enable the pathogen to evade the immune response of the infected organism. We are also interested in specific Yop chaperone (Syc) proteins that are required for the secretion of some effectors. Our long-term goal is to facilitate the design or discovery of effective countermeasures for this potential agent of bioterrorism. In addition, since many important animal pathogens employ a similar virulence strategy (e.g., *Shigella spp.*, enteropathogenic *Escherichia coli, Salmonella spp., Pseudomonas aeruginosa, Chlamydia psittaci*, and *Bordetella spp.*), we expect that some of these proteins will be appropriate targets for general "antipathogenicity" drugs. At the same time, we are also trying to identify the targets of *Y. pestis* effectors in mammalian cells so that we may begin to understand how this infamous bacterium disarms the immune system and multiplies in the lymphoid tissues of its host.

Recent Publications:

Evdokimov AG, et al. *J Mol Biol* 2001;305:891–904.

Kapust RB, et al. *Protein Sci* 1999;8:1668–74.

Evdokimov AG, et al. *Acta Crystallogr D* 2001;67:793–9.

Fox JD, et al. *Protein Sci* 2001;10:622–30.

Laboratory of Medicinal Chemistry

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The Laboratory of Medicinal Chemistry (LMC) is a multidisciplinary laboratory, which emphasizes chemical science at the interface of chemistry and biology. A major focus of the LMC is the discovery of new anticancer and anti-AIDS drugs based on biomechanistic rationale and the structural optimization of newly discovered lead compounds. A diverse group of organic chemists, analytical chemists, and molecular modeling experts interacts closely to create a research environment that maximizes new drug discovery. Approximately 50 percent of the LMC staff are postdoctoral training fellows with recent Ph.D. degrees. Close collaborations also are developed with non-LMC biological scientists studying specific enzymes as potential drug targets. Research projects are initiated by medicinal chemists and/or biologists, with each playing a critical role in all stages of research.

The LMC has four principal investigators (PIs) who carry out independent work in the areas of organic chemistry and medicinal chemistry, peptide chemistry, and analytical chemistry. Each individual PI functions as a section head and is responsible for the administration of his group and the hiring of visiting fellows to carry out his or her research. The LMC has, in addition, MiniCore facilities in the areas of NMR spectroscopy and Computer Aided Drug Design (CADD).

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John Driscoll	Special Volunteer
David Johns	Scientist Emeritus

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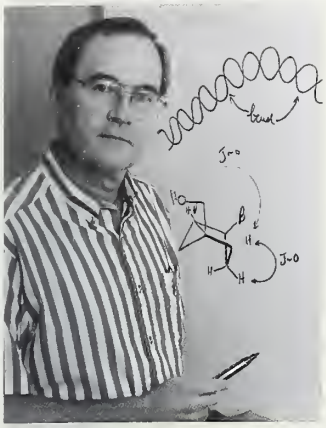

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Biography: *Dr. Marquez received his Ph.D. in medicinal chemistry from the University of Michigan in 1970. After 1 year of postdoctoral training at the NCI, he worked in private industry for 5 years in Venezuela. He rejoined the NCI in 1977 as a visiting scientist and was awarded tenure in 1987 after becoming a naturalized citizen. His main research interests are nucleoside chemistry and synthetic organic chemistry as tools for the rational design of antitumor and antiviral agents. Dr. Marquez has authored or coauthored more than 210 publications and has received 19 U.S. patents.*

Laboratory of Medicinal Chemistry
Rational Design of Antitumor and Antiviral Agents

Keywords:

antiviral
chemotherapy
DNA binding proteins
HIV
molecular modeling
nucleic acid chemistry
nucleosides
nucleotides
protein kinase C
receptors
synthetic organic chemistry

Research:

Ultrapotent Ligands for PKC and Other Phorbol Ester Receptors

One of the principal goals of this research is the understanding of the geometry of bioequivalent pharmacophores—present in diacylglycerol (DAG) and in the more potent phorbol esters—that appear to be responsible for binding to the regulatory C1 domains of protein kinase C (PKC) and other target proteins. These domains, which act as a molecular hydrophobic switch, represent regulatory modules in several families of proteins involved in signal transduction, including the PKC, chimaerin, and RasGRP families. Many of the features that characterize the interaction between ligands and PKC can be understood at the level of the isolated C1 domain. A receptor-guided approach, which utilizes as its basis the x-ray structure of the C1b domain of PKC-delta in complex with phorbol-13–O-acetate, helped us identify the "recognition domain" for DAG which consisted of a similar network of hydrogen bonds as those utilized by phorbol. The application of this model to DAGs and to the more potent DAG-lactones that were designed later suggested that both classes of compounds can bind to the C1 domain in two distinct orientations, so-called *sn*-1 and *sn*-2 orientations. Since in each of these orientations the hydrophobic groups or "affinity domains" of the ligand are directed into rather different directions, the interactions of these groups with a cluster of conserved hydrophobic amino acids located on the top half of the C1 domain were incorporated into the model. The optimization of binding to this hydrophobic cluster has already produced ligands that display low nanomolar binding affinities for PKC and related C1 domain-containing protein targets. The first subnanomolar binding ligand for beta-2 chimaerin, which surpasses even the phorbol esters in binding potency, was developed. Furthermore, some of these novel ligands have shown potent antitumor activities in the NCI 60-cell line in vitro screen. A combination of small library approaches and molecular dynamics simulations has contributed to our understanding of the nature of these important hydrophobic interactions.

Conformationally Locked Nucleosides

Nucleosides and nucleotides are inherently flexible molecules. Hence, one of the main obstacles in interpreting structure-activity correlations has been this high level of flexibility. In 1993, our laboratory described the first synthesis of a conformationally locked bicyclo[3.1.0]hexane nucleoside. Although other types of locked nucleosides have been devised by others, only the bicyclo[3.1.0]hexane pseudosugar can successfully mimic both antipodal North and South conformations typical of conventional nucleosides. Significant differences in the biological activity of locked nucleoside antipodes, which depend on the precise interaction of these rigid molecules with specific target enzymes, have been clearly demonstrated.

The principal theme in this area is and has been the study of "shape" in the recognition of individual nucleosides and nucleotides by specific enzymes (e.g., adenosine deaminase, reverse transcriptase, HSV–1 thymidine kinase, etc.). Recently, this concept has been extended to the study of DNA segments incorporating locked nucleotide units. It is well known that the binding of proteins to DNA elicits a unique conformational response of the double helix, which is tied to the function of the associated protein. Studies with cytosine (C5)-methyltransferase, an important enzyme in the control of gene expression, revealed important differences between target abasic sites locked in both antipodal conformations. Furthermore, the incorporation of conformationally locked nucleotides into DNA is a powerful tool to reinforce or disrupt typical B– or A–DNA forms associated with South and North conformations, respectively.

Recent Publications:

Nacro K, et al. *J Med Chem* 2000;43:921–44.

Lan M, et al. *Biochemistry* 2000;39:11205–15.

Wang P, et al. *J Am Chem Soc* 2000;122:12422–34.

Nacro K, et al. *J Med Chem* 2001;44:1892–904.

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Biography: *Dr. Barchi received his Ph.D. in organic chemistry from the University of Hawaii in 1985. After 2 years as a postdoctoral fellow at Duke University, he joined the NCI as a staff fellow in 1987. He spent 2 years as a special expert and was promoted to staff scientist in 1996. His main research interests are in synthetic organic chemistry as it relates to carbohydrate-based drug design, and the high-resolution structural analysis of sugars, peptides, and glycopeptides by NMR spectroscopy.*

Laboratory of Medicinal Chemistry
Glycosylated Peptide Analogs as Modulators of Tumor Cell Adhesion and as Novel Immunogens Directed Against HIV: Synthesis and Conformational Analysis by NMR

Research: Carbohydrates are presented on the surface of cells primarily in covalent linkages to proteins (glycoproteins, proteoglycans) or lipids (glycolipids, gangliosides). These diverse groups of oligosaccharide chains function to stabilize protein structures, facilitate protein transport or clearance, and mediate cell adhesion. Cell-surface glycans may reveal or mask peptide epitopes on proteins or be recognized as immunogenic structures themselves. During oncogenesis, the cell-surface glycans on tumor cells are transformed relative to the normal phenotype through modified expression of the enzymes involved in the cell's glycoprocessing machinery. These aberrations are a hallmark of highly tumorigenic and metastatic cell types. We are interested in defining the role played by these aberrations as related to tumor progression. The first step in the cascade of events leading to tumor metastasis is the interaction of the tumor cell with the surrounding extracellular matrix (ECM). Small peptide motifs have been shown to promote or inhibit tumor cell attachment to various ECM proteins. Since both the tumor cell surface and ECM proteins involved in these events are usually highly glycosylated, it follows that a glycosylated peptide segment which more closely resembles the actual protein ligand/receptor may potentiate the biological properties of the naked peptide. We have shown that glycosylation may also reduce cell attachment activity of a peptide sequence which promotes melanoma attachment to laminin. In addition, preliminary results indicate that simple sugars may cause cell attachment and spreading of the same cell line when presented on a polymeric backbone.The cell surface of HIV–1 is also replete with glycosylated proteins, in particular the *env* gene product surface glycoprotein gp120. This protein contains ~50 percent carbohydrate by weight presented as both N- and O-linked glycans. gp120 contains the principal neutralizing determinant (PND) of HIV–1 in a domain designated as V3. This highly variable domain contains potential O-linked glycosylation sites. We have prepared glycopeptides of a segment from the V3 domain to which a neutralizing antibody was mapped, and showed that

O-glycosylation of two potential sites with *-galactosamine enhanced binding to this antibody. It may be inferred from this result that the glycosylated construct more closely resembles the conformation of this segment in the native protein. We are attempting to exploit this design to build a better immunogen to be used as a novel vaccine candidate against HIV–1. We have used NMR extensively in the course of the work to define the solution conformations of the glycopeptides, and to compare the effect of glycosylation on the structures with those of their unglycosylated counterparts.

Recent Publications:

Barchi JJ Jr, et al. *Bioorg Med Chem Lett* 1995;5:711–4.

Barchi JJ Jr, et al. *J Biochem Biophys Methods* 2001; in press.

Burke TR Jr, et al. *J Med Chem* 1995;38:1386–96.

Huang X, et al. *FEBS Letts* 1996;393:280–6.

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Biography: *Dr. Burke obtained his Ph.D. under Dr. Wendel Nelson at the University of Washington, Seattle. His postdoctoral studies began at the NIH as a pharmacology research associate trainee in the laboratory of Dr. Lance Pohl, National Heart, Lung, and Blood Institute, and continued under Dr. Kenner Rice, National Institute of Diabetes and Digestive and Kidney Diseases. Dr. Burke has been a member of the Laboratory of Medicinal Chemistry, NCI, since 1989.*

Laboratory of Medicinal Chemistry
Bioorganic Medicinal Chemistry and the Modulation of Kinase-Dependent Signal Transduction

Keywords:
AIDS
breast cancer
cancer cell growth regulation
cell signaling
computer modeling
drug discovery
erbB2
growth arrest
heregulin
HIV–1 inhibitor

Continued on page 619

Research: Pharmacological agents are being developed to modulate phosphotyrosyl (pTyr)-dependent cell signaling. Emphasis is on inhibitors of pTyr-dependent binding interactions which are mediated by src homology 2 (SH2) domains and on protein-tyrosine phosphatase (PTP) inhibitors. Central to both of these efforts is the development of new pTyr mimetics which afford either increased stability toward enzymatic degradation by PTPs or increased affinity. In the SH2 domain area, development of cell-permeable growth factor receptor-bound 2 (Grb2) antagonists is being undertaken as potential new therapeutics for a variety of cancers including erbB–2- and MET-dependent cancers, including breast cancer. For this work, peptidomimetics have been designed as conformationally constrained analogs of natural Grb2 SH2 domain-bound pTyr-containing peptides.

Keywords (continued):
integrase
molecular models
organic synthesis
peptide conformation
peptide synthesis
peptidomimetics
protein kinases
protein phosphatases
protein-tyrosine kinase
protein-tyrosine phosphatase
receptor tyrosine kinase
SH2 domain
signal transduction
signaling
synthesis

In related work, a series of new pTyr-mimicking amino acid analogs have also been prepared to enhance cell permeability. Among these are medium-size, nonphosphate-containing analogs which exhibit low nanomolar Grb2 SH2 domain inhibition constants. Promising analogs exhibit potent inhibition of Grb2 binding in whole cell systems and display good cytostatic effects against breast cancer cells grown in culture or in soft agar. Studies are currently under way to examine the utility of the agents in combination therapies directed against breast cancer. Preliminary cell studies indicate that nontoxic concentrations of our synthetic Grb2 inhibitors can act cooperatively with certain standard cytotoxic chemotherapeutic agents to significantly reduce the growth inhibitory dose. In other cellular studies, our synthetic Grb2 inhibitors have been shown to inhibit human growth factor (HGF)-induced cell migration in Met containing fibroblasts. Work is currently in progress to examine these agents in whole animal metastasis models.

In the phosphatase area, a structure-based approach toward inhibitor design is being pursued. Using an epidermal growth factor receptor (EGFR)-derived pTyr-containing peptide sequence as a platform, we have examined a large number of novel nonphosphorus-containing pTyr mimetics for inhibitory potency against PTP1B. Highly potent motifs identified in this fashion have served as models for small molecule peptidomimetic design. The most potent of these low molecular weight inhibitors is currently undergoing cocrystallography with PTP1B for x-ray crystal structure determination. The aim of this work is to identify high affinity small molecule inhibitors with improved bioavailability as tools for studying cellular signal transduction and as potential therapeutic agents. Inhibitors of HIV integrase are being developed as potential anti-AIDS drugs in collaboration with the Laboratory of Molecular Pharmacology, CCR, NCI. Lead inhibitor structures have initially been derived from several sources, including three-dimensional pharmacophore searching of the more than 250,000 compounds contained within the NCI's chemical repository. Promising compounds have been systematically explored through chemical synthesis of analogs to determine structure-activity relationships (SAR) responsible for integrase inhibition. Information generated in this fashion has been applied to the design and preparation of new analogs having higher potency, reduced collateral cytotoxicity, and greater antiviral protective effects in HIV-infected cells. One lead structure in these studies has been provided by chicoric acid, which is a natural product previously reported to exhibit potent HIV integrase inhibition as well as protective effects in HIV-infected cells. Through a large number of synthetic analogs, we established important SAR parameters for this class of integrase inhibitor. In further work, we have prepared a series of sulfur-containing bisaroyl hydrazines which show potent inhibition of HIV integrase in extracellular assays and are capable of exhibiting 100 percent protection of HIV-infected cells at micromolar concentrations. Collaborative studies are under way to examine HIV integrase inhibition in whole cell systems.

In separate studies, collaborative efforts are under way to obtain x-ray structures of inhibitors bound to the HIV integrase enzyme. Information obtained from such x-ray structures should provide a starting point for the computer-assisted design of potent new inhibitors. In one approach, synthetic modification of potent inhibitors has been undertaken to render them water soluble and more suitable for cocrystallization with HIV integrase. In an

alternate approach, promising inhibitors are being synthetically modified in ways which will allow them to bind irreversibly to the enzyme active site. This has required the development of new synthetic chemistry which allows the introduction of highly reactive functionality in latent, nonreactive form, which can be unmasked to the active species in a final step prior to incubation with the enzyme.

Our collaborators are David Barford, Institute for Cancer Research, London; Don Bottaro, EntreMed Corp.; Yves Pommier, NIH; Dajun Yang, Georgetown University; and Zhong Yin Zhang, Albert Einstein Medical School.

Recent Publications:

Burke TR Jr, et al. *Biopolymers* 2001;60:32–44.

Burke TR Jr, et al. *Bioorg Med Chem* 2001;9:1439–45.

Burke TR Jr, et al. *J Org Chem* 2000;65:6288–92.

Gao Y, et al. *Org Lett* 2001;3:1617–20.

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Laboratory of Medicinal Chemistry
The Analytical Chemistry of New Antitumor and Anti-AIDS Agents

Keywords:
AIDS
chemotherapy
mass spectrometry
molecular structure
pharmacology

Research: The past decade has witnessed a revolution in the scope and power of analytical chemistry to characterize biological samples. Nowhere has this been more evident than in mass spectrometry where new ionization techniques, new methods of mass analysis, a more efficient integration of separation methods, and a steady increase in computerization have created an exceedingly powerful tool for bioanalysis. It is our goal to employ these new separations and mass spectrometric tools to devise solutions to the myriad bioanalytical challenges and problems that arise during drug discovery and new agent development. To this end, we are addressing questions dealing with the physicochemical and structural characterization of new agents and their metabolites, devising and applying innovative methodology for the measurement of these compounds in biological matrices, and answering questions relating to the disposition, pharmacokinetics, and metabolism of new agents in vitro and in vivo, including in

humans. At the present time, our research efforts are concentrated in two areas: (1) development and application of microcolumn liquid chromatography-tandem mass spectrometry (μLC/MS/MS) and capillary electrophoresis-mass spectrometry (CE/MS) systems for the analysis of biological samples, and (2) development of a general, physiologically-based pharmacokinetic model as a tool for the pharmacological evaluation of nucleoside analogs that are potential anticancer or antiviral drugs.

Lodenosine (2′–β–Fluoro-2′,3′-dideoxyadenosine, F-ddA) is a Laboratory of Medicinal Chemistry-designed and synthesized HIV reverse transcriptase inhibitor that has been evaluated in phase I and II clinical trials. F-ddA has served as a model compound for the development of multifaceted methodologies that are suitable for the analysis of other nucleoside analogs in biological samples through our extensive studies of the its analytical chemistry. Electrospray ionization and MS/MS in combination with narrow-bore HPLC have been used to provide rapid quantitative analysis of F-ddA and its primary deaminated metabolite F-ddI in biological matrices through selective reaction monitoring of the collision-induced dissociation of molecular ions. This general LC/MS/MS approach allows a structure-specific analysis that is rapid, sensitive, and requires minimal sample handling. Compound-specific fluorogenic derivatization has been utilized in conjunction with paired-ion, reversed-phase HPLC separation and fluorescence detection to develop a method capable of measuring nanomolar levels of intracellular F-ddATP, the active phosphorylated metabolite, in patient peripheral blood mononuclear cells. Currently, alternate strategies involving separation by capillary electrophoresis and detection with mass spectrometry are under investigation for the determination of these intracellular nucleotide metabolites.

We have used the above methodology to examine the disposition and pharmacokinetics of lodenosine in AIDS patients, in nonhuman primates, and in rodents. In order to understand the factors determining the disposition, metabolism, and central nervous system penetration of lodenosine, a physiologically based pharmacokinetic model has been constructed using plasma and tissue concentration data obtained after both intravenous and oral administration of both F-ddA and F-ddI in rats. This model is being used to investigate and predict the effects of various transport and metabolic parameters that regulate the oral bioavailability and tissue uptake of F-ddA and other potential nucleoside drugs. The goal of our studies is the development of a general model for nucleoside analogs that will allow prediction of oral bioavailability and metabolism after interspecies scaling. We hope that such a model can be used as a tool for new drug design by identifying the optimal structural properties and pharmacological parameters for maximum enhancement of drug bioavailability and activity.

Our collaborators are Robert Dedrick, Richard Little, Robert Lutz, Terry Phillips, Joseph Tomaszewski, and Robert Yarchoan, NIH.

Recent Publications:

Dai F, et al. *Anal Biochem* 2001;288:52–61.

Zhang H, et al. *J Pharm Biomed Anal* 2001;25:285–97.

Roth JS, et al. *J Mass Spectrom* 2000;35:1313–9.

Roth JS, et al. *Drug Metab Dispos* 1999;27:1128–32.

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Laboratory of Medicinal Chemistry
Computer-Aided Drug Design

Keywords:
ab initio calculations
drug design
HIV–1
inhibitor development
integrase
molecular modeling
NCI database

Research: The Computer-Aided Drug Design (CADD) MiniCore Facility is a research unit within the Laboratory of Medicinal Chemistry (LMC) that employs, analyzes, and develops computer-based methods to aid in the drug discovery, design, and development projects of the LMC and other researchers at the NIH. We split our efforts about evenly between support-type projects and research projects initiated and conducted by CADD staff members.

Increasingly, we are implementing projects, and making available resources developed by the MiniCore, in a Web-based manner. This innovation offers three advantages: (1) it frees all users, including the group members themselves, from platform restraints and the concomitant expenses for specific software/hardware, (2) it makes resources and results immediately available for sharing among all collaborators regardless of their location, and (3) at the same time, without additional effort, it helps further the mission of the NCI as a publicly funded institution by providing data and services to the public in the most economical way for both users and developers.

Enhanced NCI Database Browser

One such project is the Enhanced NCI Database Browser used to search the 250,000-compound Open NCI Database. This dataset is the publicly available part of the half-million structure collection assembled by the NCI's Developmental Therapeutics Program during the program's 45 years of screening compounds against cancer and, more recently, AIDS. In collaboration with researchers at the Computer Chemistry Center of the University of Erlangen-Nuremberg, we have implemented a Web-based graphical user interface for searching the structure and data in the Open NCI Database. This interface offers the user powerful tools for searching,

analyzing, and displaying search results. With this interface in place, it is now easier to add large amounts of additional, mostly calculated, data to the pool of searchable information. In collaboration with a group at the Russian Academy of Medical Sciences in Moscow, predictions are now included for more than 500 different types of biological activities for most of the quarter-million structures in the database. A three-dimensional (3D) pharmacophore search feature has also been implemented. Furthermore, hyperlinks to additional services allow users immediate access to further processing of individual structures or hit sets in a wide variety of ways. The CADD group's home page is found at **http://cactus.nci.nih.gov**; the NCI Database Browser service is at **http://cactus.nci.nih.gov/ncidb2/**. We hope this tool will be useful in drug design for researchers both inside and outside the NCI.

HIV Integrase

The second main interest of our group is HIV integrase (IN) as a drug development target. This enzyme catalyzes the integration of the viral DNA into the human DNA, which is an essential step in the viral replication cycle. HIV has been shown to develop rapid resistance to current inhibitors of protease and reverse transcriptase. Because there is no known analog for IN in human cells, IN is thought to be an ideal target for anti-AIDS drug discovery. NMR and/or x-ray crystallography techniques have determined the structures for the separate domains of HIV–1 IN. However, an experimental structure of the full-length protein remains unavailable. We have therefore initiated a project that aims to utilize all the currently available experimental results, structural, mechanistic, and otherwise, to build a model of the full-length HIV IN protein complexed with (the ends of) the viral DNA and, possibly, also with model stretches of host DNA. Using this model as a starting point, we plan to conduct 3D pharmacophore searches and docking studies with the goal of finding compounds that are potent in an assay using preintegration complexes of IN and DNA.

Among our collaborators are Bernard Brooks and Yves Pommier, NIH; Wolf-Dietrich Ihlenfeldt, University of Erlangen-Nuremberg, Germany; Neamati Nouri, University of Southern California; and Vladimir Poroikov, Russian Academy of Medical Sciences, Moscow.

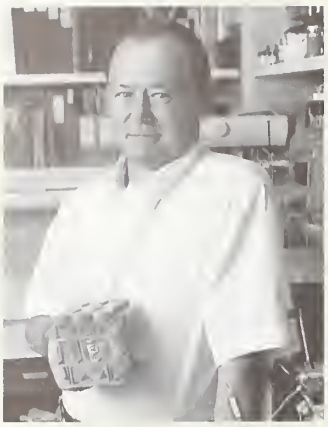
Recent Publications:

Voigt JH, et al. *J Chem Inf Comput Sci* 2001;41:702–12.

Nicklaus MC, et al. *J Chem Inf Comput Sci* 1998;38:893–905.

Nicklaus MC, et al. *J Med Chem* 1997;40:920–9.

Nicklaus MC, et al. *Bioorg Med Chem* 1995;3:411–28.



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Biography: *Dr. Roller has been with the Laboratory of Medicinal Chemistry for the past 11 years, heading the Peptide Chemistry Group. He obtained his Ph.D. from the Chemistry Department of Stanford University, with Professor Carl Djerassi. He completed his postdoctoral training at the University of Hawaii and at the University of Virginia. In 1972, he joined the Division of Cancer Etiology, NCI, to carry out studies on the structure and mechanism of both carcinogen action and oncogenic proteins.*

Laboratory of Medicinal Chemistry
Polypeptide Modulators of Cellular Signal Transduction and Cell Cycle Progression

Keywords:
cancer metastasis
cell signaling
drug discovery
erbB receptor family
hepatocyte growth factor (HGF)
human breast cancer
ligand binding
molecular interactions
molecular structure
organic synthesis
peptide synthesis
peptidomimetics
phosphorylated peptides
protease inhibitors
protein-protein interaction
receptor tyrosine kinase signaling
SH2 domain

Research: We have focused our attention on developing experimental therapeutic agents that are targeted to modulate key cellular signal transduction processes. Uncontrolled cellular proliferation is a characteristic of cancerous cells. Cell proliferation and differentiation are in part mediated through extracellular receptors initiating a cascade of events in the cell. There are also a number of intracellular factors that control, and also provide a checkpoint for, the replication of the genetic material and thus of cell division. In these efforts we have designed and synthesized a number of peptidic agents and peptidomimetically modified analogs, and these have been evaluated for controlling their specifically targeted biochemical processes.

The Grb2 protein is an adaptor molecule that functions in transmitting growth factor receptor-mediated signaling through its Grb2–SH2 domain and its two SH3 domains. Binding of Grb2 to phosphotyrosine (pTyr) motifs on growth factor receptors such as EGFR and erbB2 leads to downstream activation of the ras signaling pathway. These receptors are mutated or overexpressed in a number of tumors, including breast and ovarian cancers. Inhibition of this signaling provides for a promising target to develop anticancer agents for tumors overexpressing growth factor receptors. Based on an earlier identified phage library-derived prototype peptide, we developed a variety of cyclic peptide analogs that do not rely on phosphotyrosine moiety or its structural mimic for high affinity binding to the Grb2–SH2 domain. We have carried out extensive structure/activity studies on these novel agents to optimize the structural motifs required for inhibitory action. NMR solution-phase studies indicate a rigid, wide loop structure for the active peptides. These agents provide for an exquisitely honed multipoint contact within the Grb2-binding pocket and are expected to be specific antagonists of Grb2–SH2 domain function. They inhibit erbB2 receptor/Grb2 protein association in the 0.3 micromolar concentration range in cell homogenates, and their cell permeabilized analogs are effective in inhibiting MAP kinase activation in cultures of breast cancer cells.

A more recently initiated project focuses on developing matriptase inhibitory agents as antimetastatics. Matriptase enzyme is an epithelial cell-derived, integral membrane serine protease. This enzyme was isolated by our biologist collaborators at the Lombardi Cancer Center several years ago from human breast cancer cells in culture, and has been implicated in breast cancer invasion and metastasis. It was also demonstrated that matriptase can convert hepatocyte growth factor/scatter factor to its active form and it can activate c-Met tyrosine phosphorylation in A549 human lung carcinoma cells, and thus function in extracellular matrix degradation and epithelial cell migration. With the aim of developing selective inhibitors of matriptase, we have taken two approaches. Using homology modeling we are designing potential matriptase inhibitory peptides based on the Bowman-Birk soybean trypsin inhibitor and the Kunitz inhibitory segment structures. In a parallel effort we have synthesized for the first time a bicyclic cage-like conformationally restricted cyclic peptide, recently isolated by others from plants. This bicyclic peptide is proteolytically stable on account of its rigidity. Recent assay results by our collaborators on this synthetic peptide show that it inhibits matriptase at subnanomolar concentrations. This agent and its analogs may well be suited for inhibitory studies of metastatic tumors in animal models also.

We have unique capabilities to synthesize a variety of peptides, cyclic and constrained variants, polymerized in a linear or branched manner, or incorporating unique amino acid functionalities and conjugating groups. Structure activity studies are also served by applying circular dichroism spectroscopy and other spectral techniques to study the structure, conformation, and interaction of peptides and relevant proteins.

Collaborators on this research include Robert Dickson, John Reichert, Shaomeng Wang, and Dajun Yang, Lombardi Cancer Center, Georgetown University Medical Center; and Yves Pommier and Adrian Senderowicz, NIH.

Recent Publications:

Long YQ, et al. *Bioorg Med Chem Lett* 1999;9:2267–72.

Long YQ, et al. *Biochem Biophys Res Commun* 1999;264:902–8.

Lung FD, et al. *J Pept Res* 2001;57:447–54.

Jin S, et al. *J Biol Chem* 2000;275:16602–8.

Laboratory of Metabolism

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The Laboratory of Metabolism conducts research in the areas of chemical carcinogenesis, mammalian development and gene control, and cell cycle control.

There are six sections in the laboratory: (1) the Nucleic Acids Section is involved in the study of drug and carcinogen metabolism and the mechanism of action of genotoxic and non-genotoxic carcinogens. Chemical carcinogens are actively metabolized and the extent of this metabolism is believed to be the major determinant in the carcinogens' effects on organisms. They can be either neutralized to nonhazardous derivatives or activated to metabolites that can damage DNA, mutate genes, and sometimes transform cells. The pathways of metabolism and the enzymes involved in rodent model systems and humans are under investigation. Carcinogen metabolizing enzymes and receptors that mediate the biological activity of genotoxic and nongenotoxic carcinogens, respectively, are being studied; (2) the Endocrinology Section is engaged in the regulation of thyroid hormone synthesis and the mechanisms of organogenesis. The thyroid enhancer-binding protein (T/EBP) is involved in organogenesis of the lung, thyroid, pituitary, and hypothalamus, and in tissue-specific gene control. The pathways involved in programmed development are under study; (3) the Chemistry Section examines the mechanisms of cell cycle regulation with emphasis on the polo kinase, a novel enzyme involved in the control of mitosis. Yeast and higher eukaryotic cells are used as model systems; (4) the Protein Section studies the high-mobility group proteins (HMGs) associated with chromatin and their effects on transcription, replication, recombination, and repair. Structural biology, cell biology, and gene knock-out mice are used to examine the function of these abundant nuclear proteins; (5) the Gene Regulation Section investigates the biology of mammalian B–ZIP transcription factors. Probes are designed to inhibit the activity of these proteins and tested using transgenic mouse models and adenoviral delivery systems; (6) the Metabolic Control Section investigates the physical chemistry and activities of P450s. Monoclonal antibodies are being used as probes to investigate the role of P450s in human drug metabolism.

Laboratory of Metabolism Staff

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Biography: *Dr. Gonzalez received his Ph.D. at the University of Wisconsin in Madison in 1981 and was a staff fellow for 2 years at the National Institute of Child Health and Human Development prior to joining the NCI. A member of the Senior Biomedical Research Service, he received the Rawls Palmer Progress in Medicine Award from the American Society for Clinical Pharmacology, the John J. Abel Award from the American Society of Clinical Pharmacology and Therapeutics, and the George Scott Award from the Toxicology Forum. He twice received the Federal Technology Transfer Award, was a visiting professor in Taiwan, Japan, and Thailand, and has delivered numerous keynote and special lectures at national and international symposia. His studies are largely focused on the role of cytochromes P450 and xenobiotic receptors in chemical carcinogenesis.*

Laboratory of Metabolism
Function and Regulation of Cytochromes P450 and Xenobiotic Receptors

Research: The general theme of our Nucleic Acids Section is to understand the mechanisms of carcinogenesis and cancer susceptibility. There are two general types of carcinogens, the genotoxic carcinogens, which require metabolic activation to electrophilic derivatives capable of binding to cellular macromolecules including DNA, and the nongenotoxic carcinogens, which are not metabolically activated but cause cell transformation by perturbing the cell cycle. Xenobiotic receptors are thought to mediate the mechanism of action of nongenotoxic carcinogens. These chemicals include dioxins such as 2,3,7,8-tetrachlorodibeno-p-dioxin (TCDD) and peroxisome proliferators. They bind to the aryl hydrocarbon receptor (AHR) and peroxisome proliferator-activated receptor α (PPARα), respectively.

The function, regulation, catalytic activities, and polymorphisms in enzymes involved in drug and carcinogen metabolism are also being investigated. Among the enzymes under study are several forms of cytochromes P450 responsible for carcinogen inactivation, including CYP1A1, CYP1A2, CYP1B1, and CYP2E1. Phase II metabolic enzymes that are studied include microsomal and cytosolic epoxide hydrolases, NAD(P)H:quinone oxidoreductase, and UDP-glucuronosyltransferases. In contrast to the P450s, these latter enzymes are generally involved in inactivation of toxic and carcinogenic metabolites and therefore could be determinants of cancer and toxin susceptibility.

To determine the functions of xenobiotic-metabolizing enzymes and xenobiotic receptors in mammalian development and physiology and their role in toxicity and carcinogenesis, gene nullizygous mice have been produced using targeted gene disruption in embryonic stem cells. Phenotypes observed in mice lacking expression of an enzyme or receptor offer clues to their endogenous functions. Null mice have proven to be of tremendous value in determining the mechanism of action of toxins and

carcinogens and the role of the xenobiotic receptors and xenobiotic-metabolizing enzymes in cancer susceptibility. These mice can also be used to validate the use of rodent models in human risk assessment.

Efforts are also ongoing to identify human polymorphisms in xenobiotic-metabolizing enzymes and xenobiotic receptors since these are thought to be involved in cancer susceptibility. Genes encoding xenobiotic receptors and P450s are sequences, and exon-crossing primers are generated for use in screening human populations for novel functional mutations.

The roles of receptors and hepatocyte-enriched transcription factors in expression and regulation of genes encoding xenobiotic-metabolizing are being investigated by standard DNA binding studies and reporter gene transfections. In addition, conditional gene knock-out experiments using the Cre-LoxP system are being used to disrupt genes encoding transcription factors to study their physiological functions and determine the spectrum of their target genes in liver and other tissues.

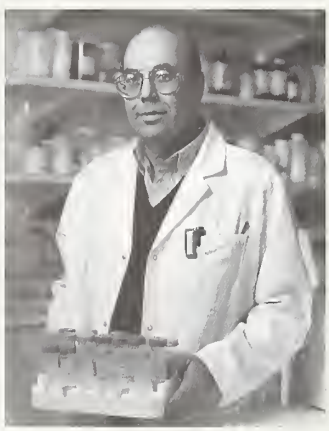
Recent Publications:

Idle JR, et al. *Lancet* 2000;355:319.

Sata F, et al. *Clin Pharmacol Ther* 2000;67:48–56.

Corton JC, et al. *Mutat Res* 2000;448:139–51.

Elizondo G, et al. *Mol Pharmacol* 2000;57:1056–63.



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Biography: *Dr. Bustin is chief of the Protein Section in the Laboratory of Metabolism. He received his Ph.D. from the University of California–Berkeley. He was a research associate at the Rockefeller University, New York, an associate professor at the Weizmann Institute, Israel, and an adjunct professor at Georgetown University. He is the recipient of the Janet and Samuel Lubell Prize from the Weizmann Institute, the 1994 Tosse Prize for Kinderrheumathology, and a 1997 awardee of the Humboldt Prize for senior scientists.*

Laboratory of Metabolism
Chromosomal Proteins and Chromatin Function

Keywords:
cancer cause
chromatin
chromosomal proteins
gene discovery
histones

Research: Precise and specific interactions between chromosomal proteins and DNA are key elements for proper packaging of the DNA into chromatin and are necessary for the orderly progression of complex processes such as transcription, replication, recombination, and repair. We study the chromatin organization and cellular function of histones and HMG proteins. HMG proteins serve as architectural elements which affect the structure of both the DNA and the chromatin fiber. We focus on the HMG–14/–17 subgroup which are the only nuclear proteins that bind

Continued on page 633

Keywords (continued):
HMG proteins
nuclear proteins
nucleosome

specifically to nucleosomes and are involved in the generation, or maintenance, of the chromatin structure of transcriptionally active genes. Using a variety of approaches, we study the structure of the proteins, their organization in nucleosomes, their expression during the cell cycle and differentiation, the manner in which they assemble into the chromatin fiber, and their role in transcription and cellular differentiation. These studies will help to elucidate the effect of chromatin structure on gene expression.

Our collaborators are H. Clarke, McGill University; Rober Hock and Ulrich Scheer, Wurzburg University, Germany; Caril Prieves, Columbia University; and H. Shirakawa, Tohoku University.

Recent Publications:

Herrera JE, et al. *Mol Cell Biol* 2000;20:523–9.

Misteli T, et al. *Nature* 2000;408:877–81.

Bergel M, et al. *J Biol Chem* 2000;275:11514–20.

Bustin M. *Trends Biochem Sci* 2001;26:43–7.

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Biography: *Dr. Friedman received his Ph.D. in chemistry from Columbia University in New York in 1979. He came to the Laboratory of Chemical Biology in the National Institute of Diabetes and Digestive and Kidney Diseases, NIH, as a staff fellow in 1979. In 1982, he joined the Laboratory of Molecular Carcinogenesis in the NCI as a senior staff fellow, and became a senior investigator in 1986. He joined the Laboratory of Metabolism in 2000 and is currently chief of the Metabolic Control Section.*

Laboratory of Metabolism
Structure/Function of Cytochrome P450

Keywords:
cytochrome P450
drug metabolism
molecular models
protein structure

Research: The cytochrome P450s are a superfamily of hemeprotein enzymes which catalyze the oxidation of a wide variety of xenobiotic and endogenous compounds. Biochemical, biophysical, and computational approaches are applied to examine the structure/function relationships which govern the interactions of P450s with substrates, inhibitors, membrane lipids, and microsomal proteins. Since these interactions modulate P450 activity, elucidation of their molecular mechanism will aid in (1) clarifying the mechanism of P450-mediated drug and carcinogen metabolism; (2) defining the role of individual P450s in the metabolism of endogenous and environmental chemicals; and (3) development of specific P450 inhibitors.

The binding kinetics of CO ligand to P450 is a valuable and unique probe of P450 conformation and dynamics. To apply this approach, we constructed a laser flash photolysis apparatus and developed appropriate signal processing and data analysis techniques. This approach was used to define the effect of substrates on specific P450s, either in the microsomal state or expressed in cells. Our results indicate that substrates can sterically hinder ligand binding and/or accelerate binding via conformational effects. Experiments were performed with several human P450s. We found that P450 3A4, which metabolizes many important drugs, is composed of different conformers with distinct substrate specificities, thus providing a basis for its recognition of structurally diverse substrates. In contrast, the alcohol inducible and carcinogen metabolizing P450 2E1 is conformationally homogenous, and ethanol increases its rigidity. These findings show that fundamental P450-ligand and P450-substrate interactions can be defined in a natural membrane environment and that P450s can assume a variety of conformational states.

We employed homology modeling to generate a structure for rat P450 2B1. When known substrates were docked into its substrate-binding site, the observed substrate-P450 interactions were consistent with the known substrate specificity of this P450. In addition, a model of human P450 1A2 was constructed. The interactions of several flavones with this P450 were modeled and found to be consistent with their inhibitory potencies. In conjunction with our previous work, P450 homology models have successfully predicted both reductase and substrate-binding domains of mammalian P450s. The models are currently being used to screen small molecule databases to discover specific P450 inhibitors.

Recent Publications:

Dai R, et al. *Cell Mol Life Sci* 2000;57:487–99.

Pincus MR, et al. *Cancer Invest* 2000;18:39–50.

Smith SV, et al. *Biochemistry* 2000;39:5731–7.

Omata Y, et al. *J Protein Chem* 2000;19:23–31.

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Biography: *Dr. Shioko Kimura obtained her Ph.D. in chemistry in 1979 at Hokkaido University in Sapporo, Japan. After 3 years of postdoctoral study at Queen's University, Kingston, Ontario, she joined the National Institute of Child Health and Human Development as a visiting fellow. In 1986, she moved to the Laboratory of Molecular Carcinogenesis at the NCI. Since 1996, she has been head of the Endocrinology Section, Laboratory of Metabolism.*

Laboratory of Metabolism
Role of Thyroid-Specific Enhancer-Binding Protein in Development

Keywords:
brain
development
DNA binding protein
gene targeting
homeobox genes
lung
organogenesis
thyroid

Research: Our research interest is to understand the mechanism of thyroid hormone synthesis and thyroid development. Current efforts are focused on understanding the role of the thyroid-specific enhancer-binding protein (T/EBP) in transcription, physiology, and development. T/EPB is a homeodomain transcription factor that regulates expression of thyroid and lung-specific genes, including those encoding thyroid peroxidase, thyroglobulin, TSH receptor, and the Na/I symporter in the thyroid, and surfactant proteins A, B, and C, and clara cell secretary protein in the lung. T/EBP is expressed in the thyroid, lung, and ventral forebrain during early embryogenesis, suggesting that T/EBP may play a role in genesis of these organs. A mouse lacking T/EBP expression is missing the thyroid and pituitary and has severe defects in lung and hypothalamus. The establishment of a *T/ebp*-null mouse line, therefore, unequivocally demonstrated that T/EBP is essential during development and provides an excellent animal model to study how the lung, thyroid, pituitary, and hypothalamus are formed during development.

Based on the detailed analyses of the defects in each organ, T/EBP appears to qualify as a master regulatory gene involved in morphogenesis of the thyroid, lung, and pituitary, which either activates or suppresses downstream target genes, ultimately leading to organ development. Our efforts have focused on identifying genes that are regulated by T/EBP during mammalian development and that are involved in organogenesis. To this end, we are carrying out PCR-based subtraction library screening and microarray analysis using lungs obtained from wild-type and *T/ebp*-null mouse embryos. Several genes have been characterized that are expressed under the control of T/EBP in the lung during development. Efforts are also ongoing to produce a conditional *T/ebp* gene knock-out mouse line using the Cre-Lox P system in order to study physiological functions of T/EBP in the thyroid and lung in relation to genesis and diseases in these organs.

Recent Publications:

Minoo P, et al. *Dev Biol* 1999;209:60–71.

Sussel L, et al. *Development* 1999;126:3359–70.

Tichelaar JW, et al. *J Histochem Cytochem* 1999;47:823–31.

Yuan B, et al. *Dev Dyn* 2000;217:180–90.

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Laboratory of Metabolism
Regulatory Mechanisms in G2/M Phase of the Cell Cycle

Keywords:
cell cycle
mitosis
polo kinase

Research: Our research focuses on the mechanisms controlling the G2/M phases of the cell cycle, which are comprised of a series of regulatory biochemical steps and coordinated cellular events to ensure the faithful partitioning of genetic and cytoplasmic components. The polo subfamily of protein kinases has been identified in various eukaryotic organisms, and many members play pivotal roles during the late G2 and M phases of the cell cycle. Members of this subfamily include mammalian Plk, Snk, and Fnk/Prk, *X. laevis* Plx1, *Drosophila* melanogaster polo, *Schizosaccharomyces pombe*, and *S. cerevisiae* Cdc5. A feature of the polo subfamily members is the presence of a distinct region of homology in the C terminal noncatalytic domain called the polo-box.

Genetic and biochemical analyses have revealed that polo kinases participate in many regulatory pathways, such as activation of Cdc2 through Cdc25C phosphatase, DNA damage checkpoint adaptation, and regulation of the anaphase-promoting complex. They also appear to regulate diverse cellular events, such as centrosome maturation and bipolar spindle formation. In addition, polo kinases have been shown to induce cytokinesis-associated septal structures.

We have previously shown that the mammalian polo-like kinase, Plk, is a functional homolog of *S. cerevisiae* Cdc5. In wild-type yeast, ectopic expression of Plk can induce abnormally elongated buds with additional septal structures. Recently we demonstrated that Plk localizes at the spindle poles, cytokinetic neck filaments, and ectopic septal structures. Without impairing kinase activity, a conservative mutation in the polo-box disrupts the capacity of Plk to complement the defect associated with a cdc5–1 temperature-sensitive mutation, to induce elongated buds, and to localize to

the subcellular structures. Our data provide evidence that the polo-box plays a critical role in Plk function, likely by directing its subcellular localization. The identification of polo-box-interacting proteins and additional Plk substrates will provide a better understanding of the mechanisms by which Plk and other polo kinases function during mitosis.

Recent Publications:

Lee KS, et al. *Proc Natl Acad Sci USA* 1998;95:9301–6.

Lee KS, et al. *Proc Natl Acad Sci USA* 1999;96:14360–5.

Song S, et al. *Mol Cell Biol* 2000;20:286–98.

Song S, et al. *J Cell Biol* 2001;152:451–70.

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Biography: *Dr. Charles Vinson received his Ph.D. from the University of Virginia studying* Drosophila *developmental genetics with Dr. Paul Adler. He subsequently carried out postdoctoral work at the Carnegie Institution in Baltimore, MD, with Dr. Steven McKnight, and joined the NCI as a tenure track fellow in 1991.*

Laboratory of Metabolism
Gene Regulation and Function: The bZIP Proteins

Keywords:
bZIP protein
dominant-negatives
drug resistance
oncogenes
transcription factor
transgenic mouse

Research: The regulation of gene expression underlies all cellular processes, including cancer. We are developing new gene-based protein methods to regulate genes, including (1) dominant-negatives (DNs) that inhibit the DNA binding of endogenous transcription factors, resulting in modulation of gene expression, and (2) gain-of-function genes that bind new DNA sequences, resulting in new gene expression.

We have developed dominant-negatives to the dimeric B–ZIP (CREB, PAR, AP–1, and C/EBP) and B–HLH–ZIP (USF, Myc, and Mi) transcription factors. These dominant-negatives contain the dimerization domain of the transcription factor and an acidic protein sequence that replaces the basic region. The dominant-negatives heterodimerize with the endogenous transcription factors and prevent DNA binding.

We are studying in detail the structural rules that regulate leucine zipper dimerization specificity and sequence-specific DNA binding. Our recent work suggests that intracellular regulation of magnesium has profound effects on the sequence-specific DNA binding of B–ZIP proteins and highlights the possibility that magnesium may be an intracellular second messenger, similar to its larger cousin, calcium.

We have expressed a dominant-negative that inhibits both the C/EBP and JUN family of transcription factors in fat tissue. The resulting mouse is "fatless" and has severe diabetes. Using the new microarray technology, we are characterizing the genes that are misregulated. We are starting a project to examine the blood serum from these mice to identify missing peptides as possible hormones secreted from fat. This could have profound implications for the regulation of energy homeostasis.

We are expressing these DNs in an inducible manner in transgenic mice to generate new phenotypes and identify transcriptional targets. Preliminary results obtained in collaboration with others indicate that expression of a dominant-negative to Jun (A–Fos) inhibits tumor formation.

We have also placed these DNs into adenoviral vectors. We have shown that expression of A–Fos potentiates killing of the chemotherapeutic resistant cell lines. We are extending these studies to determine if expression of A–Fos selectively kills cancer cells.

Our collaborators include Ira Pastan, M. Reitman, and Stuart Yuspa, NIH.

Recent Publications:

Moitra J, et al. *Genes Dev* 1998;13:168–81.

Moll J, et al. *Protein Sci* 2001;10:649–55.

Moll J, et al. *J Biol Chem* 2000;275:34826–32.

Bonovich M, et al. *Cancer Gene Ther*2001; in press.

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The Laboratory of Molecular Biology uses genetics, molecular biology, cell biology, and molecular modeling to examine and solve a broad range of important biological problems. In the Molecular Biology Section, one major program is directed at designing, producing, and testing new drugs (immunotoxins) to treat cancer. These drugs are genetically modified forms of *Pseudomonas* exotoxin A that are targeted to cancer cells by Fv fragments of antibodies. Five recombinant immunotoxins that have been developed in the laboratory are now in clinical trials. Two of these have activity in hairy cell leukemia, chronic lymphocytic leukemia, Hodgkin's disease, and other malignancies. Design of these molecules is greatly aided by molecular modeling. A new bioinformatics group collaborates with Byungkook Lee in the Molecular Modeling Section to discover new genes in prostate and breast cancer as targets for immunotherapy.

The Clinical Immunotherapy Section group runs clinical trials of recombinant immunotoxins in patients with cancer and studies issues related to these Fv-toxin molecules in the lab. Ongoing clinical trials include targeting CD22 and CD25 in patients with leukemia, lymphoma, and Hodgkin's disease with the recombinant immunotoxins BL22 and LMB–2, both of which have induced complete remissions; and targeting the antigens Lewis-y and mesothelin on solid tumors (carcinomas). Laboratory projects deal with mechanisms of toxin effectiveness and action, and also study mechanisms and prevention of toxicity to normal tissues.

The Biotherapy Section group studies the structure and function of *Pseudomonas* exotoxin (PE) and applies this knowledge to the design of recombinant immunotoxins and vaccine proteins. An immunotoxin directed to CD22 is currently being evaluated for the treatment of B cell malignancies. A chimeric protein composed of nontoxic PE and the adhesion protein pilin is being developed as a vaccine to prevent the establishment of *Pseudomonas* infections in cystic fibrosis patients.

Researchers in the Molecular Modeling Section (1) study the structure of globular protein molecules and the forces that determine the structure, stability, and interaction of these molecules, (2) design mutations that will alter/improve properties of a class of anticancer immunotoxins and of other specific protein molecules, and (3) analyze the expressed sequence tag (EST)

DNA sequence database to discover genes that are specifically expressed in a particular organ or tumor. The product of such genes can potentially be used as a target for delivery of antitumor agents and for tumor imaging.

In the Molecular Genetics Section, investigators create and exploit genetically engineered mice as models for human cancer. Their research emphasizes the role of aberrant tyrosine kinase receptor signaling in the genesis and progression of cutaneous malignant melanoma. Mouse models of melanoma are currently being employed to rigorously assess genetic and environmental melanoma risk factors and to develop effectual sun protection strategies and antimelanoma therapeutics.

In the Molecular Biology Unit C, the research focus is to identify and characterize transcription factors that regulate epidermal growth factor receptor (EGFR) expression. Investigators there have identified a novel repressor protein that decreases EGFR expression and may repress the activity of viral promoters, including the HIV–LTR.

In the Gene Regulation Section, the in vivo actions of thyroid hormone receptors are being studied using receptor knock-out and mutant knock-in mouse models of human receptor diseases. DNA microarrays are used to profile genomic alterations during development of diseases and to discover novel thyroid hormone responsive genes. Using mouse models, the modifier role of thyroid hormone in carcinogenesis will be explored by studying the interaction of thyroid hormone receptors with the Wnt signaling pathway.

In the Developmental Genetics Section, regulation of gene transcription is studied with particular emphasis on the mechanisms by which activators and repressors exert their modulatory effects. Another set of studies is focused on developing bacterial viruses as agents to cure bacterial infections in animals and humans. The goal of the research of Ding Jin is to understand the transcriptional machinery and its mechanisms using *E. coli* as a model system. To that end, Jin has taken two approaches: (1) study RNA polymerase (RNAP) and RNAP-associated proteins using genetics and biochemistry; and (2) analyze the effects of mutations in RNAP and key transcription factors on defined steps in transcription and on global expression, both in vivo and in vitro.

The Biochemical Genetics Section is focused on studies of novel regulatory mechanisms and their use in complex regulatory circuits. In particular, regulation of substrate selection by energy-dependent proteolysis, and the regulation and mode of action of small regulatory RNAs in *E. coli* are investigated.

Investigators in the DNA Molecular Biology Section study ATP-dependent molecular chaperones, proteins that facilitate protein folding, unfolding, and remodeling. They are currently exploring the mechanisms by which chaperones and proteases act together to regulate protein activity and degradation They are investigating how *E. coli* C1pA and C1pX, chaperones belonging to the ubiquitous family of Clp/Hsp 100 ATPases, recognize specific substrates, catalyze protein unfolding, and then translocate the unfolded substrates to the proteolytic chambers of their associated proteases.

In the Genetics Section, researchers are studying the role of cyclic nucleotides in gene regulation in bacteria by genetic and biochemical methods. One project involves understanding the molecular mechanisms of each of the steps by which cAMP modifies the properties of its receptor protein (CRP) to activate transcription at *E. coli:* allosteric changes, DNA binding, RNA polymerase interaction, and DNA melting. Another project studies the synthesis and biological role of cGMP in *E. coli.*

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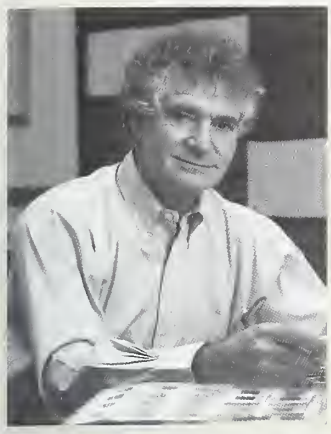

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Laboratory of Molecular Biology
New Approaches to Cancer Treatment: Recombinant Immunotoxins

Keywords:
breast cancer
colon cancer
gene therapy
immunotoxins
leukemia
prostate cancer
protein engineering

Research: Currently, 1.2 million Americans develop cancer each year and about 500,000 die from the disease, because most cancers cannot be cured once they have metastasized. To develop a new treatment for metastatic cancer, we have used genetic engineering to modify a powerful bacterial toxin, *Pseudomonas* exotoxin A (PE), so that instead of killing normal cells it selectively kills cancer cells. PE is a three-domain protein composed of 613 amino acids. Anticancer agents are produced by deleting its binding domain (aa 1–252) and replacing it with the Fv fragment of an antibody or with a growth factor that binds to cancer cells. These agents are termed "recombinant toxins." We have made recombinant toxins that target Le^y present on colon, breast, lung, and other epithelial cancers (B3(ds Fv)-PE38); mesothelin present on ovarian cancer and mesothelioma (SS1P); the normal EGF receptor overexpressed on glioblastomas and squamous cell carcinomas (TGFα-PE38); mutant EGF receptors present on glioblastomas (MR1(Fv)-PE38); the IL–2 receptor present on many leukemias and lymphomas (anti-Tac (Fv)-PE38); and CD22 present on many B cell leukemias and lymphomas (BL22). These agents are produced in *E. coli* because large amounts can be readily purified from this source. When administered to mice with the appropriate human cancer xenograft, all these recombinant toxins produce complete tumor regressions.

We are currently conducting clinical trials in patients with colon, breast, and other epithelial cancers using immunotoxin LMB–9(B3(dsFv)-PE38) and ovarian cancer and mesothelioma (SS1P). We are also conducting clinical trials with LMB–2 (anti-Tac(Fv)-PE38) in patients with CD25-positive leukemias and lymphomas, and with BL22 in CD22-positive leukemias. Both agents show promising antitumor activity. BL22 has produced many complete responses in hairy cell leukemia and both LMB–2 and BL22 have activity in chronic lymphocytic leukemia.

An ideal immunotoxin should be very active so that only small amounts need to be given to cause tumor regressions, small in size so that it can penetrate into cancers, stable so it remains functional during the 5–10 hrs required to reach the interior of a cancer, and nonimmunogenic so it can be

given repeatedly. We have designed and produced recombinant immunotoxins with these properties. Size was decreased and antitumor activity increased by deleting unnecessary sequences in the toxin. Stability has been increased by designing disulfide-linked Fv fragments that are connected by cysteines engineered into the Fv framework regions. These dsFv immunotoxins are up to 100-fold more stable than single-chain immunotoxins and have higher antitumor activity.

Efforts are currently focused on using phage display to identify new antibodies and to produce new immunotoxins directed at various solid tumors (breast, ovary, prostate, lung, colon) as well as lymphomas and leukemias. We have developed a computer-based method to identify new genes expressed in prostate and breast cancer and are characterizing these as new therapeutic targets. We have shown that we can use DNA immunization of mice to generate new antibodies. This eliminates the need to produce and purify proteins for immunization. Antibody titers of over 1:1 million have been obtained by this procedure. The mouse spleens are used to prepare libraries of Fv-phage directly.

Collaborators on this research include Darell Bigner, Duke University Medical Center; Jorge Carrasquillo, David FitzGerald, Robert Kreitman, Byungkook Lee, and Thomas Waldmann, NIH.

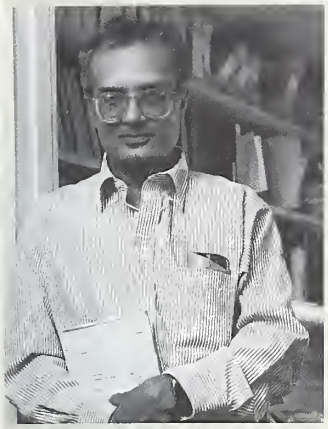
Recent Publications:

Tsutsumi Y, et al. *Proc Natl Acad Sci USA* 2000;97:8548–53.

Kreitman RJ, et al. *J Clin Oncol* 2000;18:1622–36.

Kreitman RJ, et al. *New Engl J Med* 2001; in press.

Wolfgang CD, et al. *Proc Natl Acad Sci USA* 2000;97:9437–42.



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Biography: *Dr. Sankar Adhya received his Ph.D. from both the University of Calcutta and the University of Wisconsin. He was a research associate at the University of Rochester and Stanford University. He joined the Laboratory of Molecular Biology at the NCI in 1971. In 1994, Dr. Adhya was elected a member of the National Academy of Sciences. In addition, he has been an adjunct professor in the Department of Genetics at George Washington University since 1987.*

Laboratory of Molecular Biology
Regulation of Gene Transcription

Keywords:
activators
bacteriophages
gene regulation
repressor
transcription

Research: Virtually every adaptation and developmental process originates at the level of gene regulation by transduction of extra- or intracellular signals. Transcription is the major target of regulation of gene expression. Our research interest covers modulation of transcription by DNA control elements and regulatory proteins—for example, repressors, activators, terminators, and antiterminators and their signal molecules. We have previously demonstrated transcriptional regulation both at the level of initiation by activators and repressors and at the level of elongation by terminators and antiterminators in the *gal* operon which encodes enzymes of D-galactose metabolism in *E. coli*. Presumably because of the amphibiotic nature of the biochemical pathway in carbon metabolism, the operon shows a multitude of controls even at the level of transcription initiation, and has been a paradigm system for studying control of transcription. The operon is transcribed from two promoters which are subject to both negative and positive control by at least four proteins, Gal repressor, Gal isorepressor, cyclic AMP receptor protein, and bacterial histone like protein, HU. The major highlight of our work in the past 2 years is the revelation that GalR alone exerts control on the two promoters in a multivalent way.

- *Regulation by DNA looping*. Synergistic repressor binding to two operators, O_E and O_I, encompassing the promoters *P*1 and *P*2, creates a DNA loop which inhibits transcription initiation from the *gal* promoters. A topologically closed loop of 11 helical turns, which is inflexible to torsional changes, disables the promoters by resisting DNA unwinding by RNA polymerase needed for open complex formation. Interaction between two proteins bound to different sites on DNA, modulating the activity of the intervening segment toward other proteins, may be a common mechanism of regulation in DNA-multiprotein complexes.

- *Requirement of HU*. Concurrent repression of the *gal* promoters by GalR needs another factor, which has been purified and identified to be the bacterial histone-like protein HU, and a supercoiled DNA template. Footprinting experiments show that HU binds to *gal* DNA in a site-specific way, and HU binding is entirely dependent upon binding of GalR to both O_E and O_I. HU, in concert with GalR, forms a specific nucleoprotein higher

order complex containing a DNA loop. This way, while remaining sensitive to inducer, HU deforms the promoter to make the latter inactive. The *gal* repression system provides a model for studying how a "condensed" DNA becomes available for transcription.

- *Atomic force microscopic observation of DNA loop.* We successfully applied atomic force microscopy (AFM) imaging to visualize *gal* DNA loops when complexed with GalR and HU. Supercoiling of DNA, which is critical for GalR action, stabilizes the DNA loops by providing an energetically favorable geometry of DNA.
- *Regulation by DNA unlooping.* Repressor binding to the upstream operator O_E alone, in the absence of DNA looping, represses one promoter *P*1 and activates the other *P*2. We have shown that both inhibition and stimulation of transcription requires the presence of specific regions of the alpha subunit of RNA polymerase. Our results suggest that Gal repressor inhibits or stimulates transcription initiation by disabling or stimulating RNA polymerase activity at a postbinding step by directly or indirectly altering the specific domain of alpha to an unfavorable or to a more favorable state, respectively.
- *A new enzyme.* We have discovered a new enzyme, aldose-1-epimerase, which interconverts the two optical anomers of the inducer, D-galactose.
- *Inducer-repressor interaction.* By genetic and biochemical studies, we have identified several residues in the Gal repressor which interact with the inducer.

Currently, we are (1) studying the biochemical nature of the different nucleoprotein complexes that control transcription initiation in *gal*; (2) investigating the role of HU in repression; (3) detecting both genetically and biochemically various protein-protein and DNA protein contacts in the different complexes; and (4) identifying whether one or both of the optional anomers of the D-galactose is the real inducer.

Recent Publications:

Aki T, et al. *EMBO J* 1997;16:3666–74.

Roy S, et al. *J Biol Chem* 1998;273:14059–72.

Adhya S, et al. *Cold Spring Harb Symp Quant Biol* 1999;63:1–10.

Geanacopoulos M, et al. *Genes Dev* 1999;13:1251–62.

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Biography: *Dr. Cheng obtained her Ph.D. from the University of California–San Francisco Medical Center. She received her postdoctoral training at the University of Chicago and the National Institute of Diabetes and Digestive and Kidney Diseases. She joined the NCI as a senior investigator in 1979 and was promoted to section chief in 1991. Dr. Cheng is a recipient of the NIH Merit Award for outstanding achievements and the Scientific Achievement Award from the Chinese Medical and Health Association.*

Laboratory of Molecular Biology
Molecular Mechanisms of Thyroid Hormone Action

Keywords:
carcinogenesis
genomics
microarrays
mouse models
mutant receptors

Research: The aim of our research has been to understand the molecular mechanisms of thyroid hormone action. Thyroid hormone, 3, 3′, 5-triiodo-L-thyronine (T3), exhibits diverse biological activities. In mammals, including humans, T3 is essential for normal growth, differentiation, and development. Thus, lack of T3 during the neonatal period causes irrevocable defects in development, resulting in growth retardation and cretinism.

The mechanisms by which T3 induces these various effects remain unclear, although most appear to involve the interaction of thyroid hormone nuclear receptors (TRs) with specific response elements in the promoter regions of T3 target genes. Four TR subtypes, $\alpha 1$ and $\alpha 2$, derived from the α gene, and $\beta 1$ and $\beta 2$, derived from the β gene, have been identified. Our efforts to understand the mechanisms of T3 action have focused on three areas.

Elucidation of the Structure, Regulation, and Function of TRs
We have developed several high-affinity monoclonal antibodies specific for different regions of human TRs in order to facilitate the study of the regulation and functions of TRs. We hypothesize that TRs mediate the diverse biological effects of T3 through interaction with a large network of regulatory proteins. In our search for such regulators, we have recently identified several TR-interacting proteins: the tumor suppressor p53, Ear-2, an orphan nuclear receptor, and a homolog of branched-chain amino transferase. These proteins act as negative regulators of TR signaling pathways, thereby strengthening the hypothesis that the diverse effects of T3 are mediated by crosstalks of TRs with a large network of coregulatory proteins.

Furthermore, for the first time, we have shown that the stability of TRs regulates the transcriptional activity of TRs. We elucidated that T3-induced downregulation of TRs is via the proteasome degradation pathway. Thus, this pathway plays an important role in the gene regulatory activity of TRs.

Development of Animal Models to Characterize the Roles of TRs in Disease Pathogenesis

Resistance to thyroid hormone (RTH) is a syndrome characterized by insensitivity of target tissues to the actions of T3. This disease is caused by mutations of the TRβ gene. We have developed transgenic and mutant knock-in mouse models to understand the molecular basis of this disease. In addition, we are also studying the role of TR coactivators in the pathogenesis of RTH by crossing mutant knock-in mice with mice deficient in steroid receptor coactivator-1 or -3. These mouse models are being characterized to develop better strategies for the treatment of patients.

The role of thyroid hormones in carcinogenesis has been controversial. Importantly, we have identified high frequencies of mutations of TRα and/or TRβ genes in human hepatocellular carcinoma, renal clear-cell carcinoma, and papillary thyroid carcinoma. We therefore have developed knock-in mice harboring mutant TRα or TRβ genes to test the hypothesis that mutant TRs act as modifiers in the development of human cancer. Knock-in mutant TR mice are being crossed with other mouse models of human cancer. Characterization of the phenotypes of the offspring will shed new light on the role of thyroid hormones in human cancer.

Use of Microarrays to Profile Genomic Changes During T3-Induced Cell Proliferation

Despite recent progress in the understanding of the transcriptional regulation of TRs by T3, the molecular basis of the growth promoting effect of T3 remains unknown. We have recently shown that the retinoblastoma protein plays a key role in T3-induced cell proliferation. To further identify genes associated with T3-induced cell proliferation, we used cDNA microarrays to profile genomic changes in a model cell line, GC cells. Of 358 responsive genes identified, 88 percent were not previously reported to be transcriptionally or functionally modulated by T3. Analyses of these genes revealed that T3-induced cell proliferation requires activation of multiple cellular pathways including glucose metabolism, biosynthesis, transcriptional regulation, protein degradation, and detoxification in T3-induced cell proliferation. Of particular significance was the finding that T3 rapidly repressed the expression of key regulators of the Wnt signaling pathway and suppressed the transcriptional downstream elements of the β-catenin/TCF complex. These results indicate that thyroid hormone-induced cell proliferation is accompanied by a complex coordinated transcriptional reprogramming of many genes in different pathways and that early silencing of the Wnt pathway is critical to this event. The critical role of the Wnt signaling pathway in T3-induced cell proliferation under normal and pathological conditions is currently being examined using mouse models.

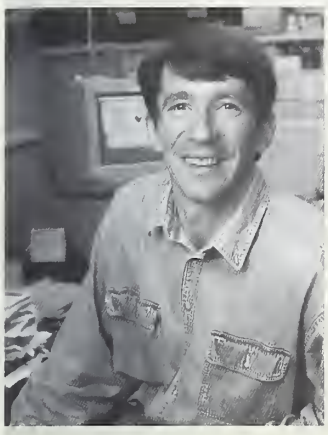
Our collaborators are Carrolee Barlow, Salk Institute; Ed Liu and Lance Miller, NIH; and Bert O'Malley, Baylor College of Medicine.

Recent Publications:

Cheng S–Y. In *Reviews in Endocrine and Metabolic Disorder.* Le Roith D, editor. Boston: L. Kluwer Academic Publishers, 2000.

Zhu XG, et al. *Mol Cell Biol* 2000;20:2604–18.

Kaneshige M, et al. *Proc Natl Acad Sci USA* 2000;97:13209–14.



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Biography: *Dr. FitzGerald is chief of the Biotherapy Section, Laboratory of Molecular Biology, in the NCI. He received his Ph.D. in 1982 from the University of Cincinnati Medical School, Department of Microbiology. The focus of his Ph.D. dissertation was the endocytic uptake of* Pseudomonas *exotoxin. He has a longstanding interest in bacterial toxins and their use as therapeutic agents.*

Laboratory of Molecular Biology
Toxin-Based Treatment of Cancer and Vaccine Development

Keywords:
dendritic cells
immunotoxin
lymphoma
pain
pilin
Pseudomonas exotoxin
recombinant antibodies
vaccine
vaccine design
vaccines

Research: Bacterial protein toxins are profoundly damaging for mammalian cells. Interestingly, a subset of these toxins interact with very well-conserved and universally expressed cellular proteins. Among the toxins exhibiting these characteristics are *Pseudomonas* exotoxin (PE) and diphtheria toxin (DT), both of which inhibit protein synthesis. By removing either toxin's binding domain and replacing it with sequences encoding recombinant antibodies or ligands that bind cell surface receptors, it has been possible to make novel fusion proteins termed "recombinant immunotoxins." Immunotoxins are targeted to kill cancer cells expressing particular surface antigens or receptors. PE binds and enters cells using the low-density lipoprotein receptor-related protein (LRP) as its surface receptor. The toxin is delivered to an acidic endosomal compartment where it is cleaved by a furin-like protease to generate two large fragments. The N terminal fragment (28 kD) of PE is comprised primarily of the toxin's binding domain, while the C terminal fragment (37 kD) has the ADP-ribosylating activity and is translocation-competent. Translocation requires the reduction of the disulfide bond linking the two fragments and also the presence of a KDEL-like sequence at the C terminus. Currently, we are trying to understand the mechanism of cell-mediated toxin reduction. Our preliminary data indicate that furin-cleaved toxin must be unfolded before reductants can gain access to a key disulfide bond linking cyteines 265 and 287. Several projects are under way that use toxin sequences for the development of therapeutic agents. To produce *Pseudomonas* vaccine for cystic fibrosis patients, we are using nonlethal PE as an immunogenic carrier for the C terminal loop of pilin. Neuropeptides, including substance

P, conjugated to truncated PE are being used to target neurons involved in the transmission of chronic pain signals.

Collaborators on these projects include Guojun Bu, Washington University, St. Louis; Ralf Hertle, Institute for Microbiology, Turbingen, Germany; Mike Iadarela, NIH; Jonathan LaMarre, Guelph; and Randall Mrsny, Genentech Inc.

Recent Publications:

Mansfield E, et al. *Blood* 1997;90:2020–6.

McKee M, et al. *Biochemistry* 1999;38:16507–13.

FitzGerald DJ, et al. *J Biol Chem* 1998;273:9951–8.

Mrsny RJ, et al. *Vaccine* 1999;17:1425–33.

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Biography: *Dr. Garges received her Ph.D. in microbiology in 1983 from the University of Maryland. She carried out her postdoctoral research at the NIH and has remained at the NCI. Dr. Garges serves on the editorial board of the* Journal of Bacteriology.

Laboratory of Molecular Biology
Genetic Regulatory Mechanisms in *E. coli* and Its Bacteriophage

Keywords:
bacterial genetics
protein structure
transcription
transcriptional regulation

Research: RprA acts by a mechanism similar to that of DsrA in stimulating RpoS synthesis, but is regulated not by low temperature but by a two-component regulatory system responsive to cell surface status. Thus, these two small RNAs allow two very different environmental signals to be sensed for increased RpoS synthesis.

With our collaborators, we have undertaken a genome-wide search for other small regulatory RNAs. We find that highly conserved stretches with the intergenic regions are reliable hallmarks of small RNAs. This study led to the identificationof 17 novel small RNAs as well as 6 new, short RNAs. The function of some of these new small RNAs is under study; a substantial number of them appear to be involved in translational regulation. This work as well as our previous studies of DsrA and RprA suggest that small RNAs are important and underappreciated components of many regulatory circuits.

Collaborators on this research include Michael Maurizi, Gisela Storz, and Sue Wickner, NIH; Carsten Rosenow, Affymetrix; and Thomas Silhavy, Princeton University.

Recent Publications:

Ryu S, et al. *J Biol Chem* 1995;270:2489–96.

Flatow U, et al. *J Bacteriol* 1996;178:2436–9.

Jakubczak J, et al. *Proc Natl Acad Sci USA* 1996;93:9073–8.

Roy S, et al. *J Biol Chem* 1998;273:14059–62.

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Biography: *Dr. Gottesman received her Ph.D. from the Department of Microbiology at Harvard University and became a postdoctoral fellow at the NIH. She then went to the Massachusetts Institute of Technology (MIT) as a research associate and returned to the NIH in 1976 as a senior investigator in the Laboratory of Molecular Biology, where she has remained. She was elected to the National Academy of Sciences in 1998 and the American Academy of Arts and Sciences in 1999 in recognition of her work on energy-dependent proteolysis.*

Laboratory of Molecular Biology
Energy-Dependent Proteolysis and Small RNAs: Novel Modes for the Regulation of Gene Expression

Keywords:
ATPase
bacterial genetics
E. coli
gene regulation
proteases
proteolysis
regulatory RNA
Rpos

Research: We have used the ATP-dependent proteases of *E. coli* and their targets as models for the study of the mechanism of proteolysis, the basis for target selection, and the ways in which unstable proteins are used in regulatory cascades. The primary cytoplasmic proteases in both prokaryotes and eukaryotes are energy dependent. These proteases play essential roles in the degradation of abnormal and misfolded proteins and in the setting of appropriate levels for critical, short-lived regulatory proteins. We have focused on the Lon protease and the Clp family of proteases. Clp proteases are made up of multiple copies of an ATPase subunit (ClpX or ClpA) and a protease subunit (ClpP). Changing the ATPase subunit of the complex changes the substrate specificity, suggesting that the selection of substrates is mediated through the ATPase subunit and presumably is regulated in part by ATP hydrolysis. After substrate selection, the ATPase domain unfolds the substrate and translocates it to the protease domain. In Lon mutants that are unable to degrade substrates, this results in a sequestration phenotype, in which the substrate is protected by Lon from other proteases.

RpoS, a stress sigma factor of *E. coli*, is rapidly degraded during exponential growth by the ClpXP protease; this degradation is in turn regulated by the response regulator protein RssB. We have found that RssB affects degradation only of RpoS, and not of another ClpXP substrate, lambda O protein. This suggests that environmental and cell cycle regulation via changes in protein degradation may operate by modifying substrate availability rather than protease activity. In vitro collaborative studies with Dr. Sue Wickner demonstrate that RssB acts directly, first interacting with the substrate and

then delivering it to the protease. Thus, regulated degradation probably is dependent on the activation of RssB, which appears to require phosphorylation. The signaling pathway for phosphorylation is under investigation.

In examining the regulation of genes encoding the Lon substrate RcsA, we identified a novel 85 nucleotide stable RNA, DsrA. DsrA is synthesized preferentially at low temperatures (<30 °C) and is necessary for the low-temperature expression of RpoS. DsrA modulates RpoS synthesis by positively affecting translation of this protein by pairing with parts of the RpoS untranslated leader. A second small RNA regulator of RpoS, RprA, has also been identified. RprA acts by a mechanism similar to that of DsrA in stimulating RpoS synthesis, but is regulated not by low temperature but by a two-component regulatory system responsive to cell surface status. Thus, these two small RNAs allow two very different environmental signals to be sensed for increased RpoS synthesis.

In collaboration with others, we have undertaken a genome-wide search for other small regulatory RNAs. We find that highly conserved stretches with the intergenic regions are reliable hallmarks of small RNAs. This study led to the identification of 17 novel small RNAs as well as 6 new, short mRNAs. The function of some of these new small RNAs is under study; a substantial number of them appear to be involved in translational regulation. This work as well as our previous studies of DsrA and RprA suggest that small RNAs are important and underappreciated components of many regulatory circuits.

Collaborators on this research include Michael Maurizi, Gisela Storz, and Sue Wickner, NIH; Carsten Rosenow, Affymetrix; and Thomas Silhavy, Princeton University.

Recent Publications:

Majdalani N, et al. *Mol Microbiol* 2001;39:1382–94.

Repoila F, et al. *J Bacteriol* 2001;183:4012–23.

Zhou Y, et al. *Genes Dev* 2001;15:627–37.

Wassarman KM, et al. *Genes Dev* 2001;15:1637–51.

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Laboratory of Molecular Biology
Transcription Machinery and Its Mechanism in *E. coli*

Keywords:
bacterial genetics
E. coli
gene expression
protein chemistry
RNA polymerase
transcription
transcription factors

Research:

Overview

The goal of our research is to understand the *E. coli* transcriptional machinery and its mechanism. Regulation of transcription is a key step in controlling gene expression in all cells. The basic structure and function of RNA polymerase (RNAP)/RNAP-associated proteins are conserved throughout evolution. The sophisticated genetics and advanced biochemistry make *E. coli* RNAP an ideal model system. To that end, *E. coli* RNAP and its associated proteins will be studied by combining biochemical and genetic approaches. Important domains in RNAP will be identified by isolating and characterizing mutant RNAPs that have altered particular functions in transcription. Transcription mechanisms will be dissected by comparing biochemical properties of wild-type and mutant RNAPs at specific steps in the transcription process. Currently, we have focused on the following studies.

The Mechanisms of the Stringent (Nutrient Starvation) Response

Under optimal growth conditions, rapidly dividing *E. coli* cells transcribe a set of genes at a very high rate. These genes engage most of the RNAP molecules in the cell, although they constitute only a small fraction of the genome. In contrast, nutrition-limiting conditions, such as amino acid starvation, cause a rapid accumulation of the guanine derivative, ppGpp, and a dramatic reduction in expression of these genes, a process called the stringent (nutrient starvation) response. Recently, we have identified a unique feature governing the interaction between RNAP and a class of promoters that are sensitive to the nutrient starvation response. The initiation complexes of these stringent promoters are intrinsically unstable, and can alternate between relatively stable and metastable states depending on the superhelicity of the DNA template. We hypothesized that modulation of the stability of open complexes at these promoters is a regulatory step and proposed a model to link transcription and the stability of the initiation complexes at these promoters. To test this model we have determined roles of the *cis* and *trans* elements that regulate the expression of these genes coordinately. In addition, we have continued to isolate and

analyze RNAP mutants that altered interaction with stringent promoters to identify the sites in RNAP that are important in the process.

Interaction Between Core RNAP and Sigma Factors

Since the binding of core RNAP with different sigma factors is, operationally, the first step in transcription initiation, it is a critical step in controlling global gene expression. We have studied this interaction with an emphasis on the role of core RNAP. We have determined the elements that influence the interaction between core RNAP and sigma factors and developed genetic systems to identify the interface between core RNAP and sigma factors. Several sigma mutations that were defective in core RNAP binding conferred temperature sensitive growth phenotype. We have isolated second site mutations in core RNAP that conferred temperature resistant growth phenotype of these sigma mutants. We have continued to characterize these suppressor mutations in core RNAP.

Functions of RNAP-Associated Proteins

Recently, we identified a novel RNAP-associated protein, an ATPase named "RapA." The RapA protein is a homolog of the SWI/SNF family of eukaryotic proteins. We showed that RapA forms a stable complex with RNAP holoenzyme as if it were a subunit of RNAP, and the ATPase activity of the holoenzyme RNAP–RapA complex is stimulated when compared to the RapA protein alone. We have studied further the interaction between RapA and RNAP (core and holoenzyme) and performed cross-linking experiments to identify the subunit(s) of RNAP that interact with the RapA protein. In addition, we have analyzed the expression of the *rapA* gene under different physiological conditions aiming at understanding the function of RapA inside the cell, and studied the interaction between RNAP and the *rapA* promoter in vitro.

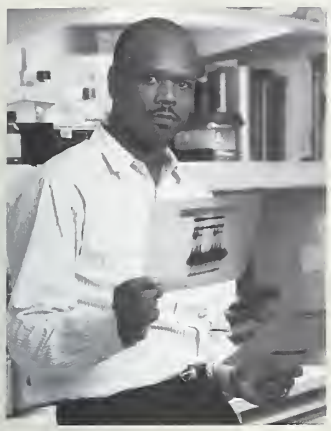
Recent Publications:

Joo DM, et al. *J Bacteriol* 1998;180:1095–102.

Zhou YN, et al. *Proc Natl Acad Sci USA* 1998;95:2908–13.

Sukhodolets MV, et al. *J Biol Chem* 1998;273:7018–23.

Sukhodolets MV, et al. *J Biol Chem* 2000;275:22090–7.



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Laboratory of Molecular Biology
Transcription Factors Regulating Expression of the Epidermal Growth Factor Receptor Gene

Keywords:
cell growth regulation
transcription factor
transcriptional regulation
transcriptional repressor

Research: Growth factor receptors are involved not only in promoting the proliferation of normal cells but also in the aberrant growth of many types of human tumors. For example, the epidermal growth factor receptor (EGFR) is found to be mutated and/or overexpressed in human carcinomas. The expression of specific genes is dependent on the interaction of nuclear proteins with the promoter regions of the genes. Our research goal is to identify and characterize transcription factors that regulate the expression of the EGFR gene. This will aid in understanding how EGFR levels are elevated in human cancers.

The regulatory region of the EGFR gene contains binding sites for a large number of transcription factors including SP1, AP2, and p53. EGFR gene expression is modulated by a variety of agents such as phorbol esters and interferons. We have shown that induction of EGFR gene transcription by phorbol-12-myristate-13-acetate (PMA), a phorbol ester, is mediated by an increase in binding of transcription factor AP2 to five sites in the EGFR promoter and AP1 binding to at least seven sites. This leads to a five-fold increase in EGFR gene transcription. Interferons also enhance EGFR gene expression. We have shown that interferon-regulated factor–1 (IRF–1) binds to an upstream region of the EGFR promoter and increases promoter activity. IRF–1 can increase endogenous EGFR levels 10-fold in transfection experiments. These results indicate that regulation of EGFR promoter activity involves the direct binding of specific transcription factors that may be activated by different types of agents.

We have recently identified and initiated characterization of a repressor of EGFR promoter activity that we have termed "GCF2" (GC-binding factor 2). GCF2 has homology to the DNA-binding region of GCF, a previously characterized transcriptional repressor. GCF2 binds to the EGFR promoter and represses activity of the promoters for SV40, RSV and EGFR. We have recently shown that GCF2 binds to and represses the promoter of the platelet-derived growth factor A-chain promoter. The mechanism of repression is consistent with competition with activator proteins for binding to the promoter region. Mutation of the GCF2-binding site in the EGFR

promoter abolishes the ability of GCF2 to repress the promoter activity. Thus, GCF2 may be important for downregulation of promoter activity. GCF2 is also able to repress the transcriptional activity of the HIV–LTR.

The GCF2 cDNA hybridizes to an mRNA of 4.2 kb that has high levels of expression in peripheral blood leukocytes and Burkitt's lymphoma cell lines. In heart and skeletal muscle, the GCF2 mRNA has a size of 2.9 kb. GCF2 mRNA is expressed at almost undetectable levels in brain and testis. Although multiple mRNA species are detected in RNA isolated from a number of tissues, only the 4.2 kilobase mRNA is found in cancer cell lines. GCF2 produced in vitro or detected in extracts from cells migrates as a 160 kDa protein during SDS-polyacrylamide gel electrophoresis and is found in higher levels in lymphoma cell lines.

We have recently isolated and cloned the rat EGFR promoter. The rat promoter is less GC-rich as compared to the human promoter but does contain binding sites for Sp1. The rat and human promoters both contain TCC repeats and multiple transcriptional initiation sites. We have shown that the TCC repeats in the rat promoter bind WT1 (Wilms' tumor gene product) and loss of WT1 binding mediates downregulation of EGFR upon nerve growth factor treatment of PC12 cell.

Our analysis indicate that regulation of EGFR promoter activity is complex and involves the interaction of many transcription factors. Elucidating the role of specific transcription factors in modulating EGFR gene expression in normal and cancer cells should provide insight as to how overexpression of the EGFR gene occurs in some cancers without gene amplification.

Collaborators on this research include Michael Birrer and Gordon Guroff, NIH; Levon Khachigian, University of New South Wales, Sydney, Australia; and Masato Takimoto, Hokkaido University School of Medicine, Hokkaido, Japan.

Recent Publications:

Khachigigan L, et al. *Circ Res* 1999;84:1258–67.

Johnson AC, et al. *Mol Med* 2000;6:17–27.

Liu X–W, et al. *J Biol Chem* 2000;275:7280–8.

Liu X–W, et al. *J Biol Chem* 2001;296:5068–73.

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Biography: *Dr. Kreitman received his M.D. from Ohio State University in 1985 and obtained his internal medicine residency training at Duke University from 1985 to 1988. He received his medical oncology fellowship training at the NIH from 1988 to 1991, has been working in the immunotoxin field since 1989, and has been directing clinical trials with immunotoxins since 1996. He won Federal Technology Transfer awards in 1994 and 1999 and the Commendation Medal from the U.S. Public Health Service in 1999.*

Laboratory of Molecular Biology
Development of Recombinant Toxins for Hematologic Malignancies

Keywords:
Burkitt's lymphoma
cancer immunotherapy
clinical trial
cytokines
DNA immunization
HTLV–1
immunotherapy
immunotoxins
lymphoma
monoclonal antibodies
monocytes/macrophages
multiple myeloma
Pseudomonas exotoxin
recombinant antibodies
T cells
TNF/LT
tumor antigens
vaccinia virus

Research: Recombinant toxins are proteins made in bacteria and they contain two parts, one for binding selectively with certain types of cells and one that kills the cell. We have been developing recombinant toxins which bind selectively to malignant hematopoietic cells, such as leukemias, lymphomas, Hodgkin's disease, and multiple myeloma. Some also bind to solid tumors such as glioblastoma, lung, breast and gastrointestinal carcinomas. Several of the agents developed in our lab have been tested in patients with hematologic malignancies, and in these trials exciting clinical remissions have been observed. Being board-certified and NCI-trained in medical oncology has allowed us to direct of several of these trials at the NIH clinical center. This allows our research lab to become focused on relevant clinical issues for improvement of immunotoxin effectiveness, and provides excellent access to the patient samples needed for successful laboratory research studies. One clinical phase I trial is with anti-Tac(Fv)-PE38 (LMB–2), a recombinant toxin which has shown promising preclinical activity and clinical responses against malignant cells displaying interleukin 2 receptors, including B and T cell leukemias and Hodgkin's disease. We have also initiated a phase I clinical trial of RFB4(dsFv)-PE38 (BL22) for patients with CD22+ lymphomas and leukemias. BL22 is the first agent since the standard purine analogs which is capable of inducing complete remissions (CRs) in the majority of hairy cell leukemia (HCL) patients treated, and is the only agent with a high CR rate in purine analog-resistant HCL, including a poor prognosis variant HCL-v. Sparing of resting T cells allows both repeated cycles and induction of cytotoxic T cells, a major advantage over purine analogs. In addition, a circularly permuted interleukin 4 toxin designed in the laboratory is being tested outside the NIH in patients with glioblastoma multiforme. A fourth molecule created in our lab, DTGM, is a fusion toxin containing human GM–CSF and truncated diphtheria toxin. DTGM is currently undergoing phase I testing outside the NIH for relapsed acute myelogenous leukemia. Our group has recently taken over the direction of NCI clinical trials of immunotoxins targeting solid tumors, such as breast, ovarian, colon, gastric, pancreatic, head and neck, and bladder cancers, as well as mesotheliomas.

A clinical trial in patients with mesothelioma and ovarian cancer has been initiated with an antimesothelin recombinant immunotoxin administered by continuous infusion to improve delivery of the protein toxin molecules to solid tumor cells.

Our recombinant immunotoxins target mutated forms of *Pseudomonas* exotoxin to bind to the surface of cancer cells. After internalization, a smaller fragment is translocated to the cytosol, resulting in cell death by inhibition of protein synthesis and apoptosis. Since the toxin ADP ribosylates elongation factor-2 catalytically, one molecule is sufficient to kill a cell. The mutated toxin is fused to a binding domain, either a growth factor or a single-chain antibody, which binds selectively to cancer cells. Cells with several hundred or thousands of binding sites per cell may internalize enough so that one or several molecules reach the cytoplasm. Normal cells, which have much fewer binding sites per cell, are protected. Recombinant toxins are made by using recombinant DNA techniques to design a plasmid encoding the protein molecule, expressing the plasmid in bacteria and then purifying the protein so that it binds selectively to cancer cells. The cytotoxic activity is determined by incubating the recombinant toxins with tumor cells in tissue culture, and determining cell killing or inhibition of protein synthesis.

Recombinant toxins with good cytotoxic activity are then tested in mice bearing human tumor cells to see if the mice can be cured at safe doses. If so, the lab consults the FDA and CTEP to decide which safety studies should be done to determine if it is safe to administer the drug to people. We are uniquely positioned to test the most promising candidate agents here at the NIH to determine their effectiveness in humans.

A major emphasis deals with basic science questions related to intracellular metabolism and transport of toxins, and recombinant antibody engineering. The lab has recently reported the isolation of new single-chain antibodies by phage-selection after DNA immunization of mice with the target antigen. The antigens targeted include those present on a wide range of myeloid and lymphoid leukemias, as well as Hodgkin's disease.

Recently, major emphasis has been placed on defining the etiology and prevention of the cytokine release syndrome, also called systemic inflammatory response syndrome (SIRS), which is a side effect of many recombinant toxins and monoclonal antibodies given to patients with leukemias. Methods to prevent SIRS are now being used in patients treated under our protocols.

One of our most exciting questions to research involves expanded cytotoxic T cell clones observed in patients with hairy cell leukemia. It is possible that they are an important immune response of patients against their leukemia which is blunted by the disease burden and by chemotherapy, but which is not blocked by immunotoxins. Laboratory studies should help explain how expansions of cytotoxic T cells are related to responses in patients.

Our lab has taken advantage of the potency of recombinant immunotoxins toward chemotherapy-resistant cancer cells to both successfully treat patients with these malignancies and also to utilize both clinical and basic science

resources to study what these interesting proteins are doing after injection into patients. Answers to these questions should lead to improved clinical approaches both within and outside the immunotoxin field.

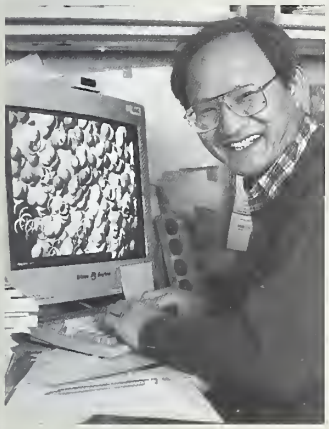
Recent Publications:

Kreitman RJ, et al. *Blood* 1999;94:3340–8.

Kreitman RJ. *Curr Opin Immunol* 1999;11:570–8.

Kreitman RJ, et al. *J Clin Oncol* 2000;18:1622–36.

Robbins DH, et al. *Clin Cancer Res* 2000;6:693–700.



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Biography: *Dr. Lee received his Ph.D. from Cornell University in 1967 and studied at Yale University. From 1970 to 1980 he taught at the University of Kansas. He has been at the NIH since 1981 and in his present position since 1991.*

Laboratory of Molecular Biology
Protein Structure Modeling and Bioinformatics

Keywords:
antibody engineering
bioinformatics
gene discovery
immunotoxin
protein folding
protein stability
protein structure

Research: We (1) study the structure of globular protein molecules and the forces that determine the structure, stability, and interaction of these molecules, (2) design mutations that will alter/improve properties of a class of anticancer immunotoxins and of other specific protein molecules, and (3) analyze the expressed sequence tag (EST) DNA sequence database to discover genes that are specifically expressed in a particular organ or tumor. The product of such genes can potentially be used as a target for delivery of antitumor agents and for tumor imaging.

Following is a more specific description of the current research projects and results: (1) We found by a theoretical analysis of the hydration thermodynamics of aromatic compounds that the substantial solubility of these compounds in water is due to the strong van der Waals interaction between water and the aromatics rings. The hydrophobic and hydrophilic dual character of these molecules explains the abundance of aromatic amino acids in membrane proteins at water-membrane interface and in globular proteins at protein-protein interface. (2) Two new protein conformational search procedures have been designed for use in ab initio protein structure prediction. One method was used to discover a couple of serious defects in the statistical "threading potential" when it is used in an ab initio folding process. The second combines a novel fast local move in the dihedral angle space, a "window growth" procedure, and an evolutionary algorithm. We are

currently developing a potential function that will go with this latter search procedure. (3) We designed two protein structure comparison procedures, one for the global and the other for the local comparisons. We used the former procedure to automatically cluster all protein structures. We are currently using the second to find domains and to develop a new set of "threading potential." (4) In collaboration with the Molecular Biology Section, we developed procedures for identifying mutation sites that, when altered, will increase the stability or reduce the nonspecific toxicity of immunotoxins. The procedures have been successfully tested experimentally on several specific immunotoxin species. (5) We developed a new procedure for clustering EST sequences that take full advantage of the draft human genome sequence. We also developed a local genome browser that is patterned after the "Golden Path" browser of Jim Kent at the University of California at San Diego and which is particularly convenient for spotting organ-specific EST clusters. We expect to be able to find new prostate- and breast-specific genes more easily using these tools.

For more information and for information on the software that we developed, please visit our web site at **http://www.lmbbi.nci.nih.gov.**

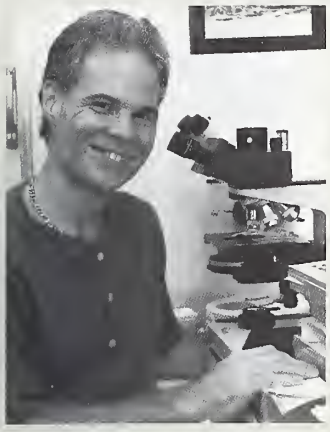
Recent publications:

Jung J, et al. *Protein Sci* 2000;9:1576–88.

Liu XF, et al. *Cancer Res* 2000;60:4752–5.

Yerushalmi N, et al. *Gene* 2001;265:55–60.

Liu XF, et al. *Prostate* 2001;47:125–31.



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Biography: *Dr. Merlino received his Ph.D. in 1980 from the Department of Cellular and Molecular Biology at the University of Michigan. He currently serves as the NIH ombudsman, and is on the editorial board of the* American Journal of Pathology.

Laboratory of Molecular Biology
Receptor Tyrosine Kinase Signaling in Mouse Models of Human Cancer

Keywords:
animal models
autocrine
dominant-negative receptor
growth factors
melanoma
metastasis
receptor tyrosine kinase signaling
transgenic mice

Research: Eukaryotic cells communicate through an intricate network of signaling pathways designed to exquisitely regulate cellular activities such as growth, differentiation, migration, and apoptosis. Signaling, and therefore biological response, can be initiated when specific peptide growth factors interact with their high-affinity cell surface receptors, many of which are powerful tyrosine kinases (RTKs) encoded by proto-oncogenes. RTK signal transduction is frequently dysregulated in tumorigenesis. The main aim of the Molecular Genetics Section is to create genetically engineered mice that serve as models of human cancer and to use these models to determine how RTK function is subverted in tumor formation and progression. We have chosen the melanocyte as a model system to study RTKs because it is unusual in its dependency for normal development and function on a multitude of tyrosine kinase receptors, including the hepatocyte growth factor/scatter factor (HGF/SF) receptor c-Met. Moreover, melanocytic tumors deserve extraordinary attention because they are arising with increasing incidence and are among the most aggressive types of human cancer. If untreated, virtually every malignant melanoma has the potential to metastasize, after which patient prognosis is extremely poor.

HGF/SF is a multifunctional factor capable of eliciting mitogenic, motogenic, and morphogenic responses in various c-*met*-expressing epithelial cells and in melanocytes. Furthermore, c-*met* is essential for normal embryogenesis, and has been implicated in the development of many human tumors, including malignant melanoma. We have determined that overexpressing an HGF/SF transgene in mice perturbs normal melanocyte development, and induces in aged animals cutaneous malignant melanoma with metastatic capability, a phenotype that is exceedingly rare in mice. Typically, these tumors showed both high expression of transgenic HGF/SF and enhanced c-Met expression and kinase activity, indicating that the creation and selection of cells harboring HGF/SF–Met autocrine signaling loops is the mechanistic basis of tumorigenesis in this transgenic model. We subjected this transgenic mouse model to various regimens of ultraviolet (UV) irradiation to determine experimentally the role of solar radiation in melanomagenesis. We found that chronic adult suberythemal UV irradiation failed to accelerate melanoma

formation in HGF/SF-transgenic mice. In contrast, a single dose of neonatal erythrogenic UV radiation induced highly penetrant cutaneous melanoma, arising with a histopathologic and molecular pathogenetic profile remarkably similar to human melanoma. Our data present the first experimental validation of epidemiological evidence that childhood sunburn poses a significant risk for malignant melanoma.

To determine if melanomagenesis can be facilitated by the absence of the *INK4a* tumor suppressor locus (encoding both 16INK4a and p19ARF), we placed the HGF/SF transgene on an *ink4a* null background. Unexpectedly, we found that virtually all mice expressing the HGF/SF transgene and deficient for *ink4a* developed highly invasive, multicentric skeletal muscle tumors by 3.3 months of age. These neoplasms resembled human rhabdomyosarcoma (RMS) morphologically and at the molecular level; both mouse and human RMS demonstrated disruption of the c-*MET*, *pRB*, and *p53* pathways. This mouse model has provided genetic evidence that c-*MET* and *INK4a/ARF* pathways represent critical, synergistic targets in RMS pathogenesis, and that simultaneous disruption of myogenic growth and differentiation drives rhabdomyosarcomagenesis.

Our collaborators include Ronald DePinho, Dana-Farber Cancer Institute; Lee Helman, NIH; and Frances Noonan, George Washington University.

Recent Publications:

Otsuka T, et al. *Cancer Res* 1998;58:5157–67.

Merlino G, et al. *Oncogene* 1999;18:5340–8.

Otsuka T, et al. *Mol Cell Biol* 2000;20:2055–65.

Noonan F, et al. *Cancer Res* 2000;60:3738–43.

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Biography: *Sue Wickner received her Ph.D. from Albert Einstein College of Medicine where she studied biochemical mechanisms involved in DNA replication with Dr. Jerard Hurwitz. She continued working on DNA replication as a postdoctoral fellow with Dr. Martin Gellert at the NIH, then moved to the Laboratory of Molecular Biology in the NCI. She received an NIH Award of Merit in 1995.*

Laboratory of Molecular Biology
Mechanisms of Action of Molecular Chaperones in DNA Replication and Proteolysis

Keywords:
heat shock proteins
molecular chaperones
proteases

Research: Our section studies ATP-dependent molecular chaperones, proteins that facilitate protein folding, unfolding, and remodeling. Chaperones are present in all organisms and are highly conserved throughout evolution. Many are induced by environmental stresses such as heat shock. They participate in various cellular processes including DNA replication, regulation of gene expression, cell division, protection and recovery from stress conditions, membrane translocation, and protein degradation. Not only are chaperones of biological importance, but they also are medically relevant because a number of diseases, such as Alzheimer's, Parkinson's, and Huntington's disease, and systemic amyloidoses, are caused by aberrant protein folding reactions that result in protein aggregation.

Several groups, including ours, have demonstrated that a large ubiquitous family of homologous proteins, referred to as Clp or Hsp100 proteins, have ATP-dependent molecular chaperone activity. While some Clp proteins function exclusively as chaperones, others act both as chaperones and as regulatory components of two component energy-dependent proteases. Structural studies have shown that the proteolytic sites of the protease component are sequestered within a chamber formed by stacked rings of protease subunits and capped on either end by the Clp chaperone component. The Clp component acts as a gatekeeper regulating entry into the chamber.

We are currently exploring the mechanisms by which Clp chaperones and proteases act together to regulate protein activity and degradation. More specifically we are investigating the role of ClpA and ClpX, two *E. coli* Clp proteins and the regulatory components of the ATP-dependent ClpAP and ClpXP proteases, in protein remodeling and degradation. Using GFP fusion proteins containing specific recognition signals, we are elucidating how Clp chaperones recognize and bind substrates, how they catalyze protein unfolding, and how unfolded substrates are translocated from their binding sites on a Clp chaperone to the cavity of the proteolytic component.

Another important question we are addressing is how regulatory proteins act to target specific substrates for degradation. We are studying the regulation of degradation of the *E. coli* stationary phase sigma factor, sigma S, by a response regulator protein, RssB, in conjunction with the ClpXP protease. In vitro, ClpXP alone degrades sigma S poorly and the addition of RssB greatly stimulates the reaction. Phosphorylation of RssB is required for its stimulatory activity. We are currently dissecting the reaction into partial reactions to understand how this unique targeting protein, whose own activity is regulated by phosphate transfer reactions, regulates proteolysis by catalyzing the presentation of a specific substrate to a specific protease.

Collaborators on this research include Susan Gottesman, Joel Hoskins, Soon Young Kim, Michael Maurizi, and Suveena Sharma, NIH.

Recent Publications:

Wickner S, et al. *Science* 1999;286:1888–93.

Hoskins JR, et al. *Proc Natl Acad Sci USA* 2000;97:8892–7.

Hoskins JR, et al. *J Biol Chem* 2000;275:35361–7.

Zhou Y, et al. *Genes Dev* 2001;15:627–37.

Laboratory of Molecular Cell Biology

The Laboratory of Molecular Cell Biology (LMCB) investigates basic cellular processes of homeostasis, growth, division, and differentiation, with an emphasis on the molecular biology of chromosomes and the cell nucleus. The laboratory combines biochemical and genetic approaches in an interactive environment to investigate areas of chromosome biology and related topics in current biochemistry and molecular biology.

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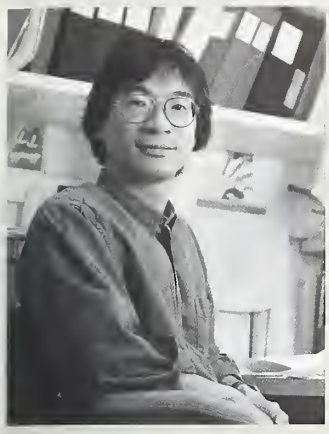

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Biography: *Dr. Wu obtained his Ph.D. in 1979 and conducted postdoctoral research at Harvard University. In 1982, he moved to the Laboratory of Biochemistry at the NCI. He was appointed chief of the Laboratory of Molecular Cell Biology in 1996. Dr. Wu received the 1987 Outstanding Young Scientist Award from the Maryland Academy of Sciences and the 1992 Young Investigator Award from the American Society of Biochemistry and Molecular Biology. He was elected to the American Academy of Arts and Sciences in 1998.*

Laboratory of Molecular Cell Biology
Eukaryotic Chromatin Structure and Gene Regulation

Keywords:
chromosome
Drosophila
gene
heat shock
nucleosome
transcription

Research: The packaging of eukaryotic DNA in nucleosomes and the condensation of nucleosome arrays in chromatin restrict access to the genome. Our group is interested in the function of large, multiprotein complexes that use the energy of ATP hydrolysis to unravel chromatin and facilitate access to DNA.

Our early studies revealed the presence of local, highly accessible (DNase I hypersensitive) sites in cellular chromatin at the start of *Drosophila* heat shock genes. Analysis of the substructure of DNase hypersensitive sites demonstrated the first in vivo footprints of transcription factors and led to the suggestion that transcription factor binding could be responsible for changing chromatin architecture. We purified the heat shock transcription factor, one of several factors involved in activating transcription of heat shock genes, and showed that its activity is induced by a monomer-trimer transition in response to stress signals.

By studying how transcription factors remodel chromatin to increase accessibility, we discovered that nucleosome organization itself is highly dynamic. In a cell-free chromatin assembly system, reconstruction of DNase hypersensitivity required not only binding of a sequence-specific transcription factor (the GAGA zinc finger protein), but also a novel, ATP-dependent activity. Purification of the activity revealed a four-subunit protein complex called the nucleosome remodeling factor (NURF). We cloned genes corresponding to each NURF component and identified ISWI, a member of the SW12/SNF2 family of ATPases, as the energy consuming subunit. NURF induces structural changes in nucleosomes to increase their mobility on DNA. Nucleosome mobility appears to be important for the establishment of a gap to allow recruitment of the transcription machinery. We are currently studying the genetics, biochemistry, and biology of NURF, and are especially interested in understanding how ATP-hydrolysis by NURF is coupled to conformation changes of nucleosomes.

We have recently identified a new, ATP-dependent chromatin remodeling complex from budding yeast called the INO80 complex. The ~12-subunit INO80 complex was found to contain a number of interesting polypeptides, including actin, actin-related proteins, and proteins that show homology to the bacterial RuvB helicase. Genetic and biochemical studies indicate that the INO80 complex is involved in transcription and, directly or indirectly, in DNA repair. We are presently studying the structure and function of the INO80 complex in yeast and other model organisms.

Recent Publications:

Hamiche A, et al. *Cell* 1999;97:833–42.

Shen X, et al. *Nature* 2000;406:541–44.

Wu C, et al. ATP-dependent chromatin remodeling by the ISWI complexes. In *Chromatin Structure and Gene Expression*. Elgin SCR, et al., editors. London: Oxford University Press, 2000.

Mizuguchi G, et al. *J Biol Chem* 2001;276:14773–83.

Laboratory of Molecular Immunoregulation

The Laboratory of Molecular Immunoregulation performs fundamental research studies of the role of cytokines and chemokines in inflammation, immunity, HIV–1 infection, angiogenesis, hematopoiesis, and cancer. LMI scientists engage in the discovery and characterization of new cytokines, study the regulation of cytokine production at the gene level (promoters and transcription factors), the action of cytokines on target cells (studies of receptors, second and third messengers), and cytokine regulation of pathophysiological processes.

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The lab consists of nine interactive groups:

- The Cytokine Molecular Mechanisms Section (CMMS), headed by Dr. William Farrar, studies cytokine receptor-initiated signal transduction mechanisms and the crosstalk between cytokine transcription factors and nuclear receptor families.
- Dr. Sergei Nedospasov heads the Cytokine Targeting and Gene Regulation Group, which studies gene regulation and the physiological role of the TNF/LT family of cytokines using a variety of knock-out models including "conditional" inactivation of genes in selected tissues and gene profiling techniques.
- Dr. Oppenheim, along with Drs. Zack Howard and Oleg Chertov, is studying the structure/function relationships of the family of chemoattractant cytokines. They are investigating the role of such chemokines in inflammation, immunity, and downstream effectors such as defensins and cathepsin G. Dr. Oppenheim is also investigating the regulation of angiogenesis by chemokines and identifying chemokine inhibitors.
- Dr. Scott Durum, head of the Immunological Cytokine Research Group, is investigating the role of cytokines in the development of T lymphocyte and natural killer cell lineages, the potential mutagenic role of cytokines, and their role in terminal differentiation and apoptotic cell death.
- Dr. Kathrin Muegge, head of the Lymphocyte DNA Repair Research Group, studies the effect of cytokines on T cell receptor gene rearrangement and the regulation of chromatin accessibility by genomic methylation and histone acetylation.
- Dr. Jonathan Keller, head of the Hematopoiesis and Gene Therapy Group, is engaged in studies of the biological and molecular mechanisms that regulate the survival, growth, and differentiation of stem cells in the cloning

of novel genes in the hematopoietic pathway and in applying this data to improve gene transfer.

- Dr. Ji Ming Wang, head of the Chemoattractant Receptor and Signal Group, is engaged in studies on the role of chemoattractant receptors in host defense against HIV–1 infection and the pathogenesis of neurodegenerative diseases.
- Dr. Teizo Yoshimura, head of the Immunobiology Group, is investigating the inductive signals and role of neutrophil-derived chemokines and other gene products, as detected by gene chip arrays, in the development of chronic (delayed-type hypersensitivity) inflammation.
- Dr. William J. Murphy, head of the Transplantation Biology Group, is engaged in examining means to improve the efficacy of bone marrow transplantation (BMT) for the treatment of cancer and other disease states. Murine models are used to develop means to enhance immunotherapeutic approaches in BMT.

Laboratory of Molecular Immunoregulation Staff

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Cheryl Nolan	Support Staff

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You Hong Cui	Special Volunteer
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Steve Stull	Technician
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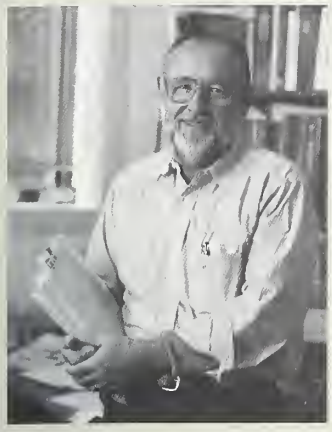

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Biography: *Dr. Oppenheim obtained his M.D. degree from the Columbia College of Physicians and Surgeons, New York, trained as a clinical associate at the National Cancer Institute (NCI), Bethesda, Maryland, and was a postdoctoral fellow at the University of Birmingham, England, in immunology. He returned to the National Institute of Dental Research and subsequently headed the Section of Cellular Immunology there and, since 1983, has been head of the Laboratory of Molecular Immunoregulation, NCI–Frederick.*

Laboratory of Molecular Immunoregulation
The Role of Cytokines in Host Defense and Repair

Keywords:
chemokines
cytokines
defensins
receptors

Research: The largest subfamily of cytokines, known as chemokines, specializes in directing the migration and homing of inflammatory leukocytes, immunocompetent lymphocytes, endothelial cells, and dendritic cells. Chemokines have been shown to contribute to fetal development, hematopoiesis, inflammation, immune responses, angiogenesis, and lymphoid tissue organogenesis. They utilize seven transmembrane G-protein coupled receptors. We have analyzed the effects of naturally occurring mutants of CCR5 and found that a point mutation in the extracellular region near the membrane still permits HIV–1 invasion but no longer conveys chemotactic signals. Other more distal mutations in the N terminus block both HIV–1 invasion and chemotactic responses, while point mutations in the transmembrane region actually make the receptor more sensitive to chemokines without affecting HIV–1 invasiveness. Thus, natural mutants provide a good means of evaluating receptor structure/function relationships and signal transduction pathways.

Chemokines also promote the adhesiveness of target cells and regulate angiogenesis and may therefore promote tumor growth. We demonstrated that CXCR4 is the only chemokine receptor that is upregulated on endothelial cells (EC) by angiogenic factors such as VEGF and bFGF. The ligand for CXCR4, SDF–1, was found to be chemotactic for EC and to be angiogenic in a number of in vitro and in vivo assays. We are finding that about 50 percent of human melanomas and breast cell cancers spontaneously produce SDF–1 and will investigate whether they use SDF–1 to induce their own blood supply. Similarly, we have documented that MCP–1 and eotaxin are angiogenic. We have also observed that IL–8 induction of neutrophil degranulation results in the release of several immunostimulating molecules. This includes the α-defensins, which have been found to be chemotactic for a subset of T lymphocytes and immature dendritic cells. The epithelial cell-derived β-defensins have been shown to use the chemokine receptor for LARC, CCR6, as their receptor. Consequently, although the defensins are relatively small 3.5–4.0 kD molecules with direct antimicrobial activity, they can also function as "microchemokines" and act as signals from the innate host

response that galvanize adaptative immune responses. Another antimicrobial peptide cathelicidin similarly has been shown to activate immune responses.

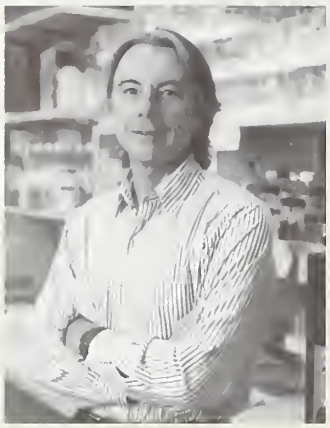
Recent Publications:

Rogers T, et al. *Ann NY Acad Sci* 2000;917:19–29.

Yang D, et al. *J Immunol* 2001;166:4092–8.

Yang D, et al. *J Leukoc Biol* 2001;69:691–7.

Salcedo R, et al. *J Immunol* 2001;166:7571–8.



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Biography: *After receiving his Ph.D. in 1978, Dr. Durum received his postdoctoral training in immunology at the National Jewish Hospital in Denver and at Yale Medical School. Dr. Durum joined the Laboratory of Molecular Immunoregulation in 1982.*

Laboratory of Molecular Immunoregulation
Cytokines and T Cell Development

Keywords:
apoptosis
cytokine
interleukin 7
signal transduction

Research: Our group has studied various aspects of cytokine function in T cell development. Lately we have focused on IL–7 because it has been clearly established in humans and mice that this cytokine is required for normal development of T cells in the thymus. Our goal is to understand the basis of this IL–7 requirement.

We have shown that IL–7 supports VDJ recombination in several different ways: IL–7 induces expression of the Rag proteins (which cleave DNA), it acts as a trophic survival factor for pro-T cells, protecting them from cell death during the VDJ recombination events, and, for some loci, IL–7 signals the opening of chromatin, rendering the locus accessible to cleavage. The trophic effect of IL–7 is a major area of current interest. This has been explored using pro-T cells, which die in the absence of IL–7, as well as in a newly developed thymocyte line that also dies upon withdrawal of IL–7. We have related this trophic effect to the synthesis of Bcl-2 family members and to blocking the translocation of Bax from cytosol to membrane fractions of the cell. Understanding the trophic activity of IL–7 may shed light on a general mechanism for the action of other trophic cytokines such as hematopoietins and neutrophins.

The signal transduction cascade leading from the IL–7 receptor to the gene rearrangement and trophic responses is being explored in our lab. At the receptor level, we have used knock-out mice to show that IL–7R, c and Jak3

are required for signaling the gene rearrangement. Current studies are being undertaken to identify the regions of the IL–7R intracellular domain that initiates different types of signals and to identify the proteins that bind to these regions. The substrates of the Janus tyrosine kinase Jak3 will be sought (these were previously thought to be Stat5α/β but this has recently been ruled out). To study the IL–7 receptor and postreceptor events, we developed a retroviral transfection system for introducing constructs into the IL–7-dependent thymocyte line and into lymphoid progenitors. These methods and approaches will allow us to address the question of whether the unique physiological requirement for IL–7 receptor signals is due to a unique signal transduction cascade.

We have collaborated in this research with O. Dammann, Harvard University; M. Dizdoroglu, National Institutes of Standards and Technology; L. Fliegel, University of Calgary; W. Leonard, NIH; and C. Thompson, University of Pennsylvania.

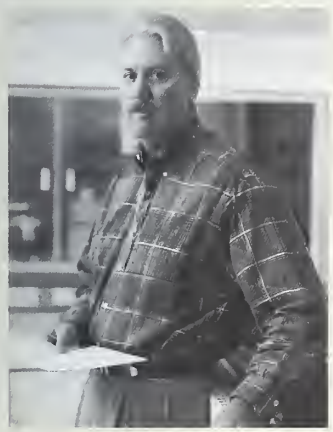
Recent Publications:

Hofmeister R, et al. *Cytokine Growth Factor Rev* 1999;10:41–60.

Khaled AR, et al. *Proc Natl Acad Sci USA* 1999;96:14476–81.

Benbernou N, et al. *J Biol Chem* 2000;275:7060–5.

Khaled AR, et al. *J Biol Chem* 2001;276:6453–62.



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Biography: *Dr. Farrar is the head of the Cytokine Molecular Mechanisms Section, Laboratory of Molecular Immunoregulation. He received his Ph.D. degree from Virginia Polytechnic Institute and State University. He came to the National Institutes of Health as a postdoctoral fellow in 1978 where he developed an interest in defining the role of cytokines in the development of the immune response. Since 1983, he has continued his studies in the lab, elucidating signal transduction pathways associated with cytokines and other endocrine factors.*

Laboratory of Molecular Immunoregulation
Molecular Interactions of Cytokine and Nuclear Receptor Signal Transduction

Keywords:
androgens
cancer cell growth
cell signaling

Research: The Cytokine Molecular Mechanisms Section has been involved in the establishment of models in which to characterize the intracellular biochemical signals stimulated by a number of potent biological factors that regulate the mature immune response and other extraimmune functions. We have focused on studies that involve the crosstalk between distinct transcription factor signaling systems. It has become increasingly clear from our work and that of others that growth factor signal pathways may be

communicating and interacting with numerous members of the steroid and nonsteroid nuclear receptor families. We have investigated the transcriptional crosstalk and regulation of three nuclear receptors, peroxisome proliferator-activated receptor-γ (PPARγ), androgen receptor (AR), and estrogen receptor (ER). We have identified a form of PPARγ receptor that, when activated by ligand in human T lymphocytes, potently blocks the transcription of IL–2. The mechanism apparently includes physical association with the NF–AT transcription factor and blockade of NF–AT function. Another unusual observation has been that IL–6 stimulates the growth of the human prostate cell line LNCaP. IL–6 apparently activates the AR in the absence of steroid by the activation of the JAK2–STAT3 pathway. In this instance, STAT3 was found to complex with AR and to activate AR dependent genes and cellular proliferation. These observations have far-reaching implications concerning the effects of inflammatory molecules on steroid-sensitive tumors and demonstrate a novel molecular mechanism for activating steroid receptors. Interestingly, many if not all multiple myeloma tumors express ER and respond to IL–6. We tested the effects of estrogen agonists on several MM tumor cell lines and found that they impair cell proliferation by a mechanism that may include cell death. Activated ER was found to block the IL–6 pathway by blocking the molecular action of STAT3. Investigations are under way to map the interactive domains of the described nuclear receptors and the STATs or cytokine transcription factors and to determine the exact molecular nature of the cross modulation of NR and these cytokine signal transcription factors.

The areas of transcriptional control have received increasing attention by our laboratory during the last few years. We have studied, to a large degree the upstream events and have now focused on the identity and consequences of transcriptional complexes that may contain generic transcription factors, coactivators, and possibly tissue-specific modifiers. We have developed a project area for the purpose of identifying and characterizing previously unknown transcription complexes.

Novel Transcription Factors in Cytokine-Signaling and Coactivators for Steroid Receptors

This project includes two different transcriptional activating systems. P48 is an important component of the IFN-γ signal pathway. P48 complexes with STAT1 and STAT2 to form the ISGF–3 complex that mediates many genes involved in the antiviral, antitumor, and immune responses mediated by IFN-γ. Little is known about the regulation of the p48 molecule itself, except that it too is transcriptionally regulated by IFNs. We have identified a unique region of the IFN-γ inducible promoter of the p48 gene, called GATE. Employing a yeast one hybridization system containing copies of the GATE promoter, we have screened a human monocyte cell line library for the expression of proteins that may activate the GATE promoter. Five isolated clones showed homology to three different genes. Two of the isolated genes revealed a 100 percent identical sequence with C/EBP protein family members. Currently, we are studying one member of the C/EBP family that appears to function in the GATE complex, and experiments with this transcription factor in knock-out mice are planned.

Our endeavor to study transcriptional complex regulation is not only limited to cytokine inducible genes, but also to our lateral and overlapping paradigm that involves nuclear receptors. Using a yeast model, we have screened for genes expressed by the human LNCaP cell line that will coactivate the AR. A few potential clones have been isolated and are under study. The importance of this cannot be underestimated since many breast carcinomas that are steroid independent have been shown to overexpress coactivators of the endogenous ER. None have been found for prostate cancer to date and this project is consistent with our observation that prostate cancer cells may have AR activated by steroid-independent means. It is clear that in addition to "generic" transcription factors, tissue-specific modifiers must be operating to allow tissue-specific functioning of genes, and we hope that by screening promoter activation in our yeast models against tissue-selected genes we may be able to identify tissue-specific components of these targeted transcription complexes and reveal their biological functions.

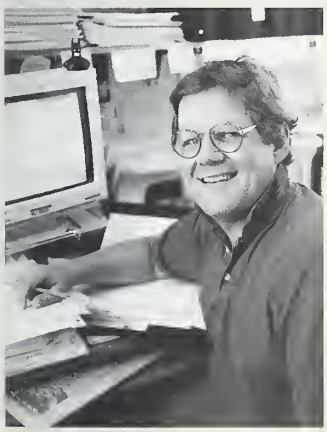
Recent Publications:

Wang LH, et al. *Blood* 2000;95:1249–57.

Chen T, et al. *Cancer Res* 2000;60:2132–5.

Wang LH, et al. *Blood* 2000;95:3816–22.

Kirken R, et al. *J Immunol* 2000;165:5097–104.



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Biography: *Dr. Keller obtained his Ph.D. under the direction of James Ihle at George Washington University, where he characterized and purified interleukin 3. He has a longstanding interest in the molecular and cellular regulation of hematopoietic cell growth and differentiation.*

Laboratory of Molecular Immunoregulation
Molecular and Cellular Characterization of Hematopoietic Cell Growth/Differentiation—Gene Therapy Applications

Keywords:
bone marrow cells
cell growth
gene therapy
vectors

Research: Constitutive hematopoiesis is supported by the proliferation and differentiation of a limited number of pluripotential stem cells. This process is tightly regulated by hematopoietic growth factors and the micro environment. Understanding this process has led to improved strategies for (1) radio-protection, (2) chemoprotection, (3) bone marrow transplantation, (4) gene transfer, and (5) a better understanding of the leukemogenic process. We are studying the biological and molecular mechanisms, which regulate the survival, growth, and differentiation of stem cells, and applying this data to improve gene transfer.

Hematopoietic Stem Cells

A number of pluripotential hematopoietic stem cells (PHSC) have been purified based on the expression of cell surface antigens including Thy-1, Sca-1, and CD–34. These cells coexpress c-Kit, proliferate in response to multiple cytokines, form splenic colonies when transplanted into irradiated mice CFU-s, radioprotect lethally irradiated recipients (short-term reconstituting cells, STRC), and contain pluripotential stem cells (long-term reconstituting cells, LTRC). We have identified a c-Kt negative (c-Kit-neg) stem cell that does not respond to combinations of known cytokines and does not have CFU-s or STRC activities but has LTRC activity. Further, c-Kit-neg cells can give rise to c-Kit-pos PHSC when transplanted in vivo. Taken together, we have hypothesized that steady-state hematopoiesis is supported by PHSC that express c-Kit, and that c-Kit-neg PHSC represent a quiescent stem cell population (c-Kit maturation pathway). Future studies involve further purification of this stem cell population and characterization of the signals required for the growth and differentiation of this quiescent stem cell population.

Molecular Regulation of Hematopoietic Stem Cell Growth and Differentiation

The molecular events that regulate lineage commitment and terminal differentiation of PHSC are largely unknown. Using two cell lines that are blocked at different stages of hematopoietic cell differentiation (representative of normal multipotential and committed progenitor cells), and differential display PCR, subtractive cloning and micro array analysis, we have identified several novel genes regulated during hematopoietic cell differentiation. One of these genes is identical to p205, a previously described gene of unknown function; that is, a member of the interferon-inducible p200 gene family (IFI–200). p205 expression is induced in normal PHSC under conditions that promote myelomonocytic cell differentiation (stem cell factor, SCF, plus interleukin 3, IL–3). p205 encodes a nuclear protein that inhibits IL–3-dependent progenitor cell proliferation and serum-induced NIH–3T3 cell proliferation. Current studies are directed at better understanding the potential tumor suppressive activities of this family of proteins in hematopoietic cells and their role in regulating differentiation and apoptosis. Finally, we are collaborating with Dr. Peter Johnson to better understand the functional role of the transcription factor family of CCAAT-enhancer binding proteins (C/EBP) in hematopoietic cell growth and differentiation.

Novel Vector and Approaches for Gene Transfer

Successful gene transfer to hematopoietic cells has been limited by the inability to transduce PHSCs. To overcome this, we have developed vectors to target stem/progenitor cells that express c-Kit, the gene encoding the receptor SCF. These include nonviral molecular conjugate vectors and adenoviral vectors. These vectors specifically target c-Kit-positive hematopoietic cells, infect up to 95 percent of target cells, and yield high levels of transient gene expression. We are currently exploring methods to target gene delivery to hematopoietic cells in vivo and have developed a method to deliver gene therapy vectors to bone marrow cells by direct intrafemoral injection. We are currently testing whether this approach can be used to correct monogenic disorders SCID and whether it might have broader applications to cancer and AIDS.

Collaborators on this research include Peter Johnson, NIH, and Sally Spence, SAIC–Frederick.

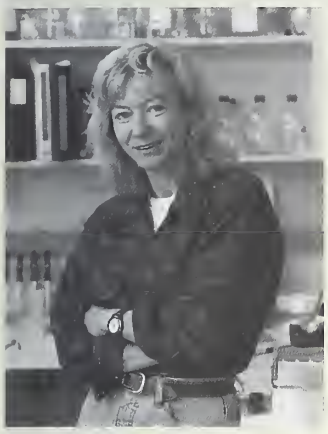
Recent Publications:

Smith JS, et al. *Proc Natl Acad Sci USA* 1999;96:8855–60.

Ortiz M, et al. *Immunity* 1999;10:173–82.

Du Y, et al. *J Biol Chem* 2000;275:6724–32.

Keller JR. *Mol Aspects Immun* 2001;1:217–20.



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Biography: *Dr. Muegge obtained her M.D. degree at the Medical School Hannover, Germany. During her postdoctoral period she worked on cytokines and T cell development with Dr. Scott Durum. As an SAIC scientist, she now investigates the control of chromatin accessibility during lymphoid development and V(D)J recombination.*

Laboratory of Molecular Immunoregulation
Chromatin Remodeling and Lymphocyte Activation

Keywords:
chromatin
lymphocytes
receptors

Research: The focus of our research is to understand how chromatin remodeling specifically affects cellular activation and differentiation of lymphocytes. We are currently working on two models: (1) We examine how the cytokine interleukin 7 controls chromatin accessibility to allow for V(D)J recombination of T cell receptor genes, and (2) we study the biologic effect of Lsh, a novel SNF2/helicase family member, in lymphoid development and the molecular mechanism of chromatin remodeling in lymphocytes. These studies should contribute to our understanding of how chromatin controls cellular processes such as recombination, DNA repair, replication, and transcription.

V(D)J recombination is a site-specific recombination process that is essential for the assembly of the T cell receptor (TCR) and immunoglobulin genes. This process is tightly regulated in lymphoid precursor cells to permit the generation of functional immune receptors while protecting the integrity of the rest of the genome. Targeting of the recombination process to a specific immune receptor locus is thought to be controlled by a change in chromatin accessibility. We identified interleukin 7 (IL–7) as an extrinsic signal that is crucial for gene rearrangement at the TCRγ locus. In the absence of the IL–7 signal the chromatin at the TCRγ locus is repressed and unaccessible for the RAG V(D)J recombinase. We are currently testing whether genome methylation and alteration in histone acetylation specifically control accessibility of the TCR locus for recombination induced by IL–7. Elucidation of the molecular

mechanism of this pathway whereby IL–7 alters chromatin accessibility for gene rearrangement may provide insight into a number of cellular processes controlled by chromatin accessibility such as DNA repair and recombination.

We have recently cloned a novel member of the SNF2/helicase family from rearranging thymocytes and termed it "Lsh" (lymphoid specific helicase). Lsh homologs of the SNF2 family are frequently involved in transcriptional regulation via remodeling of chromatin. Some SNF2 members are able to disrupt mononucleosome structures in vitro and/or can associate in larger complexes with histone modifiers such as histone acetylases. Lsh is preferentially expressed in lymphoid tissue and its expression is closely correlated with the onset of S-phase after pre-TCR or TCR signaling in T cells. Deletion of Lsh by homologous recombination in mice results in early death of the newborns. Hematopoietic precursors from Lsh–/– embryos show a defect in lymphoid development, and mature T cells fail to proliferate without Lsh. Thus Lsh is a nonredundant member of the SNF2 family, and furthermore it plays a crucial role in lymphocyte proliferation and/or protection from apoptosis. Our goal is to define the physiologic role of Lsh in lymphocytes and to elucidate the molecular mechanism by which it specifically alters chromatin structure in lymphocytes.

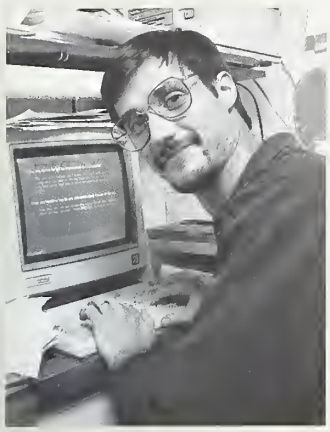
Recent Publications:

Geiman TM, et al, *Proc Natl Acad Sci USA* 2000;97:4727–77.

Schlissel MS, et al. *J Exp Med* 2000;191:1045–50.

Huang J, et al. *J Leukoc Biol* 2001;69:907–11.

Geiman TM, et al. *Biochim Biophys Acta* 2001;1526:211–20.



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Biography: *Dr. Murphy obtained his Ph.D. in immunology while at the University of Texas Southwestern Medical Center in 1989 under the supervision of Dr. Michael Bennett. He became a postdoctoral fellow under Dr. Dan Longo of the Biological Response Modifiers Program at the NCI–Frederick before becoming head of the Transplantation Biology Laboratory in 1993. He is currently in the Intramural Research and Support Program, SAIC–Frederick. He is also an adjunct research professor at Hood College.*

Laboratory of Molecular Immunoregulation
Immunobiology of Bone Marrow Transplantation

Keywords:
adoptive immunotherapy
animal models
bone marrow
cancer immunotherapy
immunotherapy
interleukins
natural killer cells
NK cells

Research: Our laboratory is interested in examining issues in bone marrow transplantation (BMT) and its use for the treatment of cancer and other disease states. BMT, both allogenic and autologous, is currently used for the treatment of a variety of disease states ranging from aplastic anemia to cancer, but significant obstacles limit the efficacy of this procedure—these include marrow graft failure, graft-versus-host disease (GVHD), immune deficiency following the transplant, and, when used for the treatment of cancer, relapse of the tumor. Natural killer (NK) cells have been shown to be responsible for mediating the specific rejection of bone marrow cell (BMC) allografts in lethally irradiated mice. However, little is known concerning the nature of these cells and BMC rejection which leads to marrow graft failure. We have found that NK cell subsets exist that are responsible for mediating the specific rejection of BMC from mice bearing the appropriate MHC molecules. In addition, these NK subsets also play an important role in the normal homeostasis of hematopoiesis, suggesting that it is one of their normal physiologic functions. We are currently examining the differentiation of these various subsets.

We are also using activated NK cells as a means of providing additional antitumor effects when BMT is used with tumor-bearing mice. We found that adoptive transfer of NK cells can provide significant antitumor effects while at the same time promote hematopoietic engraftment and prevent GVHD in mice. We are currently examining the mechanism(s) underlying these effects. Our laboratory has also been examining means to accelerate immune and hematopoietic reconstitution following BMT. This would also be of use in other instances where immune recovery is desirable, such as in AIDS. We have been focusing on the use of neuroendocrine hormones such as growth hormone (GH) and prolactin. They are attractive since they are relatively nontoxic when given systemically and can exert pleiotropic effects. We have found that GH can exert significant hematopoietic growth-promoting effects after in vivo administration. GH can also accelerate immune and hematopoietic reconstitution after BMT in mice. Using a human/mouse chimera model, we have found that these hormones can improve human T cell trafficking and function in vivo. Based on our studies, clinical trails are

currently under way evaluating these hormones in patients with AIDS. We are also examining the physiological role of these neuroimmune interactions.

Another problem with BMT is an EBV-induced B cell lymphoma that can arise in immunodeficient individuals. We have found that stimulation of CD40, a molecule present on B cells critical for their development and function, can promote B cell recovery after BMT. Using a human/mouse chimera model, we have also found that CD40 stimulation can prevent the occurrence of this EBV-induced lymphoma in vivo. Thus, CD40 stimulation after BMT may accelerate immune recovery and prevent lymphoma generation. We are currently examining the role of CD40 in hematopoiesis both in vitro and in vivo.

Collaborators on this research include Michael Bennett, University of Texas Southwestern Medical Center; William Fanslow, Immunex Corporation; Dan Longo and John Ortaldo, NIH; and Susan Richards, Genzyme Corporation.

Recent Publications:

Welniak LA, et al. *J Immunol* 2001;166:2923–8.

Koh CY, et al. *Blood* 2001;97:3132–7.

Welniak LA, et al. *Transplant Proc* 2001;33:1752–3.

Salcedo R, et al. *J Immunol* 2001;166:7571–8.

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Biography: *Dr. Sergei Nedospasov obtained both Ph.D. and D.Sci. degrees from the Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, and he continues to be a member of the Institute. He is also a professor and head of molecular immunology at Moscow State University. Dr. Nedospasov joined the Laboratory of Molecular Immunoregulation (LMI) in 1991 where he has been a principal investigator since 1996.*

Laboratory of Molecular Immunoregulation
Regulation and Physiological Functions of Tumor Necrosis Factor and Lymphotoxins

Keywords:
cancer cell growth
Cre recombinase
cytokine
gene targeting
immune response

Research: TNF and lymphotoxins (LT) are potent immunoregulatory cytokines capable of signaling activation, proliferation, and apoptosis of cells of various lineages. Genes encoding TNF, LT-α, and LT–β are tightly linked in a compact 12-kb TNF/LT locus within MHC.

In order to address the biological functions of the TNF/LT subfamily of cytokines, we used Cre-loxP technology and have generated a panel of

knock-out mice with single (LT–β), double (LT–β–TNF), and complete (triple) deficiency in this locus. This allowed us to reveal distinct and overlapping contributions of TNF and LT signaling into organogenesis and maintenance of lymphoid tissues. We have demonstrated that LT deficiency is associated with enhanced tumor growth and metastasis and with impairment in development and recruitment of NK cells. Cre-loxP technology was also used to generate mice with conditionally targeted TNF and LT genes, including inactivation in B cells, T cells, and macrophages.

In order to identify genes responsible for the mutant phenotype in distinct histological compartments and organs in mice with LT, TNF, or combined deficiencies, we are searching for genes whose expression is substantially altered in knock-out mice. In particular, by subtraction cloning and by comparative hybridizations to gene arrays, we have identified a number of known and novel sequences which are expressed at a much lower level in the spleens of LT-deficient mice. These genes include novel phospholipase A2, chemokines of lymphoid organs ELC, SLC, and BLC, macrophage scavenger receptors, myeloperoxidase, and others. The causative role of these genes in the development and maintenance of mutant phenotype will be addressed in transgenic studies. Our studies may also reveal the relevance of these murine models for human disease.

Our collaborators are L. Forster and K. Pfeffer, Technical University of Munich, Germany; P. Johnson, J. Keller, W. Murphy, N. Rice, C. Stewart, L. Tessarollo, R. Wiltrout, and H. Young, NIH; D. Kuprash, Engelhardt Institute of Molecular Biology, Moscow; D. Kwiatkowski, Oxford University, UK; M. Marino, Ludwig Institute for Cancer Research, New York; A. Nordheim, University of Tuegingen, Germany; K. Rajewsky, University of Cologne, Germany; and B. Ryffel, University of Cape Town, South Africa.

Recent Publications:

Kuprash DV, et al. *J Immunol* 1999;162:4045–52.

Ito D, et al. *J Immunol* 1999;163:2809–15.

Kuprash DV, et al. *J Immunol* 1999;163:6575–80.

Shakov A, et al. *Genes and Immunity* 2000;1:191–9.

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Biography: *Dr. Ji Ming Wang, head of the Chemoattractant Receptor and Signal Group, is engaged in studies on the role of chemoattractant receptors in host defense against HIV–1 infection and the pathogenesis of neurodegenerative diseases.*

Laboratory of Molecular Immunoregulation
The Role of Chemoattractants and Receptors in Host Defense

Keywords:
chemotactic peptide
HIV–1
inflammation
neurodegeneration

Research: Human leukocytes express a number of seven-transmembrane (STM), G-protein coupled receptors, which recognize exogenous or host derived chemotactic factors. Some such receptors, notably the chemokine receptors CCR5 and CXCR4, are also used by HIV–1 as coreceptors to infect human cells. Dr. Wang's group found that both CCR5 and CXCR4 can be deactivated when human cells are exposed to certain peptides that use different receptors such as FPR and FPRL1, two receptors for the bacterial or synthetic chemotactic peptides. The deactivation of CCR5 and CXCR4 not only impairs their function as receptors for cell migration but also inhibits HIV–1 infection. Using small synthetic peptides, we have shown that the deactivation of CCR5 and CXCR4 could be achieved in blood mononuclear cells, which resulted in inhibition of HIV–1 infection. Further research is focused on the possibility of using HIV–1 coreceptor deactivation as a approach to the development of additional anti-HIV–1 agents.

Dr. Wang's studies have been expanded to the role of chemoattractant receptors in the pathogenesis of neurodegenerative diseases. Aβ42 is a 42 amino acid form of the β amyloid peptide and is a key causative factor of Alzheimer's disease (AD). In addition to its reported direct toxic effect on nerve cells, Aβ42 has been found to activate mononuclear phagocytic cells in the brain that causes inflammation and release of neurotoxic mediators. Dr. Wang's group has identified a STM receptor, FPRL1, to be used by Aβ42 to induce mononuclear phagocyte migration and activation. In addition, they detected a high level of expression of the FPRL1 gene in CD11b positive phagocytic cells surrounding and infiltrating the lesions in the brain tissues of AD patients. These results suggest an active involvement of this receptor in the proinflammatory aspects of AD lesions. Interestingly, recent research by this group also revealed FPRL1 to be a receptor for a peptide fragment of the human prion protein, which, in its isoforms, causes "Kuru" or "Mad Cow" disease. The peptide fragment Prp10–126 forms aggregates and activates mononuclear phagocytes to release mediators toxic to neurons in vitro. Studies are being conducted to elucidate the role of FPRL1 in the pathogenesis of neurodegenerative diseases and its potential as a therapeutic target.

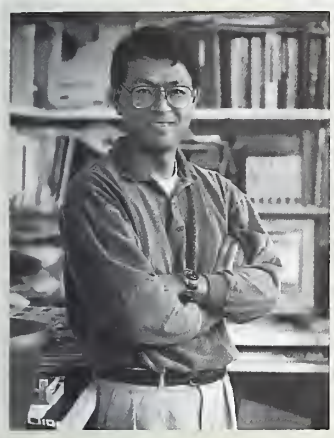
Recent Publications:

Shen W, et al. *Blood* 2000;96:2887–94.

Li B, et al. *Blood* 2001;97:2941–7.

Le Y, et al. *J Immunol* 2001;166:1448–51.

Le Y, et al. *J Neurosci* 2001;23:1–5.



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Biography: *Dr. Yoshimura obtained his M.D. degree from Kumamoto University School of Medicine, Kumamoto, Japan. He also obtained his Ph.D. in experimental pathology there, where he studied the mechanisms of macrophage infiltration into the sites of delayed-type hypersensitivity reactions with Professor Hideo Hayashi.*

Laboratory of Molecular Immunoregulation
Molecular Mechanisms Regulating the Transition from Acute to Chronic Inflammation

Keywords:
chemokine
cytokines
inflammation
interleukins
MCP–1
neutrophils
receptor tyrosine kinase
TNF

Research: The main focus of our research has been on the dissection of the molecular mechanisms regulating the transition from acute to chronic inflammation, including delayed hypersensitivity (DTH). Evidence from studies performed in animal disease models suggested the involvement of neutrophils in the development of DTH. We have recently shown that stimulation of human neutrophils with multiple cytokines could induce delayed expression of the CC-chemokine monocyte chemoattractant protein-1 (MCP–1). MCP–1 was previously cloned in our laboratory and plays a major role in the infiltration of monocytes, characteristic to the chronic phase of inflammation. The delayed expression of MCP–1 was due to a maturations step induced by unidentified factor(s), and the mature neutrophils quickly responded to TNFα for the maximal expression of MCP–1. We hypothesize that this maturation is one of the key steps providing MCP–1 for the conversion of acute into chronic inflammation and DTH. To test the hypothesis, we are in the process of producing MCP–1 conditional knock-out mice.

Although neutrophils are considered to be terminally differentiated cells with a limited capacity to synthesize proteins, our study suggests that neutrophils, after further maturation, may have a broader role in inflammation. To understand the capacity of stimulated neutrophils to express genes, we recently performed cDNA arrays and sequential analysis of gene expression (SAGE) and compared the results with those obtained from unstimulated neutrophils. Hundreds of genes were either down- or upregulated, including many uncharacterized genes, in stimulated neutrophils. We are currently analyzing the data to uncover genes responsible for novel roles of neutrophils in inflammation.

Recent Publications:

Yamashiro S, et al. *J Leukoc Biol* 1999;65: 671–9.

Yamashiro S, et al. *Immunology* 2000;101:97–103.

Yamashiro S, et al. *Blood* 2000;96:3958–63.

Yamashiro S, et al. *J Leukoc Biol* 2001;69:698–704.

Laboratory of Molecular Pharmacology

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The main focus of the Laboratory of Molecular Pharmacology (LMP) is to explore possible connections between molecular biology alterations that drive malignant cell proliferation and potential avenues for therapy. To achieve this, LMP investigators (1) dissect the molecular interactions between effective anticancer drugs and their molecular targets; (2) focus in molecular detail on chromatin, its abilities to repair DNA damage, and its responses to chemotherapeutic agents with particular emphasis on DNA topoisomerases, nucleosome roles in chromatin function, and initiation of DNA replication, and (3) investigate the emerging knowledge of the molecular regulatory networks of neoplastic cells to discover new drugs or therapeutic strategies and molecular markers that predict the response of individual tumors to particular drug therapies.

The laboratory is organized into six research groups, each focusing on specific areas of interest:

- The study of **DNA and topoisomerases as targets of anticancer drugs** probes the mechanisms of action of drugs at the molecular level and compares new compounds to those that are presently used in cancer chemotherapy. Novel agents that target DNA or its activities such as replication, recombination, and repair are investigated. Human cancer cell lines are being characterized to determine the factors and genes that govern drug susceptibility.

- **Chromatin structure and function** investigations study the molecular biology of histones and their modifications induced by oncogenic processes and by chemotherapeutic agents. Current investigations are aimed at elucidating the role of the phosphorylation of histone H2AX in response to DNA double-strand breaks.

- Analyses of **DNA replication** focus on the molecular processes that deliver information from cell cycle regulators to the DNA duplex. These studies combine biochemical analysis with recombinase-mediated gene targeting to identify DNA sequences that are essential for initiation of DNA replication and probe into cellular regulators that interact with these sequences.

• **Information-intensive strategies for drug discovery** combine experimental and theoretical methods to explore relationships between the genetic make-up of malignant cells and their response to chemotherapeutic agents. Multiple molecular databases at the DNA, RNA, and protein levels are being generated by the group and its collaborators for the 60 cell lines of the NCI Anticancer Drug Screen. Included are databases on protein expression by 2D-PAGE, mRNA expression by cDNA microarray and oligonucleotide chip, and chromosomal aberrations by spectral karyotyping and array-based comparative genomic hybridization. Novel methods of data analysis and visualization developed by the group are based on classical statistics, computer-intensive statistics, and artificial intelligence. The databases are being mined to identify compounds that can selectively inhibit particular human cancer cell types. Hypotheses generated are tested in transfected cell lines in collaboration with other LMP groups and are being considered as the basis for clinical trials.

• Evaluating the **integrated behavior of cell regulatory networks** elucidates the relationship of the cellular networks/pathways and the cellular responses to chemotherapeutic agents and proposes novel therapeutic strategies for cancer treatment and innovative drug associations.

• **HIV integrase as a target of antiviral drugs** involves a search for novel inhibitors of this essential enzyme for retroviral replication, and determines the molecular interactions between inhibitors and HIV–1 integrase.

Laboratory of Molecular Pharmacology Staff

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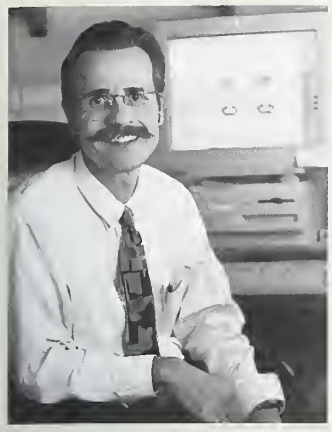
Satoshi Nishizuka	Research Fellow
William Reinhold	Technician
Margot Sunshine	Consultant

Integrated Cell Regulatory Networks

Kurt Kohn	Principal Investigator



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Biography: *Dr. Pommier received his M.D.and Ph.D.degrees from the University of Paris, France, and has been at the NIH since 1981. He received an NIH Merit Award for his role in elucidating the function of topoisomerase enzymes as targets for anticancer drugs and Federal Technology Transfer Awards for studies on HIV–1 integrase and DNA topoisomerase inhibitors. Dr. Pommier is a program committee member of the American Association for Cancer Research, and associate editor for Cancer Research, Molecular Pharmacology, Leukemia, The Journal of Experimental Therapeutics and Oncology, The International Journal of Oncology, and Drug Resistance Updates. Dr. Pommier holds several patents for inhibitors of DNA topoisomerases I and II and HIV–1 integrase inhibitors.*

Laboratory of Molecular Pharmacology
DNA, Topoisomerases, and HIV Integrase Molecular Pharmacology

Keywords:
cell cycle checkpoints
chemotherapy
DNA binding proteins
DNA damage
DNA repair
HIV
pharmacology

Research: DNA topoisomerases are essential for all DNA transactions. Topoisomerase I (top1) and topoisomerase II (top2) are targets for the most potent anticancer drugs to date. Top2 inhibitors include the widely used anticancer agents VP-16 and adriamycin. Camptothecin is a specific top1 inhibitor, and several camptothecin derivatives have recently been introduced in the clinic with promising results in solid tumors including colon and ovarian carcinomas. We study the molecular interactions between drugs, DNA and the enzyme-DNA complexes, and the cellular determinants of cytotoxicity. Evidence has been obtained that topoisomerase inhibitors block the religation of cleavage complexes. Cleavage complexes are enzyme-linked DNA breaks that correspond to topoisomerase catalytic intermediates. Using a camptothecin derivative with an alkylating group and DNA oligonucleotides, we have shown that camptothecins bind at the enzyme-DNA interface. We have sequenced top1 point mutations that render the enzyme resistant to camptothecins. Constructs are being made to further elucidate the structure of the top1-DNA-camptothecin ternary complex. We have recently found that top1-mediated DNA damage can be elicited by commonly occurring endogenous DNA modifications (mismatches, abasic sites, 8-oxoguanine, DNA breaks), as well as by carcinogenic polycyclic aromatic adducts (ethenoadenine, benzo[a]pyrene diol epoxide adducts). These observations suggest that frequently occurring DNA modifications can lead to the formation of top1 cleavage complexes. We are investigating the mechanisms of damage and repair in several ways: (1) characterization of the cellular lesions induced by top1 cleavage complexes in cancer cells (replication-mediated DNA double-strand breaks); (2) elucidation of the cellular responses/pathways elicited in response to such lesions (activation of DNA–PK, RPA phosphorylation, activation of histone phosphorylation [γ-H2AX], transcriptional responses); (3) analysis of the effects of camptothecins in mammalian cells with known genetic defects (Werner syndrome and cells deficient in PARP, beta-polymerase, XRCC1, etc.); and

(4) investigation of the biochemical processing of top1 cleavage complexes in vitro using oligonucleotides and purified repair factors (such as TDP and PNKP). To understand how the genetic makeup of human cells influences their cellular response to anticancer agents and the rationale for the selectivity of topoisomerase inhibitors toward cancer cells, we are studying cell lines from the NCI Anticancer Drug Screen and cell lines with selected gene disruptions.

Ecteinascidin 743 (Et743) is a natural product (from a Caribbean marine tunicate) remarkably active against sarcomas and presently in phase I/II clinical trials. Because of its unique activity profile, we have been interested in elucidating its mechanism of action. We recently demonstrated that Et743 alkylates guanine N2 at selective sites in the DNA minor groove. This observation sets Et743 apart from the DNA alkylating agents currently in clinical use. We recently generated Et743-resistant cells, and these cells are deficient in nucleotide excision repair (NER). Thus, Et743 is not just a top1 and a transcription inhibitor, but its antiproliferative activity appears NER dependent. To our knowledge, Et743 is the first drug with such a mechanism of action.

Our laboratory has pioneered the HIV integrase inhibitor research since 1993. The molecular interactions of drugs with retroviral integrases are investigated using recombinant integrases in biochemical assays and by exploring different steps of the integration reaction. The rationale for searching HIV integrase inhibitors is that: (1) viruses with mutant integrase cannot replicate, and (2) integrase is one of the three retroviral enzymes (with reverse transcriptase and protease) with no cellular equivalent. Our goals are to discover new antiviral agents, evaluate which steps of the integration reactions are affected by drugs, and determine the drug binding site in the HIV–1 integrase-DNA complex. Discovery and studies of HIV integrase inhibitors will provide new strategies for anti-AIDS therapy.

Recent Publications:

Takebayashi Y, et al. *Proc Natl Acad Sci USA* 1999;96:7196–201.

Wang YX, et al. *Cell* 1999;99:433–42.

Strumberg D, et al. *Mol Cell Biol* 2000;20:3977–87.

Pommier Y, et al. *Proc Natl Acad Sci USA* 2000;10:2040–5.

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Biography: *Dr. Aladjem received her Ph.D. from Tel Aviv University. She was a research associate at the Weizmann Institute of Science and then a postdoctoral fellow at the Salk Institute, where her studies focused on the initiation of DNA replication and cell cycle control in mammalian cells. Dr. Aladjem joined the Laboratory of Molecular Pharmacology in October 1999.*

Laboratory of Molecular Pharmacology
Initiation of DNA Replication in Mammalian Cells

Keywords:
cell cycle control
DNA replication and cell cycle

Research: The DNA replication group aims at understanding how information from the cell cycle machinery leads to the initiation of DNA replication. Recent studies yielded abundant information about signaling pathways that prevent normal cells from proliferating in response to developmental cues or to damage sensor signals. Cancer results from the inability to respond to these signals, leading to DNA replication and mitosis under potentially genotoxic conditions. Such replication may promote genetic instability by inducing mutations and chromosomal aberrations. Although DNA replication is the ultimate endpoint of cell cycle signaling pathways, very little is known about how signals from damage sensors and cell cycle regulators reach the DNA duplex. Our studies probe into this missing link through the analysis of molecular factors that determine the site and the timing of DNA replication.

Biochemical studies in mammalian cells revealed that DNA replication initiates from fixed genomic regions called replication origins or initiation regions (IRs). In bacteria, viruses, and yeast, these regions carry genetic information essential for the initiation of DNA replication. However, until recently it was not clear whether the initiation of DNA replication in mammalian cells similarly depends on specific DNA sequences. This question was particularly hard to analyze in mammalian cells because, unlike yeast and bacterial replication origins, mammalian DNA sequences that functioned as replication origins within their native loci did not initiate DNA replication extrachromosomally. We have developed an experimental system that circumvents this problem using an intrachromosomal replicator assay. This assay served to show that replication initiation required specific *cis*-acting genetic elements. We used this system to identify replicator sequences within the human β-globin locus and others have used the system to identify another replicator in the human c-Myc locus. We now use this system to pinpoint the essential sequences within the globin replicator. During the last year we have narrowed down the sequences essential for initiation of DNA replication by analyzing several deletion mutants and are now modifying individual sequence elements that are common to replication origins to determine whether these sequences are required for initiation.

We are also using the β-globin locus as a model system to study how replication timing and origin usage are coordinated with gene expression patterns and correlated with local chromatin features. Previous studies showed that two sequence elements were essential for initiating DNA replication: the initiation region (IR), and the locus control region (LCR) residing 50 kb upstream of the IR. The LCR is also involved in regulating globin gene expression, and in the establishment of tissue- and developmental-specific chromatin structures in the β-globin locus. The involvement of LCR in replication implies that initiation of DNA replication may require interactions between distant sequences. These observations suggested that the chromosomal environment may play an important role in determining origin activity. Results from another research group also demonstrated that such interactions at a distance were required for replication initiation in another locus (DHFR Ori-β). We have developed a system that will allow us to differentiate ES cells to hematopoietic and nonhematopoietic lineages. Since the β-globin locus is expressed only in hematopoietic cells, this system will allow us to use the β-globin locus as a model system to study how replication timing and origin usage are coordinated with gene expression patterns and correlated with local chromatin features. In addition to using the native murine locus, we use homologous recombination to insert human globin sequences into regions within murine ES cell chromosomes that differ in their expression patterns. These cell lines will afford further insight into the genomic environments that allow initiation of DNA replication from specific replicators.

A major caveat of our studies is that they all rely on data from a single locus. We are currently developing protocols for genomic hybridization on microarrays, a whole genome approach to identify multiple replication origins. Such studies could reveal "replicator consensus sequences" or indicate that replicators share common structural motifs, but not sequence homology.

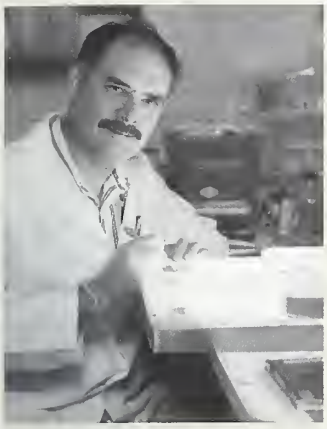
Recent Publications:

Aladjem MI, et al. *Curr Biol* 1998;8:145–55.

Aladjem MI, et al. *Mol Cell Biol* 1997;17:857–61.

Aladjem MI, et al. *Science* 1995;270:815–9.

Aladjem MI, et al. *Science* 1998;281:1005.



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Biography: *Dr. Bonner received his Ph.D. from Harvard University in biochemistry and molecular biology. He studied as a postdoctoral fellow at Oxford University and the MRC Laboratories in Cambridge, England. In 1974 he came to NIH and began his studies on histone functions as a staff fellow in the National Institute of Child Health and Human Development. Two years later he moved to NCI, continuing his studies on histone roles in chromatin in the Laboratory of Molecular Pharmacology.*

Laboratory of Molecular Pharmacology
Functions of Histone H2AX in Chromatin

Keywords:
chromatin structure
DNA double-strand breaks
histone H2AX
ionizing radiation
meiotic recombination
phosphorylation
V(D)J recombination

Research: The typical mammalian cell contains between 2 and 4 meters of DNA compacted to the 90 to 180 mm of 30 nm diameter fibers of interphase chromatin and further condensed to the 120 mm of 700 nm diameter arms of mitotic chromosomes. This compaction is accomplished by small basic proteins which complex with DNA to form nucleosomes—the structural and functional units of both interphase chromatin and mitotic chromosomes. The nucleosome comprises 145 bp DNA and eight small basic protein molecules called histones, 2 from each of the 4 core histone families, H4, H3, H2B, and H2A. A minimum of another 20 bp of DNA stretches between nucleosomes complexed with the linker histone H1.

Einstein's maxim, "Everything should be made as simple as possible, but not simpler," certainly has applied to chromatin. For years many researchers accepted this uniform nucleosome model as meeting Einstein's maxim, but with increasing cognizance of several complexities, most now recognize it as too simple. One intriguing complexity is the division of the histone H2A family into several subfamilies, each with its own conserved sequence characteristics. In mammals, the bulk H2A histones, meaning those species visible on an SDS gel of total cell extract, belong to the H2A1 subfamily while two other subfamilies, the H2AX and H2AZ, each constitutes about 10 percent of the total H2A. H2AX and H2AZ homologs are present throughout eukaryotic evolution. For example, budding yeast contains three H2A species; the two most plentiful are members of the H2AX subfamily, while the other is a member of the H2AZ subfamily.

These complexities of the H2A protein family suggest that histones may perform novel and unforeseen roles in chromatin. The human H2AX cDNA sequence revealed the C terminal protein sequence KATQAS^{139}QEY*, similar to the sequence KATKAS^{129}QEL* reported for budding yeast H2A1 (* signifies the C terminus). This finding demonstrated that higher eukaryotes contain an H2A species with a C terminus very different from that of their bulk H2A species but at the same time strikingly homologous with that of bulk yeast H2A. We then found that mammalian H2AX is extensively phosphorylated within minutes after the introduction of DNA double-strand

breaks (DSBs) into cells by ionizing radiation. We named this phosphorylated mammalian H2AX species "γ-H2AX," localized its phosphorylation site to serine 139, and determined that hundreds to several thousand H2AX molecules become phosphorylated in the chromatin equivalent of at least 2 megabases of DNA per DSB.

To determine where γ-H2AX molecules are located in cells containing DNA DSBs, we generated an antibody specific to the C terminus of human γ-H2AX (anti-γ). With anti-γ we demonstrated that the phosphorylation of the serine four residues from the C terminus in response to ionizing radiation is conserved throughout eukaryotic evolution, occurring not only in mammalian species but also in the nonmammalian species, *Xenopus*, fruit fly, and budding yeast. γ-H2AX appears as small punctate foci within the nuclei of mammalian cells exposed to ionizing radiation. The foci appear within 1 min of irradiation and some foci grow for about 30 mins to diameters of 0.5 mm. There do not appear to be large changes in the number of foci during the first 60 mins after irradiation, but after that there are noticeable and continuing decreases in numbers of foci. The numbers of γ-H2AX foci obtained after subjecting cells to ionizing radiation is consistent with the predicted range of numbers of slowly-rejoining DNA double-strand breaks. IMR90 cells subjected to 0.6 Gray (an amount of radiation chosen because it is below the D0 [the dose permitting 37 percent clonal survival] for most mammalian cell types while inducing a countable number of γ-H2AX foci in a maximum projection) would be expected to contain numbers of DNA double-strand breaks consistent with a Poisson distribution peaking at 7 slowly-rejoining breaks. In such IMR90 cell cultures, the number of γ-H2AX foci was measured to be 10.1 ± 3.9 after 15-min recovery, 11.6 ± 5.3 after 30-min recovery, and 11.4 ± 6.1 after 60-min recovery, values close to the predicted value of slowly-rejoining DNA double-strand breaks.

Using muntjac (a small deer) cells with giant chromosomes, we found that γ-H2AX foci also form on metaphase chromosomes with about the same kinetics as in interphase nuclei. On metaphase chromosomes the foci assume disk-like shapes which resemble chromosome bands. Strikingly, the vast majority of γ-H2AX disks appear in apparently intact chromosome arms even 90 mins after irradiation during anaphase when the sister chromatids were being pulled apart. However, whenever abandoned chromosome arm fragments are present, one end is covered with a γ-H2AX focus.

In collaboration with Drs. Tanya Paull and Martin Gellert, we asked whether proteins involved in DNA DSB repair colocalize with γ-H2AX foci in vivo and whether foci formation by these proteins depend on γ-H2AX foci formation. We demonstrated the utility of targeted DNA DSBs with the LaserScissorsTM as a means of studying the relocalization of proteins to those lesions in vivo. RAD50, MRE11, and other proteins were found to localize to γ-H2AX foci. Wortmannin prevented γ-H2AX foci formation if added before irradiation, but not when added after irradiation. When γ-H2AX foci were absent, foci of other DNA repair proteins were also absent, suggesting that prior formation of γ-H2AX foci was necessary for subsequent formation of these other foci Paull et al., 2000).

In collaboration with Dr. Andre Nussenzweig's and Dr. Thomas Ried's laboratories in NCI, we asked whether or not γ-H2AX is involved in V(D)J recombination in vivo. We found that histone γ-H2AX foci are also found at sites of V(D)J recombination-induced DSBs. In developing thymocytes, γ-H2AX foci colocalize with the T cell receptor α locus in response to recombination activating gene (RAG) protein-mediated V(D)J cleavage.

We had observed that testes from unirradiated mice contain considerable amounts of γ-H2AX by two-dimension gel analysis and that γ-H2AX is present in testes sections. Thus we strongly suspected that γ-H2AX may be involved in meiotic recombination and such was shown to be the case in great detail. During leptotene and zygotene, γ-H2AX antibody labels multiple chromatin domains that encompass those stretches of axial elements that have foci of the recombinase DMC1. These leptotene/zygotene γ-H2AX signals are *Spo11*-dependent and disappear as synapsis progresses. These data provide compelling evidence that, as in yeast, recombination in the mouse is initiated by Spo11-dependent DSBs that form during leptotene, that the processing of the breaks is closely co-coordinated with synapsis, and that the X non-PAR axis is not protected from these breaks. The intriguing and controversial point to come from this work was the finding that spo11$^{-/-}$ mice, which lack the enzyme for making meiotic DNA DSBs, still showed γ-H2AX staining over the sex body.

An additional collaborator in this work was Paul Burgoyne, National Institute for Medical Research, The Ridgeway, Mill Hill, London.

Recent Publications:

Rogakou EP, et al. *J Cell Biol* 1999;146:905–16.

Paull TT, et al. *Curr Biol* 2000;10:886–95.

Chen HT, et al. *Science* 2000;290:1962–4.

Mahadevaian K, et al. *Nat Genet* 2001;27:271–6.

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Biography: *Dr. Kohn received an M.D. degree from Columbia College of Physicians and Surgeons in 1956 and a Ph.D. in biochemistry and molecular biology from Harvard in 1965. He came to the NCI as a clinical associate in 1957 and has served as chief of the Laboratory of Molecular Pharmacology since 1968. His major area of investigation has been the mechanisms of action of DNA-targeted anticancer drugs. He developed the DNA filter elution methodology to measure DNA damage in mammalian cells and showed that DNA topoisomerases are targets of action of clinical anticancer drugs. His recent research interest is to apply the emerging knowledge of cell cycle control networks to possible new modes of therapy.*

Laboratory of Molecular Pharmacology
Determinants of Cancer Cell Chemosensitivity

Keywords:
cancer cell growth regulation
cell cycle
chemotherapy
theoretical biology

Research: A premise of our investigations is that the molecular regulatory defects that make cancer cells malignant also are likely to make them susceptible to specific kinds of metabolic stress. We are therefore working to understand the bases and consequences of the regulatory abnormalities and to search for new compounds or therapeutic strategies that could take advantage of those abnormalities.

Cell cycle regulation. After many years of studying the production and repair of DNA damage consequent upon treatment of cells with cancer chemotherapeutic drugs, recent progress led us to the conviction that the occasional effectiveness of these types of therapies is due to regulatory defects in cancer cells, especially in regard to cell cycle checkpoints. We are therefore now investigating the checkpoint response mechanisms in cells that have specific molecular abnormalities. We are studying the molecular and biological events that follow treatment with drugs having known mechanisms of action. As an aid to the design of functional experiments based on current knowledge of cell cycle control with its complex molecular regulatory networks, we have recently devised a self-consistent grammar of interaction diagrams that could facilitate computer simulations.

DNA topoisomerases. The concept of DNA topoisomerases as targets of anticancer drugs originated in this laboratory in the course of systematic investigations of DNA lesions produced by many different agents and were studied by means of our DNA filter elution techniques. We are investigating several aspects of the mechanism of lesion formation due to drugs that block topoisomerases on DNA as well as the consequences of such blockages to the cell. We found that the sites of lesion formation on the DNA have base sequence preferences that are characteristic for each structural class of topoisomerase-targeted drug. This led us to a structural hypothesis of the drug-DNA-topoisomerase complexes, which in turn led recently to the synthesis of a new camptothecin derivative that, in addition to complexing

with topoisomerase I, alkylates the DNA at the site of the complex. Studies of cell death caused by topoisomerase-targeted drugs recently led us to devise a subcellular system for the study of apoptosis.

HIV integrase. We have been investigating potential inhibitors of HIV integrase, an enzyme that is essential in the life cycle of the virus. We initiated this investigation because of a general analogy of mechanism between this enzyme and topoisomerases. Using an assay system devised by Dr. Robert Cragie and his coworkers, we were first to publish findings on inhibitors of retroviral integrase. Some highlights of this work are described herein by Dr. Pommier. Based on extensive structure-activity studies, we have deduced a pharmacophore basis of the inhibition that holds for several chemical classes of inhibitors.

Clues from cytotoxicity data on characterized human cancer cell lines. We are searching for correlates between cytotoxicity and molecular abnormalities existing in various human cancer cell lines. Confirmed cytotoxicity data has accumulated on several thousand compounds tested against a panel of 60 human tumor cell lines in the NCI cancer screen. The cell lines are being characterized with respect to abnormalities in proto-oncogenes, tumor suppressor genes, and cell cycle checkpoint responses. We have developed computer programs to search for possible relationships between molecular abnormalities and sensitivity to tested compounds. Drug candidates derived in this way would be predicted to act selectively against tumors that possess the specific molecular abnormalities. Internally consistent correlations have been demonstrated for compounds of related chemical structure or similar mechanism of action, thus indicating that the database is rich in information.

Recent Publications:

O'Connor PM, et al. *Proc Natl Acad Sci USA* 1994;91:9480-5.

Pommier Y, et al. *Proc Natl Acad Sci USA* 1995;92:8861-5.

Fesen MR, et al. *Proc Natl Acad Sci USA* 1993;90:2399-403.

Kohn KW, et al. Programmed cell death. In *DNA Repair Mechanisms: Impact on Human Diseases and Cancer.* Vos JMH, editor. London: Landes Co., 1994.

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Biography: *Dr. Weinstein received his M.D. from Harvard Medical School and his Ph.D. in biophysics from Harvard University. After an internship and residency in medicine at the Stanford University Medical Center, he came to the National Institute of Arthritis, Metabolic, and Digestive Diseases (now the National Institute of Arthritis and Musculoskeletal and Skin Diseases), then to the NCI. His bibliography includes over 170 manuscripts (including 9 in* Science *as first author), principally on the search for new treatments for cancer and AIDS.*

Laboratory of Molecular Pharmacology
Gene Expression Profiling and Bioinformatics in Cancer Drug Discovery

Keywords:
2D gel electrophoresis
bioinformatics
breast cancer
cancer
chemoinformatics
gene chip
gene expression
gene expression profiling
genomics
informatics
leukemia
mass spectrometry
melanoma
microarray
molecular pharmacology
neural networks
p53
prostate cancer
proteomics
statistics

Research: The twin goals of this research group at the NCI are to better understand the complex molecular pharmacology of cancer cells and to find new agents for treatment of cancer. The studies are 50 percent experimental, 50 percent bioinformatic.

Experimental: Characterization at the DNA, mRNA, and protein expression levels of cancer cells. Included are cell types used in the NCI's drug discovery program. Central methods are those of molecular biology, genomics, and proteomics.

Bioinformatics: Analysis and integration of large-scale databases on the molecular structures, potency patterns, and possible targets of compounds tested in the NCI drug discovery program. Central methods include those of classical and computer-intensive statistics, data mining, pattern recognition, artificial intelligence, and Q–SAR analysis.

Recent Publications:

Weinstein JN, et al. *Science* 1997;275:343–9.

Ross DT, et al. *Nat Genet* 2000;24(3):227–34.

Scherf U, et al. *Nat Genet* 2000;24(3):236–44.

Weinstein JN. *New Engl J Med* 2000;343:1408–9.

Laboratory of Pathology

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The Laboratory of Pathology (LP) has four interdependent missions: clinical service, research, training, and developmental molecular diagnostics.

Service: The laboratory conducts all of the diagnostic anatomic pathology for all the patients undergoing clinical trials in the NCI and in the NIH Clinical Center, for all patients being considered for entry into clinical trials, and for epidemiologic and case studies of disease pathophysiology. Clinical diagnostic services include surgical pathology, cytogenetics, cytopathology, postmortem, hematopathology, pediatric pathology, electron microscopy, flow cytometry, and specialized histology. In addition, the laboratory provides expert consultation for extramural requests in the fields of hematopathology, OB/GYN pathology, cytopathology, and pediatric pathology.

Research: The laboratory conducts basic and applied research in the following program areas: genetic mechanisms of human tumor progression, angiogenesis, gene regulation, molecular immunology, invasion and metastasis, and cellular interaction with the extracellular matrix. Discovery-oriented research in the laboratory has yielded the identification of more than 20 genes which can be mechanistically linked to cancer pathogenesis, and provide diagnostic and therapeutic strategies.

Training: The laboratory supports an acclaimed Accreditation Council on Graduate Medical Education (ACGME)-accredited 4-year program in anatomic pathology. The training program combines anatomic pathology service with experimental molecular pathology. More than 90 percent of residents over the past 10 years have gone on to a career in academic pathology. The laboratory also offers pathology fellowships in hematopathology, cytopathology, and surgical pathology.

Molecular Diagnostics: This component translates research into clinical service with an eye to the molecular pathology of the future. The laboratory has originated novel microdissection technology, including laser capture microdissection, which enables the pathologist to sample microscopic areas of tissue to obtain pure cellular samples. In parallel, the laboratory is working with collaborators to refine microhybridization array "chip" technology and apply it to microdissected RNA and protein from human tissue. The goal is to employ microdissection to sample each pathologic stage of cancer progression, or before and after treatment, and obtain a fingerprint of the fluctuating sets of genes and proteins in actual human tissue biopsies. The laboratory provides the human pathology component for the NCI Cancer

Genome Anatomy Program (CGAP). The CGAP is constructing cDNA libraries from human tissue which represent each stage of solid tumor progression. The laboratory codirects with the FDA the Clinical Proteomics Program. The goal of this program is to apply novel proteomics technology to disease profiling of patient tissue.

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Biography: *Dr. Liotta is chief of the Laboratory of Pathology and chief of the section of Tumor Invasion and Metastases in the Center for Cancer Research, NCI, NIH. He is the former deputy director for Intramural Research, NIH. He received his undergraduate degree at Hiram College in 1969 and went on to complete an M.D./ Ph.D. program at Case Western Reserve University in 1976. Dr. Liotta's Ph.D. is in biomedical engineering with cancer metastasis as his Ph.D. topic. He served his residency training in anatomic pathology at the NIH in the Laboratory of Pathology and became chief of the same laboratory in 1982. Dr. Liotta is chair of the NIH Radiation Safety Committee.*

Laboratory of Pathology
Molecular Basis of Tumor Motility and Invasion

Keywords:
invasion
laser capture microdissection
metastasis

Research: Dr. Liotta has devoted his career to the study of cancer invasion and metastasis, the major cause of cancer treatment failure. He was one of the first scientists to investigate this process at a molecular level. In the mid 1970s he proposed, and experimentally demonstrated, the linkage between angiogenesis onset and tumor invasion and metastatic dissemination. He was the first to propose that tumor cell attachment and degradation of the basement membrane, and interactions of tumor cells with the extracellular matrix, were crucial to invasion and metastasis. He found that disruption of the basement membrane is the general hallmark of the transition from in situ to invasive cancer for all human epithelial cancers. He discovered metalloproteinases which degrade the basement membrane collagen and facilitate invasion. He proposed that metalloproteinase inhibitors would be potential anticancer treatment agents. He went on to develop and lead a metastasis research group at the NCI. Scientists in the Laboratory of Pathology have discovered a series of novel genes and proteins which regulate cancer invasion and metastasis, providing new strategies for cancer diagnosis and treatment. The current focus is the proteomic crosstalk at the tumor host interface which drives the transition from premalignant epithelial proliferative lesions to invasive carcinoma.

Genetic Manipulation of Invasion Using A Novel *Drosophila Melanogaster* Cancer Model: Discovery Tool for Genes Functionally Required for Invasion and Metastasis

Dr. Woodhouse in Dr. Liotta's group has developed a novel drosophila model which can be genetically manipulated to switch on the malignant invasive phenotype in cell types that are the precursors of solid tumors. A large number of mutants could be rapidly screened to evaluate phenotypic alterations in the in vivo locomotion and invasion of genetically altered cells. They have shown that loss of function of specific *Drosophila* tumor suppressors leads to metastatic tumors. Three such mutants are *lethal giant larvae (lgl)*, *discs large (dlg)*, and *brain tumor (brat)*. These *Drosophila* tumor suppressor mutants have similar phenotypes and the proteins they encode

may have related functions. The *Drosophila* invasion model starts with flies which are lacking one copy of a tumor suppressor gene such as *lethal giant larvae (lgl)*. Larvae lacking both copies of the *lgl* gene can be generated from heterozygous parents. Loss of function of both *lgl* genes produce neoplastic growths. Brain fragments, or epithelial imaginal discs, when transplanted into adult fly hosts, yield tumor cells which are highly motile, invasive, and metastatic. Dr. Woodhouse has screened for new motility and metastasis genes by P element mutagenesis. In order to isolate mutations which affect metastasis, they have generated random mutations in an *lgl* heterozygous background by mobilization of P elements. After screening thousands of mutations which suppress or enhance the metastasis of *lethal giant larvae* brain tissue, they have identified four genes which are functionally required for different stages of the drosophila phenotype of invasion and metastasis. The genes provide new insights into the mechanism of cancer invasion in humans.

Proteomics: Novel Technology Applied to Study the Microecology of the Tumor Host Interface at the Invasion Front

Dr. Liotta and colleagues in the laboratory invented Laser Capture Microdissection (LCM), which is commercialized by Arcturus and used in more than 500 labs worldwide. The technology has enabled investigators for the first time to develop cDNA libraries of normal epithelium, premalignant precursor lesions, invasive carcinoma, adjacent stroma, and metastasis, all from the same patient. LCM has been applied to make broad discoveries in genomics, functional genetics, and is now extending into tissue proteomics. Dr. Liotta, in partnership with Dr. Emanuel Petricoin of the FDA, initiated the first joint initiative between the FDA and the NCI to develop new technology for the discovery of proteins and the profiling of signal pathways in actual human tissue. They were the first to use "artificial intelligence" type learning algorithms to discover disease-associated proteomic patterns. Dr. Liotta's protein ligand microarrays have been used to analyze the protein signal pathways that are deranged during the evolution of invasive cancer in human tissue. They have proposed that LCM combined with protein microarrays constitutes a new paradigm for studying the mechanism of action of candidate pharmaceuticals. For clinical trials in the CCR, the technology is being applied to patient tissue biopsies conducted before, during, and after experimental therapy.

Recent Publications:

Liotta L, et al. *Nature* 2001;411:375–80.

Paweletz CP, et al. *Oncogene* 2001;20:1981–9.

Liotta LA. *Nature* 2001;410:24–5.

Liotta LA, et al. *Nat Rev Genet* 2000;1:47–56.

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Biography: *Dr. Abati received her M.D. from the State University of New York at Buffalo in 1982. She completed her anatomic and neuropathology training at New York University Medical Center, with subsequent fellowships at Memorial Sloan-Kettering Cancer Center where she also served as chief fellow. Board certified in anatomic pathology, neuropathology, and cytopathology, she was named chief of cytopathology in1996 and has been the director of the NIH Fellowship in Cytopathology since 1992. Her academic interests are in the application of molecular techniques to diagnostic cytopathology. She is on the editorial advisory boards of* Cancer Cytopathology, Diagnostic Cytopathology, *and* Clinical Cancer Research, *and devotes efforts to the American Society of Cytopathology and the Papanicolaou Society of Cytopathology. Recently, Dr. Abati served as the conference director for the NCI-sponsored Breast Fine Needle Aspiration Conference, an international effort to standardize all aspects of breast fine needle aspiration. Members of Dr. Abati's laboratory have been recipients of The Papanicolaou Society's Award for Best Research Presented by a Trainee in Cytopathology at the annual meeting of the United States and Canadian Academy of Pathology from 1996 to 1999.*

Laboratory of Pathology
Cytopathology Section

Keywords:
cytopathology
immunocytochemistry

Research: Cytopathology is no longer simply a screening modality but rather provides definitive diagnoses that direct patient management and treatment. The Cytopathology Section provides complete diagnostic service in cytopathology for the NIH's Clinical Center. We specialize in the application of ancillary diagnostic techniques such as immunocytochemistry, flow cytometry, and, most recently, molecular diagnostics as applied to patient management and collaborative investigations and for the confirmation of morphologic diagnoses.

The Cytopathology Section has a distribution of specimens as follows (based on 1998 caseload): fine needle aspiration (FNA), 30 percent; CNS, 33 percent; effusions, 8 percent; respiratory, 9 percent; GU, 10 percent; cervical/vaginal, 7 percent; GI, 1 percent; miscellaneous, 2 percent. The section has a high rate of malignancy—25 percent of all accessioned specimens. Due to the nature of the specimen material, a large number of our cases require immunoperoxidase studies (20 percent). The immunosuppressed nature of the patient population dictates that a significant proportion of our cases requires special studies for pathologic organisms (10 percent). The relatively high rate of pathologic findings combined with the diversity of types of exfoliative and FNA specimens provide a broad experience in diagnostic cytopathology for residency and fellowship training.

The Cytopathology Section is involved in numerous clinically-related research projects, many of which utilize FNA with immunocytochemistry and molecular techniques to provide ancillary diagnostic information. A partial listing of such studies includes: (1) evaluation of circulating tumor cells (CTC) and minimal residual disease (MRD) in bone marrow and peripheral blood

samples of stage III and IV breast cancer patients; (2) evaluation of immunomagnetic bead technologies for the detection of CTC and MRD; (3) evaluation, quantitation, and monitoring of expression of malignant melanoma antigens (MART–1, gp100, tyrosinase) through the utilization of monoclonal antibodies in FNAs from malignant melanoma patients prior to and during treatment with vaccine therapy; (4) evaluation and quantitation of subsets of T cells during immunotherapy for malignant melanoma; (5) evaluation of FNA material for subsequent PCR, microarray, and proteomics technologies; and (6) evaluation of ovarian and renal cell carcinoma aspirates for expression of MOV–18 for possible immunotherapy.

Recent Publications:

Simsir A, et al. *Diagn Cytopathol* 2001;24(5):328–32.

Filie AC, et al. *Cancer* 1999;87(4):238–42.

Abati A, et al. *Cancer* 1999;87(4):231–7.

Abati A, et al. *Clin Lab Med* (Rev) 1998;18(3):561–83.

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Biography: *Dr. Arthur received her M.D. from Case Western Reserve University in 1976. Following training in pediatrics and pediatric hematology/oncology at the University of Minnesota, she joined the School of Medicine faculty at the same institution. She served as director of the Cytogenetics Laboratory and attending physician in pediatric hematology/oncology from 1980 to 1996. Dr. Arthur joined the NCI Laboratory of Pathology in November 1996. She has established the new Cytogenetics Section, which conducts diagnostic laboratory services, resident and fellow training, and research in cytogenetics.*

Laboratory of Pathology
Cancer Cytogenetics

Keywords:
cancer
chromosome
cytogenetics
fluorescence in situ hybridization
genetics
karyotype

Research: Dr. Arthur's past research has focused on the clinical and biological significance of acquired chromosome abnormalities in cancer, with an emphasis on hematologic malignancies. Dr. Arthur was one of the first to describe the clinical features and prognostic significance of the t(4;11)(q21;q23) in acute lymphoblastic leukemia, and she has continued to publish on rearrangements of chromosome band 11q23 with her colleagues in clinical medicine, epidemiology, and molecular biology at the University of Minnesota and in the Children's Cancer Group and Cancer and Leukemia Group B. Drs. Arthur and Bloomfield were the first investigators to report the association of abnormalities of chromosome 16 with a specific morphologic subtype of acute myelogenous leukemia, now called M4Eo, and a good prognosis. Dr. Arthur has also coauthored seven publications of one of the largest series of cytogenetic analyses of non-Hodgkin's lymphomas in the

literature, and correlations of cytogenetics with clinical, morphologic, and immunophenotypic features of these lymphomas. She has participated extensively in national and international cooperative group studies of cancer cytogenetics, including the Children's Cancer Group, the Cancer and Leukemia Group B, and the Fourth, Fifth, and Sixth International Workshops on Chromosomes in Leukemia, which have unequivocally demonstrated the diagnostic and prognostic significance of karyotype in acute leukemias, non-Hodgkin's lymphomas, and childhood solid tumors. In part as a result of the findings of these studies, diagnostic karyotyping has become the standard of care, and is now used in planning therapy for patients with these malignancies.

Since joining the NCI Laboratory of Pathology, Dr. Arthur has established her new research program in cancer cytogenetics. The focus of this research is the use of routine as well as new molecular cytogenetics techniques, including comparative genomic hybridization (CGH) and multicolor spectral karyotyping (SKY), to define chromosomal abnormalities that may be important in tumor initiation or progression.

Dr. Arthur is currently participating in two collaborative research projects. The first of these is a study of B cell chronic lymphocytic leukemia (B–CLL). To address the questions of timing of karyotypic changes in the course of B–CLL, and the possible specificity and significance of these changes, she is conducting prospective serial studies of patients with B–CLL referred to the NCI for evaluation and possible treatment. The cytogenetic- specific aims of this project are: to determine the optimal culture conditions for obtaining karyotypically abnormal mitotic cells from peripheral blood of patients with B–CLL; to determine whether or not interphase fluorescent in situ hybridization (FISH) detects clonally abnormal cells missed by G-banded metaphase analysis; to determine whether or not CGH will detect gains or losses of chromosomal material not found by metaphase or FISH analyses; to correlate cytogenetics results with clinical, morphologic, and immunophenotypic features of the disease; and to correlate karyotype with cDNA microarray analysis of gene expression. These studies have the potential to define new clinicopathological subsets of B–CLL patients, and they may reveal associations of karyotype with abnormal gene expression in these patients.

The second collaborative project is a study of the possible role of the dihydrofolate reductase (DHFR) gene in the pathogenesis of a specific myelodysplastic syndrome, the 5q– syndrome. The aims of this study are: to do high-resolution G-banding to FISH mapping of a locus-specific DHFR gene probe; to determine the frequency of loss of the DHFR gene using metaphase and interphase FISH techniques in patients who have clonal deletions of the long arm of chromosome 5 by G-banded analysis; and to investigate the possible pathogenetic role of loss of DHFR in patients with the 5q– syndrome by correlating the cytogenetics results with clinical and morphologic features, RT–PCR analysis of the DHFR gene, hematopoietic progenitor cell assays, and response to treatment with leucovorin.

Dr. Arthur has recently introduced CGH and SKY technologies into the cytogenetics research laboratory. Her future research plans include molecular cytogenetic analyses of B–CLL and laser capture microdissected adult solid

tumors to determine what the specific cytogenetic changes are in these neoplasms, and when in the course of tumor development they occur.

We have collaborated with Neil Caporaso, Lance Liotta, Margaret Rick, Candido Rivera, Louis Staudt, Maryalice Stetler-Stevenson, and Wyndham Wilson, NIH; and Gerald Marti, Food and Drug Administration.

Recent Publications:

Smith MA, et al. *J Clin Oncol* 1999;17:569–77.

Heerema NA, et al. *Leukemia* 1999;13:679–86.

Bussey KJ, et al. *Genes Chromosomes Cancer* 1999;25:134–46.

Woods WG, et al. *Blood* 2001;97:56–62.

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Biography: *Dr. Emmert-Buck received his M.D. and Ph.D. degrees from Wayne State University School of Medicine. His clinical training in anatomic pathology was conducted in the Laboratory of Pathology, NCI, and completed in 1995. In 1996, he was promoted to unit chief to head the Pathogenetics initiative.*

Laboratory of Pathology
Cancer Pathogenetics

Keywords:
CGAP
genetics
MEN1
prostate cancer
technology

Research: The aim of the Pathogenetics Unit is to investigate genetic alterations underlying tumor development and progression. Emphasis is placed on the study of human cancer as it occurs in vivo, and on the integration of basic research, clinical information, and developing technologies. The three areas of focus are: (1) technology/methodology development; (2) prostate cancer; and (3) multiple endocrine neoplasia type I (MEN1). To overcome the problems of tissue heterogeneity, the Pathogenetics Unit codeveloped both laser capture microdissection (LCM) to increase the speed and efficiency of tissue microdissection, and layered expression scanning (LES) to facilitate high-throughput molecular profiling of tissue samples. In parallel, several associated methodologies have been developed to improve the accuracy and sensitivity of molecular analysis of clinical specimens.

In the study of prostate cancer, the laboratory is taking a three-dimensional analytical approach to examine the entire prostate gland and the physical and molecular relationship of tumor progression. An important future goal is to integrate genomic and protein data with gene expression data sets such that a comprehensive analysis of the status of genes and gene products will be

possible. Much of the current work is being performed as a part of the NCI's Cancer Genome Anatomy Project (CGAP).

MEN1 is an inherited syndrome characterized by development of multiple neuroendocrine (NE) tumors in affected individuals. The responsible gene was recently discovered by the NIH MEN1 working group, including the Pathogenetics Unit and groups from the National Human Genome Research Institute, the National Center for Biotechnology Information, and the National Institute of Diabetes and Digestive and Kidney Diseases. Germline mutations in the MEN1 gene result in formation of NE tumors in a predictable manner and include tumors from multiple organs. Thus, studies of the gene and gene product (menin) are a unique and exciting opportunity to gain new insights into fundamental events and principles of tumor formation. Specific projects of the Pathogenetics Unit include characterization of MEN1 gene mutations in neuroendocrine lung tumors, and immunohistochemical evaluation of menin in human tissues.

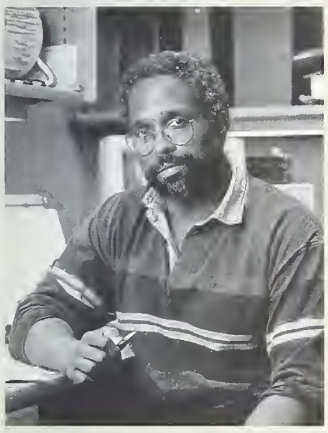
Recent Publications:

Cole KA, et al. *Nat Genet* 1999;21(1):38–41.

Strausberg RL, et al. *Trends Genet* 2000;16:106–8.

Emmert-Buck M, et al. *Am J Pathol* 2000;156:1109–15.

Englert CR, et al. *Cancer Res* 2000;60(6):1526–30.



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Biography: *Dr. Gardner received his B.S. from Yale University in 1979, and his M.D. and Ph.D. from Johns Hopkins University School of Medicine in 1989. He completed training in anatomic pathology at the NCI in 1992. Since then he has been a member of the Laboratory of Pathology at the NCI.*

Laboratory of Pathology

The Role of P300-Mediated CREB/Rel Transcription Factor Crosstalk in Regulating Lymphokine During T Cell Activation

Keywords:
ATF
CD28
costimulation
CREB
cytokine gene expression

Continued on page 716

Research: Our laboratory studies the signal transduction biology of activated T cell and T cell lymphoma. In the past 3 years, we have identified and defined a novel gene regulatory pathway during T cell activation through which multiple signal transduction pathways are integrated at the level of the interleukin 2 promoter. The target of this signal integration is a gene regulatory element within the interleukin 2 promoter that we have defined as the CD28RE–TRE. The CD28RE–TRE is a cis-element within the IL–2 promoter that mediates a crosstalk between the Rel/κβ and CREB/ATF

Keywords (continued):
IL–2
interleukin 2
leukemia
lymphocytes
lymphoma
NFκB
P300
rel
T cells
transcription regulation

family of transcription factors. Work in the laboratory has established that this crosstalk mediates the recruitment of the p300/CBP family of transcriptional coactivators to the IL–2 promoter through specific binding sites for Rel/κβ and CREB/ATF on p300/CBP. Moreover we have found that the coordinated action of p300, Rel/κβ, and p300 is targeted for upregulation by T lymphomagenic oncogenes including the Tax oncoprotein of adult T cell leukemia/lymphoma and the NPM–Alk oncogene, produced by the t(2;5) chromosomal translocation of anaplastic large cell lymphoma. Work in the laboratory has established that the mechanism of action of these important oncogenes is via their coordinated upregulation of both CREB and Rel-dependent pathways, thus establishing CREB/Rel crosstalk as a major target for oncogene action in activated T cell. Interestingly, we have found that the CD28RE–TRE is also a reciprocal target for tumor suppressor genes and factors that mediate apoptosis in T cells. Work in the laboratory has demonstrated that the tumor suppressor p53 can potently transpress the CD28RE–TRE and the interleukin 2 promoter via its interaction with the C terminal domains of p300. Notably, this repression can be nearly completely reversed by the expression of the Tax oncoprotein, and the mechanism of action involves the dual ability of Tax to both directly induce trans-action from the CD28RE–TRE and inhibit its trans-repression by p53. We have also found that CREB/Rel-mediated recruitment of p300 to the CD28RE–TRE is targeted for downregulation by the glucocorticoid receptor. These findings clearly demonstrate that the interplay of p300 and CREB/Rel crosstalk at the CD28RE–TRE element is a convergent target for the action of numerous factors that control growth, mitogenesis, apoptosis, and tumor suppression. Continued work in the laboratory is focused at identifying the molecular parameters of CREB, Rel, and p300 and the upstream signaling pathways that are important for this complex interplay of molecular signaling during normal T cell homeostasis and T cell oncogenesis.

Recent Publications:

Butscher W, et al. *J Biol Chem* 2001;276(29):27647–56.

Okamoto Y, et al. *Am J Pathol* 2001; in press.

Gardner K, et al. *Breast Cancer Res* 2001;3(1):11–3.

Wilson K, et al. *J Immunol* 1999;163(7):3621–8.

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Biography: *Dr. Jaffe completed her medical education at Cornell University and the University of Pennsylvania, receiving an M.D. from the latter in 1969. After an internship at Georgetown University, she joined the NCI as a resident in anatomic pathology, and has been a senior investigator since 1974. She has served on the editorial boards of* The American Journal of Pathology, The American Journal of Surgical Pathology, Blood, Cancer Research, *and* Modern Pathology, *among others. She has been president of both the Society for Hematopathology as well as the United States and Canadian Academy of Pathology, and was elected to the advisory board of the American Society of Hematology. In 1993, she was elected a fellow of the American Association for the Advancement of Science. She is among the 10 most highly cited researchers in clinical medicine for the field of oncology between 1981 and 1998.*

Laboratory of Pathology
Pathogenesis and Pathobiology of Malignant Lymphomas

Keywords:
chemokines
lymphoma
molecular markers
monoclonal antibodies

Research: Our work focuses on the definition of malignant lymphomas as tumors of the immune system, delineation of new disease entities, studies related to the pathophysiology of malignant lymphomas, and clinical correlations including prognosis and response to treatment. Our section also has a major commitment to diagnostic hematopathology. Our clinical duties and research activities are intertwined, with many of the research activities emanating from clinical service observations.

As one of the principal architects of the Revised European-American Classification of Lymphoid Neoplasms (REAL classification), we proposed that lymphoid neoplasms should be viewed as individual disease entities. Each disease is defined by a constellation of clinical and laboratory features including morphology, immunophenotype, genetic features, clinical presentation, and course. Prior classification schemes had attempted to arrange lymphomas according to a predetermined framework, such as clinical outcome for the Working Formulation, or lymphocyte differentiation schemes for the Kiel classification. According to the REAL classification, which has now been accepted as the international standard, each disease has its own unique biological and clinical characteristics. Our laboratory is devoted to the definition of lymphoproliferative disorders according to these established principles.

One of our major areas of interest has been the mature or postthymic T cell lymphomas. We have recognized significant differences between nodal and extranodal T cell lymphomas. In particular we observed that extranodal T cell lymphomas have a cytotoxic T cell phenotype and are often associated with necrosis and/or apoptosis. We have defined a number of clinicopathological entities within this broad group of diseases. Hepatosplenic γ/δ T cell lymphoma was characterized as a distinct clinicopathologic entity, derived from cytotoxic γ/δ T cells; associated with a specific chromosomal

abnormality, isochromosome 7q; and characterized by an aggressive clinical course with a median survival of less than 2 years. It is much more common in males than females, typically presenting in the second and third decades of life.

Subcutaneous panniculitic T cell lymphoma is another entity described in our laboratory. It presents in young adults, usually with multiple subcutaneous nodules. Patients are at risk to develop a hemophagocytic syndrome, and the disease is aggressive, with a median survival of less than 3 years. It had frequently been mistaken for benign panniculitis. The neoplastic cells uniformly express a CD8+ cytotoxic T cell phenotype. In addition, the cells are positive for the cytotoxic-associated proteins perforin and TIA–1. These proteins mediate cytotoxicity and apoptosis by T cells and NK cells, and therefore may be responsible for the cellular destruction characteristic of these lesions. We have shown that cellular destruction is apoptotic in nature, rather than mediated by other causes.

Angiocentric lymphoma or nasal T/NK cell lymphoma is another unique entity. Most cases are derived from NK cells. The tumor cells are universally associated with Epstein-Barr virus. The tumor has novel epidemiological features, being found commonly in Asians and Native American populations of the Western Hemisphere. The name "angiocentric" lymphoma derives from the propensity of the tumor cells to invade blood vessels and to be associated with extensive necrosis. We have been interested in characterizing chemokine expression in these neoplasms, and identified interferon-γ–inducible protein-10 (IP–10) and the monokine interferon-γ-induced gene (Mig) as causative factors of the vascular damage and necrosis in Epstein-Barr virus (EBV)-positive angiocentric lymphomas. Our ongoing studies of chemokine expression are elucidating other aspects of the pathophysiology of malignant lymphomas. For example, we showed that eotaxin was closely correlated with the degree of eosinophilia in Hodgkin's disease. In more recent studies, we have identified MIP1-α to be consistently overexpressed in patients with hemophagocytic syndromes, regardless of the underlying disease or precipitating event.

Other recent work relates to a delineation of the reactive and neoplastic lymphoid proliferations seen in patients with the autoimmune lymphoproliferative syndrome (ALPS). We have identified an increased incidence of B cell lymphomas in these patients and have explored possible pathophysiologic mechanisms for the abnormal B cell expansions in these patients with defective apoptosis.

Our interest in the classification of malignant lymphomas has led to our involvement in several international projects to establish an international consensus on the nomenclature and definitions of hematological malignancies. I have been directing a project for the World Health Organization (WHO) to establish a classification scheme for neoplasms of the lymphoid, myeloid, and histiocytic systems. A monograph summarizing the WHO classification was published in 2001.

Collaborating with us are Stephen Straus, Giovanna Tosato, Thomas Waldmann, and Wyndham Wilson, NIH.

Recent Publications:

Campo E, et al. *Am J Surg Pathol* 1999;23:59–68.

Teruya-Feldstein J, et al. *Blood* 1999;93:2463–70.

Quintanilla-Martinez L, et al. *Blood* 2000;96:443–51.

Straus SE, et al. *Blood* 2001;98:194–200.

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Biography: *Dr. Kleiner received his Ph.D. in 1986 in chemistry and his M.D. in 1988 from the University of Chicago. His graduate work focused on purification of Trichomonal secondary alcohol dehydrogenases, their kinetic characterization, and suicide inactivation. He came to the NIH as a resident in anatomic pathology and after completion of his training, he joined the surgical pathology staff where he has cultivated his interests in the pathology of liver disease and the biochemical mechanisms of tumor cell invasion.*

Laboratory of Pathology
Pathology of Chronic Liver Disease; Biochemistry of Matrix Metalloproteinases and TIMPs

Keywords:
liver
metastasis
proteases
virus-cell interactions

Research: Dr. Kleiner is responsible for overseeing the clinical service component of the Laboratory of Pathology. The laboratory serves as the anatomic pathology service for the NIH Clinical Center, supplying surgical pathology, hepatopathology, autopsy, cytopathology, flow cytometry, ultrastructural pathology, and cytogenetic diagnostic services. These services include both routine diagnostic services and pathology support for the many clinical research protocols carried out at the NIH.

The Postmortem Pathology Section is responsible for conducting the autopsies performed at the Clinical Center. Any patient seen on protocol at the NIH or who has a disease of significant clinical or research interest to a particular principal investigator may have an autopsy done at the NIH. The goal of the service is to answer clinical questions about the patient's disease, to determine the cause of death, to provide for hospital and protocol-related quality assurance, and to serve as a research support for investigators who would like to use tissue from autopsies or carry out autopsy-based studies. The section is also responsible for training pathology residents in autopsy pathology. A weekly CME-approved clinical-pathological correlation conference is held in the autopsy suite to review the gross and microscopic findings on our cases.

In addition to these administrative responsibilities, Dr. Kleiner has significant service responsibilities as one of the full-time surgical pathologists. Although his area of expertise is in liver disease, he reviews all general surgical pathology material when on service. He reviews all liver biopsies at a weekly liver

biopsy conference. Dr. Kleiner's other service responsibilities include immunohistochemical QA and supervising the laboratory computer system currently being installed.

Dr. Kleiner's research interests are focused on chronic liver disease and on the biochemistry of matrix metalloproteinases. Chronic liver disease is a significant health problem both in the United States and worldwide. In particular, chronic hepatitis C (CHC) has become recognized as a potentially serious chronic disease in the United States. Approximately one to two percent of the population is chronically infected with this virus and there is a wide variation in rate of disease progression. Some patients develop cirrhosis or hepatocellular carcinoma in less than 5 years while others may have chronic hepatitis for 40 years or more without significant morbidity. The focus of Dr. Kleiner's research includes the objective evaluation of pathologic changes following therapy and prediction of disease outcome based on histological parameters. In collaboration with investigators in the National Institute of Diabetes and Digestive and Kidney Diseases, we are evaluating new treatment regimens for CHC. However, in the absence of an effective therapy or vaccine, it is of paramount importance to be able to predict not only which patients may expect to benefit from therapy but also which patients are likely to progress more quickly to cirrhosis. Using a multivariate regression model, we have determined histologic factors that predict short-term progression of fibrosis. These observations may be extended to other chronic liver diseases such as chronic hepatitis B, primary biliary cirrhosis, and steatohepatitis.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endoproteases whose degradative activity is directed towards the proteins of the extracellular matrix. The tissue inhibitors of metalloproteinases (TIMPs) are a corresponding family of tight-binding inhibitors of the MMPs. Numerous studies have demonstrated an important role for MMPs in the processes of tumor cell invasion, angiogenesis, and chronic inflammatory/degenerative diseases. Our research has focused on understanding the protein-protein interactions between MMP–2 and TIMP–2 and the role TIMP–2 plays in the processes of activation and inhibition both in vivo and in vitro. The current objective is to understand the kinetics of activation, first in a chemically initiated activation and then with the putative in vivo activator membrane-type matrix metalloproteinase-1. Since regulation of activation is a key part of the control of degradative activity, understanding the kinetics of activation permits better understanding of the MMP–2/TIMP–2 interaction and gives us an experimental model for analyzing the effects of exogenous inhibitors.

In vitro, MMP–2 is activated by reaction with aminophenylmercuric acetate (APMA), which initiates a cleavage of the amino-terminal pro-domain, reducing the molecular weight of the enzyme from 72 kDa to 62 kDa. In the absence of TIMP–2, we have shown that this reaction occurs entirely intramolecularly (i.e., zero-order) and passes through a 64 kDa intermediate. The inhibitor BB–94, a MMP-specific inhibitor, inhibits only the second cleavage step, suggesting that the active site is only available to the inhibitor after the first intramolecular reaction. Addition of TIMP–2 to the reaction inhibits both intramolecular cleavage steps and changes the reaction order. We have shown that, for activation to occur, TIMP–2 must be transferred

directly from one enzyme molecule to another. We are now extending these observations to the activation of MMP–2 by recombinant MT1–MMP.

Among our collaborators are Wong-Ho Chow, Jay Hoofnagle, Alan Kirk, T. Jake Liang, Leonard Seeff, William Stetler-Stevenson, Stephen Straus, and Jo Anne Zujewski, NIH; Stanley Pillemer, NIDCFD; and Edward Tabor, Food and Drug Administration.

Recent Publications:

Kleiner DE, et al. *Cancer Chemother Pharmacol* 1999;43:S42–51.

Lau DT–Y, et al. *Hepatology* 2000;32:828–34.

Goldbach-Mansky R, et al. *Arthritis Res* 2000;2:145–53.

Yeo AET, et al. *J Viral Hepat* 2001;8:256–61.

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Biography: *Dr. Kohn is a graduate of the University of Michigan Medical School where she also completed residency training in internal medicine; she came to the NCI for medical oncology training in the Medicine Branch and then joined the Laboratory of Pathology to investigate signal transduction molecular targets in invasion and angiogenesis, and ovarian cancer. She serves on numerous NIH and NCI committees and participates in program review groups. Dr. Kohn is a participating member and protocol investigator in the Gynecologic Oncology Group, judges the Fellows Award for Research Excellence competition yearly, and is serving as a woman scientist advisor to the director, Center for Cancer Research.*

Laboratory of Pathology
Molecular Targets for Ovarian Cancer: Calcium Influx Regulated Targets and Proteomic Applications

Keywords:
angiogenesis
apoptosis
calcium
invasion
ovarian cancer
signaling transduction

Research: Novel targets for therapeutic intervention and to assist in early diagnosis in ovarian cancer and other cancers are needed. Our laboratory is focusing on two hypotheses. First, based upon our data credentialing calcium influx as a molecular target, we hypothesized that events downstream of calcium influx would be additional and potentially more powerful molecular targets in proliferation, invasion, and angiogenesis. We used CAI (carboxy-amido-triazole), a synthetic blocker of calcium influx in nonexcitable cells, to develop a tool from which to prosecute this hypothesis. We have cloned a novel gene, CAIR–1 (also cloned as BAG–3), that is a signaling intermediate, binding to partner proteins through one of three domains: PXXP, WW, or BAG. We have shown that CAIR–1 regulates availability of phospholipase C-γ, binding it in the latent stage. Current studies are addressing the effect of CAIR–1 on protection of cells from proapoptotic injury such as chemotherapeutics and anoikis.

Translational applications of this finding are under study using samples ascertained during the clinical trials described below. The study of CAIR–1 will generate new leads into molecular targeting of this pathway for therapeutic gain.

Our second hypothesis addresses the need to better understand the molecular signatures of epithelial ovarian cancer. We hypothesized that using gene and protein fingerprinting and discovery using the tumor tissue of low malignant potential and invasive epithelial ovarian tumors and from sera from patients with those diagnoses, we will uncover novel molecular targets and patterns for early diagnosis and therapeutic gain. Application of laser capture microdissection to tissues yielded generation of cDNA libraries from which gene mining led to the finding that granulin-epithelin precursor (GEP) is uniquely upregulated in invasive ovarian cancer. The function of GEP was confirmed in ovarian cancer cell lines using antisense transfection studies. Further studies characterizing the activity of GEP in ovarian cancer are under way. Proteomic approaches have uncovered differential expression of several genes that may be batched in a multiplex fashion as a putative biomarker panel. In concert with this, collaborative studies with Drs. Liotta and Petricoin of the NCI/FDA Clinical Proteomics Initiative have yielded a novel biomarker pattern that is highly sensitive and specific for epithelial ovarian cancer. This finding is being advanced at present for longitudinal studies and against nonmalignant control pathology prior to advancing to validation biomarker studies.

Together, these two lines of investigation have given us new mechanistic information into the signaling underlying progression and survival in carcinomas, focusing on ovarian cancer.

Among our collaborators are William Figg, Steven Libutti, and Lance Liotta, NIH; David Fishman, Northwestern University ; Rebecca Liu, University of Michigan; Emmanuel Petricoin, FDA; Bo Rueda, Harvard University; Russell Schilder, Fox Chase Cancer Center; Ginette Serrero, University of Maryland; and Alan Wells, University of Pittsburgh.

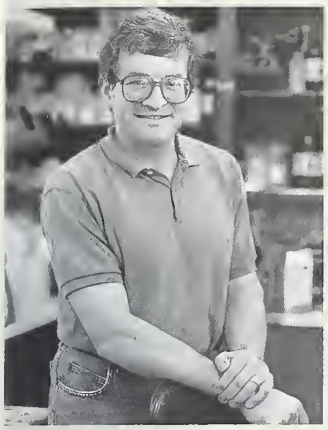
Recent Publications:

Masiero L, et al. *J Cell Sci* 1999;112:3205–13.

Doong H, et al. *Oncogene* 2000;19:4385–95.

Kohn EC. *Clin Cancer Res* 2001;7:1600–9.

Liotta LA, et al. *Nature* 2001;411:375–9.



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Biography: *Dr. Levens received his M.D. and Ph.D. from the University of Chicago. Subsequently, he completed residency training in anatomic pathology at the Laboratory of Pathology, NCI, where he is now the chief of the Gene Regulation Section.*

Laboratory of Pathology
DNA Conformation, Topology, and the Regulation of C-Myc Expression

Keywords:
c-myc
DNA topology
transcription

Research: Currently, Dr. Leven's research is focusing on the transcriptional regulation of c-myc and the elucidation of the roles of conformation and topology-selective, sequence-specific DNA binding transcription factors. The c-myc gene employs multiple cis-elements, upstream and downstream of its promoters, P1 and P2. The goal is to illuminate the mechanisms through which the input of multiple factors sets the expression level of this critical proto-oncogene. Two elements and their associated trans-factors, first described in this group, display unusual properties in vitro and in vivo which may serve to highlight important new regulatory modes.

Previous studies identified a cis-element (1500 bp upstream pf P1) that bound a factor downregulated with the cessation of c-myc transcription during promonomyelocytic leukemia cell differentiation. This cis-element, FUSE (far upstream element), was defined by deletional and mutational analysis; the 70 kDa FUSE binding protein (FBP) was purified and, following peptide cleavage and microsequencing, the cDNA was cloned. FBP possesses a DNA binding motif specific for particular sequences only when contained in single-stranded or negatively supercoiled DNA. Additional dissection of FBP revealed both a potent transcription activation domain at its carboxyl-terminus and a strong repression activity within the amino-terminus. Expression of a dominant-negative FBP composed of only its DNA binding domain, as well as antisense FBP RNA using replication defective adenovirus vectors, demonstrates that the endogenous, cellular c-myc gene is regulated by FBP. A partner protein recently identified in our group, named the "FBP interacting repressor" (FIR), inhibits activated, but not basal, transcription. Coupling these properties with the unusual DNA binding specificity indicates that FBP may participate in the regulation of c-myc by sensing conformational perturbation of FUSE in response to processes such as transcription. Thus the c-myc promoter may directly feedback-regulate itself, requiring no diffusible mediator. FBP expression is shut off during differentiation and increased when cells are induced to proliferate. Both FBP and FIR regulate transcription through TFIIH in vivo and in vitro.

The conformation of the FUSE was analyzed in vivo. One strand of the FUSE is occupied in vivo when c-myc is expressed, but is vacant when c-myc is silent. Thus the opening and closing of the FUSE (perhaps mediated through transcription-driven supercoiling) may be a key choke-point for c-myc control. The potential significance of FBP and FUSE is underscored by the discovery of two homologous proteins, FBP2 and FBP3. The mechanistic illumination of FUSE and the FBPs should yield new insights into the integration of multiple pathways onto a complex promoter.

Second, studies of the CT-element, 100 to 150 bp upstream of c-myc P1, underscore many of the principles operating at the FUSE. The CT-element, essential for P1, also stimulates expression from P2. A complex array of factors interacts with this five-times-repeated element. One of these factors is hnRNP K, which unexpectedly possesses the same general DNA binding protein architecture as FBP. hnRNP K binds to one strand of the CT-element either as a single strand or when embedded in negatively supercoiled DNA. Comparison of the CT-element/hnRNP K complex in vitro and in vivo provides strong evidence that hnRNP K is at the CT-element in living cells. Further work indicates that the CT-element is a flexural and torsional hinge in vivo and in vitro, probably reflecting intrinsic conformational instability.

To prove that hnRNP K interacts with CT-elements in vivo, the powerful VP16 activation domain was fused to hnRNP K's carboxyl-terminus. This fusion protein activates CT elements in supercoiled but not linearized plasmids when transfected into Hela cells, showing that DNA conformation and gene expression are coupled.

HnRNP K activates RNA polymerase II-mediated expression both in vivo and in vitro. Recent experiments show that hnRNP K can bind with linear DNA and modify transcription in response to ongoing transcriptionally generated torsion. The potential significance of hnRNP K is underscored by its interactions with c-src, vav, and TATA binding protein. It also binds tightly with the retinoblastoma protein. Our recent work suggests that hnRNP K interacts with major players governing the cell cycle and controlling apoptosis, and may itself participate in these processes.

The importance of factors interacting with and modifying DNA conformation in vivo is highlighted by our discovery of protein-dependent conformational marking of active genes in mitotic chromosomes. This observation suggests a mechanism for selecting genes for transcriptional restart following pan-genomic mitotic repression.

Further studies of single-stranded/topology-dependent factors, and of the rules coupling DNA conformation and topology with transcription, replication, repair, or recombination of the c-myc gene, promise to help us understand the expression of c-myc under physiological and pathological circumstances.

Recent Publications:

Michelotti EF, et al. *Nature* 1997;388:895–9.

He L, et al. *EMBO J* 2000;19(5):1034–44.

Liu J, et al. *Mol Cell* 2000;5(2):331–41.

Liu J, et al. *Cell* 2001;104:353–63.

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Biography: *Dr. Mackem undertook her graduate research on the regulation of herpesvirus immediate early gene expression by VP16 with Dr. Bernard Roizman at the University of Chicago and received her Ph.D. as an MSTP trainee in 1982. She moved to the East Coast and completed her M.D. at the Johns Hopkins University School of Medicine in 1984, and then went on to residency training in anatomic pathology at the National Cancer Institute. Since completing the residency in 1987, she has been a staff member in the Laboratory of Pathology at the NCI. Her research there has focused on the regulation of axis formation during vertebrate gastrulation and limb development.*

Laboratory of Pathology
Genes Regulating Pattern Formation During Embryonic Development

Keywords:
embryo
gastrulation
gene regulation
knockout
limb development
skeletal development
transgenic mice

Research: Our laboratory has identified several new developmental control genes (transcription factors) that appear to be involved in regulating the formation of and the pattern of structures in the primary embryonic axis and the limb axis. We are analyzing their normal function and potential role in oncogenesis. Many of the same regulatory and signaling cascades appear to operate during both primary embryonic induction and establishment of secondary embryonic fields, such as the limb. Analyzing the role that such transcriptional regulators play in different developmental contexts may offer additional insights into the sorts of basic processes that they govern in the cell. Furthermore, key processes in development, including differential cell proliferation, programmed cell death, migration, and cell-cell interactions, are recapitulated in a pathologic manner during oncogenesis and metastasis, suggesting an aberrant regulation or "reactivation" of such processes. Understanding how these events are triggered and regulated will be invaluable in deciphering tumor biology and ultimately help to identify new ways to intercept cellular targets that drive tumor cell behavior.

The major events of gastrulation include inductive interactions that establish and pattern the embryonic axis, and morphogenetic movements yielding the germ layers and shaping the embryo. We have isolated several novel homeobox and T-box genes involved in mesoderm formation during gastrulation, and we are exploring their roles in regulating mesodermal cell

fate and behavior, with particular emphasis on the coordination of organized morphogenetic movements. Both gain of function experiments in chick embryos and generation of null mutant mouse embryos are being used for functional analyses. Chick tail development is a continuation of gastrulation and we have demonstrated that the tail tip retains true Spemann-type organizer activity (induces a secondary embryonic axis) including neural induction, cell recruitment into paraxial mesoderm, and induction of gastrulation-like morphogenetic movements to produce elongated mesodermal outgrowths. Tail bud grafts to extraembryonic membranes provide an accessible and controlled experimental system to analyze inductive activities in this tissue removed from the context of other endogenous signals in the embryo.

Because the major events of gastrulation occur over a short time span and require very dynamic regulation of gene expression, a second focus of the laboratory is to decipher the mechanisms mediating these rapid expression changes; several features of one of the organizer-specific homeobox genes we are studying (Gnot1) may provide new clues to expose how such regulation works at the posttranscriptional level. Both the abundance and localization (nuclear to cytoplasmic transport) of this gene are highly regulated in the embryo.

Limb development is an intensively studied and excellent model for vertebrate pattern formation that offers a solid conceptual framework to interpret new results. In this system, patterning is tightly linked to differential growth regulation; 5′ members of the Hoxd homeobox cluster appear to regulate anterior-posterior (e.g., thumb to little finger) pattern of limb skeletal elements as downstream targets of Sonic Hedgehog signaling. Using a transgenic model, we have found that some of the 5′Hoxd genes also play a role in establishing and/or maintaining these polarizing signals in the posterior limb through a positive feedback loop with Sonic Hedgehog. Hoxd genes also appear to play key roles in regulating proliferation of chondrogenic elements in the limb, and we have identified the c-Fos oncogene as a possible target of Hoxd genes, but generally the effector target genes through which they act remain elusive. Considerably less is known regarding the relative importance of Hoxd genes at these later (fetal) stages of cartilage proliferation and potentially under pathologic conditions. We are analyzing Hoxd-12 and Hoxd-13 as prototypic examples of 5′Hoxd gene function, with a major emphasis on identifying direct targets at the molecular level and on learning more about late roles in proliferating cartilage and potential contribution to neoplastic processes using an inducible transgenic model system.

An area of emerging interest is elucidating how limb initiation and position along the body axis are regulated. Both retinoids and FGFs play critical roles in this as well as in other inductive events during embryonic development. We are developing new tools to evaluate dynamic changes in retinoid distribution and to analyze FGF signaling at localized sites in the embryo. In this context, we have evidence that the transcription factor T, or Brachyury, may also play a role in the relay of FGF signals from the embryonic midline to the periphery that is thought to initiate limb budding. Intriguingly, in some

systems, T has been shown to participate in positive feedback loops with FGFs. As part of a collaborative effort, we are developing dominant-negative FGF receptors that can be applied in soluble form to antagonize FGF signals. These will serve as useful tools to document FGF relay sites in the embryo and as an adjunct to evaluate the possible role of T as an intracellular component of an FGF relay that initiates limb budding.

Retinoids are clearly important at multiple points in development and have been implicated in positioning both sites of limb initiation and of limb polarizing regions. It remains uncertain whether these events are mediated by localized regions of higher RA or differential tissue sensitivities to RA. We are interested in developing methods to evaluate RA sources in situ during early stages of development when the embryo is rapidly changing. In a collaborative effort, we have developed a rapid in situ assay using a chimeric glucocorticoid/retinoic acid receptor/GFP fusion protein. The chimeric protein demonstrates RA-dependent nuclear translocation, providing for a rapid read-out assay by confocal fluorescence imaging of cultured living embryo slices on transfected monolayers expressing the chimera.

Collaborating with us are Chuxia Deng, Gordon Hager, and Glenn Merlino, NIH.

Recent Publications:

Knezevic V, et al. *Development* 1998;125:1791–801.

Knezevic V, et al. *Development* 1997;124:4523–36.

Mackem S, et al. *J Biol Chem* 2001; in press.

Knezevic V, et al. *Genesis* 2001; in press.

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Biography: *Dr. Merino received her B.S. and M.D. from the University of Venezuela. After graduating from medical school in 1974, she was accepted at Yale-New Haven Hospital as a resident in anatomic pathology. She remained in the Laboratory of Pathology at Yale University as chief resident and fellow in surgical pathology under the supervision of Dr. Darryl Carter. After completion of her residency, she was asked to remain as an assistant professor first and later associate professor in pathology and as a member of the surgical pathology staff at the Yale University School of Medicine. In 1985, she joined the Laboratory of Pathology at the NIH. In 1995, Dr. Merino was made a member of the Royal Academy of Medicine of Madrid, Spain.*

Laboratory of Pathology
Prognostic Markers in Breast Cancer and Premalignant Lesions

Keywords:
breast
prognostic markers

Research: The Surgical Pathology Section provides expertise and diagnostic services in the field of anatomic pathology for Clinical Center patients and collaborates with the research staff in those investigations that involve the use and study of human pathological material. Approximately 6,000 surgical specimens and biopsies (more than 60,000 slides, which include routine and a variety of special stains) are accessioned each year. These include more than 2,000 fresh human tissues.

Members of the section are primary faculty for the instruction of residents in surgical pathology. To assist in providing better patient care, the members of the section also participate in a variety of teaching programs and departmental conferences (with, for example, the Surgery Branch) in which patient diagnosis and modalities of therapy are discussed. Members also provide consultant services to the community as well as to pathologists throughout the country.

Dr. Merino's research aim is to study and investigate the role of different tumor markers as prognostic tools in the diagnosis of breast, gynecological, and thyroid cancers, as well as other endocrine tumors and soft tissue sarcomas. She and her colleagues are investigating the potential role that genetic alterations may have in recognizing premalignant lesions of the breast. The purpose of these studies is to investigate the presence of tumor suppressor genes in these locations and the possible role they may play in the development of sporadic human breast cancer. Dr. Merino is interested in studying precancerous conditions that may lead to the development of invasive cancers.

Recent Publications:

Middleton L, et al. *Am Med Surg J* 2000;24(12):1650–6.

Cardiff RD, et al. *Oncogene* 2000;19(8):968–88.

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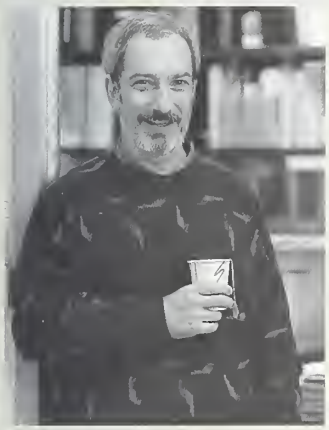

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Biography: *Dr. Raffeld received his M.D. from the Abraham Lincoln School of Medicine of the University of Illinois. He completed clinical training in pathology at the Brigham and Women's Hospital in Boston and at the Hematopathology Section of the Laboratory of Pathology (LP), NCI. He was appointed to the laboratory's permanent staff in 1989. Dr. Raffeld serves as deputy chief of the Hematopathology Section and is also responsible for the Laboratory of Pathology's Specialized Diagnostics Unit, which includes the Clinical Immunohistochemistry Unit and the Molecular Diagnostics Unit. Dr. Raffeld's research interests are concerned with the molecular pathogenesis of lymphomas and the use of molecular and immunohistochemical markers in lymphoma diagnosis.*

Laboratory of Pathology
Molecular Pathogenesis of Human Lymphomas

Keywords:
cell cycle regulation
lymphoma
molecular diagnostics

Research: The Immunohistochemistry Unit provides immunohistochemical support for all diagnostic pathology services in the laboratory using state-of-the-art automated equipment and serves as a resource for intra- and interdepartmental collaborative research. The service maintains a stock of approximately 120 antibodies for diagnostic studies and performs over 25,000 clinical stains per year.

The Molecular Diagnostics Unit performs molecular genetic tests to support the clinical and research activities of the NIH. Tests involve DNA–PCR, RT–PCR, DGGE, Southern blotting, and in situ hybridization. Clonal analysis for immunoglobulin and TCR rearrangement accounts for over 80 percent of requested tests. Other tests include analyses of Bcl-1, Bcl-2, NPM/ALK fusion transcripts, HHV–8, EBV, and p53.

Lymphomas account for about 10 percent of human neoplasms. They have a wide range of behavior even within the same histologic subtype. The laboratory's research is directed toward elucidating and understanding the molecular mechanisms underlying the pathogenesis of lymphomas, and in using this information to develop rational methods for classification and prognostication.

ALCL, a large cell lymphoma variant that primarily affects children and young adults, is characterized by the t(2;5), which results in inappropriate expression of anaplastic lymphoma kinase (ALK) through its fusion with

nucleophosmin (NPM). We have shown that NPM/ALK fusion transcripts are specific to ALCL, and do not occur in other lymphomas, including Hodgkin's disease (HD). We also showed that the immunohistochemical profile of ALCL is different from HD, in that ALCL expresses the cytotoxic granule-associated proteins TIA–1, perforin, and granzyme B. Because some cases of ALCL simulate HD, these molecular and immunohistochemical markers are useful in distinguishing the two diseases. These types of studies provide an objective basis for using molecular tests diagnostically.

We have also been interested in understanding the functional significance of recurrent coding region mutations that we identified in cases of Burkitt's lymphoma. We found that mutations surrounding a region including Thr 58 and Ser 62 interfered with phosphorylation at these sites and enhanced the transforming potency of MYC. More recently we have found a second grouping of mutations involving MYC box II that do not enhance transformation properties, but rather interfere with Myc's ability to mediate apoptosis. These studies are providing new information regarding the mechanism of action and control of this critical regulatory protein.

Our laboratory also participates in the NCI CGAP initiative through the provision of tissues for use in this gene discovery effort, and in developing immunohistochemical staining procedures for laser capture microscopy. The information generated from the CGAP effort should help in elucidating the genetic pathways involved in the genesis of different lymphomas and in lymphoma progression.

Recent Publications:

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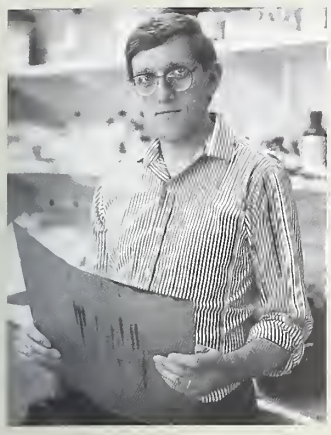
Lopatin U, et al. *Blood* 2001;97(10):3161–2.

Fend F, et al. *J Clin Pathol* 2000;53(9):666–72.

Kumar S, et al. *Mod Pathol* 2000;13(9):988–93.



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Biography: *Dr. Roberts received his B.S. from the Massachusetts Institute of Technology and his Ph.D. in biological chemistry from the University of Michigan. After postdoctoral training at Michigan and in the Laboratory of Biochemical Pharmacology of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), he became a research chemist in the NIDDK and later joined the NCI as chief of the Biochemical Pathology Section. Dr. Roberts serves on peer review panels and on the editorial boards of the* Journal of Biological Chemistry *and* Glycobiology. *His research interests include tumor cell-matrix interactions, angiogenesis, the biochemistry of cell surface carbohydrates, and host-pathogen interactions.*

Laboratory of Pathology
Regulation of Cellular Function and Gene Expression by Extracellular Matrix Components in Tumor Cells and Opportunistic Pathogens

Keywords:
adhesion molecules
angiogenesis
Candida albicans
cell signaling

Research: Cell-cell and cell-matrix interactions are important regulators of normal cell growth and differentiation and play essential roles in pathological conditions such as tumor metastasis and infection by pathogens. We are defining functions of adhesion molecules, their cell surface and matrix receptors, and the signal transduction pathways that regulate their activities in specific diseases. These studies will identify new targets for intervention and could provide a basis for designing novel pharmacological agents. The primary research projects in the laboratory are: (1) investigating the role of thrombospondin-1 (TSP1) in regulating tumor progression, and (2) defining the host-pathogen interactions that are required for establishing disseminated infections by the pathogenic yeast *Candida albicans.*

We are investigating the role of the adhesive glycoprotein TSP1 in regulating tumor growth and metastasis and the mechanism by which it inhibits angiogenesis in vitro and in vivo. Stable overexpression in tumor xenografts demonstrated that TSP1 is a suppressor of tumor progression. Although this was initially ascribed to inhibition of angiogenesis by TSP1, we now have evidence for significant direct effects of TSP1 on both tumor cells and the host immune response. We are using synthetic peptides, recombinant TSP1 fragments, and mutagenesis of the full-length protein to define the domains and specific amino acid sequences in TSP1 that mediate activities of TSP1 toward each of these cell types. We have identified peptide sequences in TSP1 that mimic the antiangiogenic activities of the whole molecule. These peptides induce programmed cell death in endothelial cells but not in tumor cells. Stable analogs of these peptides inhibit angiogenesis in several animal models and are being developing for therapeutic applications. The differential responses of tumor and endothelial cells to TSP1 arise from the utilization of distinct cell surface TSP1 receptors and result in different intracellular signals in each cell type. Recognition of TSP1 by the integrin $\alpha 3\beta 1$ on breast carcinoma cells is specifically modulated by IGF–I and CD98.

The same integrin is stimulated by EGF but not by IGF–I in small cell lung carcinoma and by VE-cadherin in endothelial cells. We are defining the cell-specific signal transduction pathways that control the activities of this and other TSP1 receptors and identifying genes whose expression is regulated by these TSP1-initiated signals using several cell types and TSP1 transgenic mice. Using DNA microarrays, we recently identified TSP1-regulated genes in T cells and demonstrated that TSP1 acts globally to suppress T cell receptor signaling. Two TSP1 receptors, CD47 and proteoglycans, mediated this activity.

Candidiasis is an increasingly common complication of cancer treatment with high morbidity and mortality. Candidemia has increased 10-fold in the past 10 years and now constitutes the third most common cause of positive blood cultures. Candidemia in neutropenic cancer patients causes septic shock and multiorgan failure. Because clinical isolates are increasingly resistant to available antifungal agents, new approaches are needed to prevent and treat these infections in cancer patients. Our current studies of the pathogenic yeast *C. albicans* are based on our discovery that hemoglobin specifically induces expression of a receptor for the extracellular matrix protein fibronectin. This response to hemoglobin is conserved among pathogenic species in the *Candida* genus but not other yeasts. Hemoglobin induces adhesion to several host matrix proteins and to endothelial cell monolayers. Both biochemical and molecular approaches are being used to identify the matrix receptors induced on *C. albicans*. We have identified a hemoglobin-induced cell wall protein that binds to fibronectin. We have cloned several novel genes that are specifically induced by hemoglobin. These genes define a new differentiation pathway by which the pathogen adapts to the vascular compartment of its host. Understanding the molecular mechanisms for regulation of this differentiation pathway could lead to new therapeutic targets to manage infections caused by these organisms.

Recent Publications:

Guo N, et al. *Cancer Res* 2000;60:457–66.

Chandrasekaran L, et al. *Mol Biol Cell* 2000;11:2885–900.

Pendrak ML, et al. *Biochemistry* 2000;39:16110–8.

Li Z, et al. *J Immunol* 2001;166:2427–36.

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Biography: *Dr. Patricia S. Steeg received her Ph.D. from the University of Maryland in 1982 and was a Jane Coffin Child Memorial Fund for Medical Research Fellow in the Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research, and the Laboratory of Pathology, NCI, where she was tenured in 1992. She became chief of the Women's Cancers Section in 1993.*

Laboratory of Pathology
Molecular Characterization of Breast Cancer Progression

Keywords:
breast cancer
metastasis

Research: The Women's Cancers Section performs basic and translational research on the molecular biology of breast cancer. Two major projects are under investigation: (1) What is the role of nm23 in breast cancer metastasis? (2) What molecular events are responsible for early neoplastic progression in the breast?

The nm23 family of genes was discovered by Dr. Steeg on the basis of its reduced expression in highly metastatic murine melanoma cell lines, as compared to related, poorly metastatic cell lines. Reduced Nm23 expression has been correlated with poor patient survival and the presence of metastases or other histopathological indicators of aggressive clinical course in breast and other cohort studies, although it does not represent an independent prognostic factor. Six transfection studies have documented that overexpression of nm23 cDNA in either breast carcinoma or melanoma cell lines results in a 50 to 90 percent decrease in tumor metastatic potential in vivo, establishing nm23 as a metastasis suppressor gene. Increased Nm23 expression induced morphological and functional differentiation in transfection studies involving breast and neuroendocrine cells.

We are investigating the relationship of Nm23 structure and function using site-directed mutagenesis. Two nm23–H1 mutations abrogated the in vitro tumor cell motility suppressive capacity of Nm23 upon transfection into breast carcinoma cells: (1) proline 96, the killer of prune mutation in the *Drosophila* nm23 homolog that can cause aberrant differentiation; and (2) serine 120, a site of mutation in human stage IV neuroblastomas and phosphorylation. Subsequently, Nm23 proteins were expressed and purified, then tested for biochemical activities in vitro. The proline 96 and serine 120 mutant proteins were uniquely deficient in aspects of histidine-dependent protein phosphotransferase pathways, leading to the hypothesis that this biochemical pathway may be responsible for the biological suppressive effects of Nm23.

Translational projects are under way in the section based on the hypothesis that elevation of Nm23 expression in micrometastatic breast and possibly other tumor cells may impact their colonization, motility, and differentiation

with a clinical benefit. We have identified the promoter for nm23–H1 and the portion which determines whether breast carcinoma cell lines of varying metastatic potentials express high or low Nm23 levels. No mutations were detected in this region. Two types of gene regulation are under study, including DNA methylation and mammary specific transcription factors.

The development of preventive strategies for women at high risk for breast cancer will require a "molecular map" of the cancer progress. In situ hybridization and immunohistochemical experiments have determined that ductal carcinoma in situ (DCIS) lesions frequently overexpress cyclin D1 and the RXR member of the retinoid family, as compared to either normal breast cells in the margin of the specimen or premalignant lesions conferring a lower risk for the development of invasive breast cancer. We have transfected cyclin D1 into a premalignant breast cell line and find that it augments colonization without full tumorigenicity; i.e., it contributes but is probably insufficient for full tumorigenicity. Interestingly, the cyclin D1 transfectants were preferentially sensitive to inhibition of colonization by both radiation and specific apoptosis inducers. The data suggest that cyclin D1 overexpressing cells may be colonization-competent and therefore moving towards malignancy, and that one prevention scheme may involve their selective apoptotic removal.

A new initiative is to identify proteins differentially expressed among human premalignant breast lesions using proteomics and mass spectroscopy sequencing. These efforts may uncover new proteins that contribute to breast oncogenesis.

Recent Publications:

Freije JMP, et al. *J Biol Chem* 1997;272:5525–32.

Zhou Q, et al. *Cancer Res* 2000;60:2611–5.

Hartsough MT, et al. *Cancer Res* 2001;61:2320–7.

Wulfkuhle, JD, et al. *Proteomics* 2001; in press.

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Laboratory of Pathology
Lymphomagenesis and Progression of Hematopoietic Neoplasms

Keywords:
apoptosis
lymphoma

Research: The Flow Cytometry Unit provides and develops specialized diagnostic procedures utilizing cytometric techniques as a clinical service. The Unit also conducts translational research into mechanisms of lymphomagenesis. Homeostasis in lymphoid tissues is maintained by close regulation of lymphoid cell proliferation and programmed cell death, or apoptosis. Inhibition of apoptosis can result in a neoplastic expansion of lymphoid cells (i.e., lymphoma) due to unchecked proliferation. In addition, genetic events leading to inhibition of programmed cell death result in resistance to numerous chemotherapeutic regimens as well as γ-radiation. Factors that inhibit apoptosis can therefore result in formation of lymphomas with poor prognosis due to resistance to treatment. The Flow Cytometry Unit is studying the contribution of tissue inhibitors of metalloproteinases (TIMPs) to lymphoid homeostasis and lymphomagenesis.

The tissue inhibitors of metalloproteinases (TIMPs) are a family of closely related proteins that were initially described as inhibitors of the matrix metalloproteinases. In addition to blocking matrix metalloproteinase activity, TIMPs also have growth factor-like activity. We have found that TIMP–1 is expressed by neoplastic B cells and this expression is associated with a more aggressive phenotype. TIMP–1 expression in B cells correlates with the germinal center phenotype that occurs with the generation of lymphoblasts, and TIMP–1-expressing lines can be described as mature, activated B cells in a preplasma cell stage. Induction of TIMP–1 expression induces further differentiation in B cells and upregulates the activation marker CD23 and the survival antigen CD40 while downregulating CD77 and surface immunoglobulin expression. CD77 expression is highly restricted to germinal-center B lymphocytes and is a neutral glycolipid expressed by a subset of B lymphocytes that readily enter programmed cell death. Apoptosis in these cells is prevented by CD40 engagement and by soluble CD23. Rescue from apoptosis by CD40 is mediated by a Bcl-2-independent mechanism. Based upon these previous studies, a model of B cell maturation has been proposed in which ligation of CD40 drives cells to loose CD77 and express membrane and

soluble CD23, which in turns acts as an autocrine factor. Thus, TIMP–1 may play a role in the normal development of the B cells in that it may provide an extracellular membrane signal to prevent programmed cell death. Further studies revealed that TIMP–1 promotes cancer cell growth through inhibition of programmed cell death. TIMP–1 expression in Burkitt's lymphoma cell lines confers resistance to Fas, radiation, cold shock, and serum starvation-induced apoptosis. TIMP–1's inhibition of both Fas-dependent and Bcl-2-dependent pathways is highly unusual and indicates a potential for far-reaching effects on cell growth and homeostasis. This antiapoptotic effect is novel, appears receptor mediated, and is not due to inhibition of matrix metalloproteinase activity. Since Bcl-2 has been shown to inhibit apoptosis in B cells, we determined whether Bcl-2 or the related proteins are implicated in the TIMP–1 protective effect. Although upregulation of TIMP–1 does not enhance expression of Bcl-2 or the Bcl-2 homolog Mcl-1, it clearly upregulates Bcl-X_L.

In addition to controlling several genes in B lymphocytes, including the immunoglobulin light-chain gene, the transcription factor NFκB has been reported to induce expression of antiapoptotic genes in B cells. We therefore studied expression of NFκB p65 (Rel A) and found no change in the cytoplasmic expression of NFκB with induction of TIMP–1 expression. However, higher expression of the NFκB inhibitor IκBα is observed, indicating a decrease in NFκB activity. Consequently, NFκB activation does not appear to be involved in the induction of antiapoptotic proteins by TIMP–1. Interestingly, this decreased NFκB activity is also supported by our results showing downregulation of immunoglobulin expression by TIMP–1. Furthermore, TIMP–1 suppression of PCD did not correlate with levels of cell surface CD95 expression. These results suggest that TIMP–1's antiapoptotic mechanism is not mediated by Bcl-2 or suppression of CD95 expression, but likely by inducing Bcl-X_L. Also, upregulation of Bcl-X_L by TIMP–1 appears to be independent of NFκB activation.

In summary, TIMP–1 expression in B cells inhibits programmed cell death by a novel mechanism and may be a negative prognostic factor in B cell non-Hodgkin's lymphoma. TIMP–1 inhibition of apoptosis may disrupt lymphoid homeostasis, resulting in development of lymphoma through uncontrolled neoplastic proliferation of lymphoid cells as well as resistance by the neoplastic cells to conventional cancer therapies. The laboratory is currently studying the putative role that TIMP–1 may play in the development of lymphoma.

Recent Publications:

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Guedez L, et al. *Am J Pathol* 2001;158(4):1207–15.

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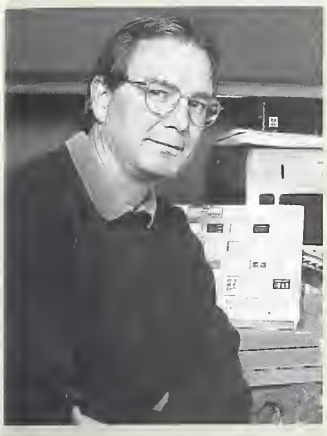
Stetler-Stevenson M, et al. *Blood* 2001;98(4):979–87.



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Biography: *Dr. William G. Stetler-Stevenson received his Ph.D. from Northwestern University in biochemistry/molecular biology in 1983 and his M.D. in 1984. He then trained in anatomic pathology at McGraw Medical Center in Chicago from 1984 to 1987, when he joined the Laboratory of Pathology as a senior staff fellow; he became a medical officer (research) in 1991 and chief of the Extracellular Matrix Pathology Section in 1993. In 1996, Dr. Stetler-Stevenson was corecipient of the Warner Lambert/Parke-Davis Award from the American Society for Investigative Pathology.*

Laboratory of Pathology
Extracellular Matrix Regulation of Cell Growth and Invasion

Keywords:
angiogenesis
cancer
cell proliferation
extracellular matrix
metalloproteinase inhibitors
metastasis
MMPIs
protease inhibitors
proteases

Research: The Extracellular Matrix Pathology Section is investigating the interaction of tumor cells and endothelial cells with the extracellular matrix during the processes of cell migration and invasion associated with cancer metastasis and angiogenesis. In particular we are interested in how these cell-matrix interactions influence cell growth and attachment, as well as the coordination of cellular events required to achieve a successfully invasive phenotype.

The role of matrix metalloproteinases (also known as the matrixins) in the progression of human cancer has been well documented in numerous reports. Studies have defined the close correlation between expression of various members of this family of closely related, zinc atom-dependent endoproteinases and patient survival in numerous cancer patient populations. Furthermore, the use of the endogenous inhibitors of these proteases, the tissue inhibitors of metalloproteinases (TIMPs), have been extremely useful in demonstrating the requirement for MMP activity in the process of tumor cell invasion, dissemination, and metastasis formation, as well as the related process of tumor-induced angiogenesis. These observations have led to the development of synthetic matrix metalloproteinase inhibitors that are currently in phase III clinical trials for cancer therapy.

However, despite demonstration of a correlation of MMP expression and invasive behavior, little is known about mechanisms that regulate MMP expression, control MMP activation, and coordinate MMP activity with other cellular processes that are required for the invasive phenotype. For example, two distinct mechanisms have been implicated in the activation of pro-MMP–2. One mechanism involves formation of a trimolecular complex of pro-MMP–2, TIMP–2, and MT–1–MMP on the cell surface and recruitment of a TIMP–2 free, active, second MT–1–MMP molecule. The other mechanism appears to involve binding of pro-MMP–2 to the ab3 integrin. We are investigating both mechanisms and the role of TIMP–2 in MMP–2 activation in endothelial cells.

The TIMPs themselves are a closely related family of low-molecular-weight proteins with four members now identified, TIMP–1, TIMP–2, TIMP–3, and TIMP–4. In addition to their function as metalloproteinase inhibitors, these proteins have been reported to have a number of other biological effects. The most studied of these, and the one of most potential utility in the cancer clinic, is the growth regulatory activity of the TIMPs. TIMP–1 and TIMP–3 reportedly stimulate cell growth of a number of cell lines in vitro following serum starvation. TIMP–2 has been reported by our laboratory to stimulate the growth of quiescent, serum starved fibroblasts and fibrosarcoma cells, as well as inhibit the growth of bFGF-stimulated human microvascular endothelial cells. These divergent effects are mediated by a cAMP-dependent mechanism involving the activation of cAMP-dependent protein kinase (PKA). Stimulation of a mitogenic response is seen in the quiescent cells and inhibition of growth is observed in the presence of tyrosine kinase-type growth factor receptor stimulation. Both effects are dependent on adenylate cyclase activity, and the outcome is thought to result from integration of the TIMP–2-initiated signaling via cAMP with other signaling pathways—the MAP kinase pathway, for example. However, what is not clear is the requirement for TIMP–2 to inhibit metalloproteinase activity in order to modulate cell growth. We have reported that synthetic MMP inhibitors and TIMP–1 do not reproduce the growth modulating effects that we observe for TIMP–2. To directly address this issue we have prepared, in collaboration with the Protein Expression Laboratory, National Institute of Arthritis and Musculoskeletal and Skin Diseases, a mutant TIMP–2 that lacks MMP inhibitor activity but retains correct tertiary structure as demonstrated by binding to pro-MMP–2. Appending a single alanine residue to the amino-terminal end of recombinant wild-type (wt) TIMP–2 generated this inactive mutant. Cleavage of the amino-terminal alanine using an aminopeptidase resulted in recovery of MMP inhibitor activity. This mutant will be useful in dissociating the growth modulating activity of TIMP–2 from the MMP inhibitor activity.

In collaboration with the Flow Cytometry Unit in the Laboratory of Pathology, we have shown that TIMP–1, but not TIMP–2, confers resistance to programmed cell death in Burkitt's lymphoma cells. In ongoing collaborative studies, we are examining the mechanism of this effect and specifically the role of inhibition of MMP activity. Like the effects of TIMP–2 on cell growth described above, this effect of TIMP–1 is not observed with synthetic MMP inhibitors and is specific for TIMP–1 (i.e., not observed with TIMP–2). The primary goal of this research program for the next 3 to 5 years is to develop our understanding of the growth regulatory effects of TIMPs and specifically the antiangiogenic/growth inhibitory properties of TIMP–2 for application in the oncology clinic. Currently, we are attempting to identify the domains within the TIMP molecules that are responsible for the growth modulating and antiapoptotic properties, as well as the cell surface receptors for the TIMPs that may modulate these effects. Identification of the peptide domains involved in TIMP–2 inhibition of endothelial cell growth would allow preparation of synthetic analogs that could be useful in cancer therapy in conjunction with other cytostatic strategies, such as MMP inhibitors described above.

We have collaborated with David Kleiner and Paul Wingfield, NIH.

Recent Publications:

Hoegy SE, et al. *J Biol Chem* 2001;276:3203–14.

Stetler-Stevenson WG. *Surg Oncol Clinics of North Am* 2001;10:383–92.

Guedez L, et al. *Am J Pathol* 2001;158:1207–15.

Alpler Ö, et al. *J Natl Cancer Inst* 2001;93:1375–84.

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Biography: *Dr. Tsokos received her M.D. from the National University of Athens, Greece, where she also worked on her postdoctoral thesis. She completed her residency training in anatomic pathology at the Laboratory of Pathology, NCI, and received training in pediatric and ultrastructural pathology at the same institution. Dr. Tsokos was named chief of the Pediatric Tumor Biology and Ultrastructural Pathology Section in 1988. In 1987, she was elected professor of pathology, School of Health Sciences of Crete, Greece. Dr. Tsokos is associate editor of the* Journal of Investigative Medicine *and serves on the editorial board of* Clinical Immunology.

Laboratory of Pathology
Programmed Cell Death in Solid Pediatric Tumors: The Fas/FasL Signaling Pathway

Keywords:
apoptosis
childhood tumors
Ewing's sarcoma family of tumors (ESFT)
neuroblastoma
rhabdomyosarcoma

Research: The Pediatric Tumor Biology and Ultrastructural Pathology Section provides diagnostic services in pediatric tumor pathology and electron microscopy and studies mechanisms of programmed cell death (apoptosis) in solid pediatric tumors. In recent years, specific translocations and oncogene patterns have emerged as markers of clinical significance in solid pediatric tumors. The section incorporates novel approaches such RT–PCR and in situ hybridization in the diagnosis and classification of these tumors and studies the significance of molecular markers in their biology and prognosis. The goal of our research is to study signaling pathways that govern the expression of apoptosis-related molecules such as Fas and Fas ligand (FasL) and their biologic significance in tumors of the Ewing's sarcoma family (ESF), neuroblastoma, and rhabdomyosarcoma. With presently available therapeutic approaches, the prognosis of recurrent pediatric sarcomas is dismal. It is important to identify novel agents or strategies that would eradicate chemotherapy-resistant tumor cells. We elected to study the mechanisms that control the machinery of programmed cell death, hoping to understand reasons for chemotherapy failures and ways to overcome them.

FasL is a transmembrane protein of the tumor necrosis factor (TNF) family, which induces apoptosis upon crosslinking with the Fas receptor. It is cleaved by an unknown metalloproteinase into a soluble form, which is a less potent inducer of apoptosis. FasL-expressing tumor cells use FasL as a cytolytic effector molecule to kill Fas-expressing activated lymphocytes (counterattack model). Previous studies from our laboratory have established the presence

of Fas and FasL in tumors of ESF. We have also shown that FasL is expressed at a statistically significant higher frequency in metastatic ESF tumors and is functional in vitro. These data support the association of FasL with a more aggressive phenotype in ESF tumors. However, the very same molecule can be detrimental to ESF tumors if they have a functional Fas pathway. Indeed, we found that synthetic metalloproteinase inhibitors increase the levels of FasL and Fas on the surface of ESF tumor cells by inhibiting cleavage of FasL. This leads to apoptosis of Fas-sensitive cells. Fas-resistant ESF tumor cell lines did not respond to the metalloproteinase inhibitors, but underwent apoptosis with a newly described molecule, the TNF-related apoptosis-inducing ligand (TRAIL). These studies suggest that alternative treatments with apoptosis-inducing molecules may be possible in the near future for tumors of the ESF. Because FasL, in contrast to Fas, shows only limited expression in normal cells despite its universally highly expression in tumors, we plan to study mechanisms of upregulation of FasL by tumor-specific proteins or oncogenes.

The pathway by which the apoptotic signal is transmitted after Fas/FasL ligation involves several steps that may vary among the various cell types. In general, an initial death-inducing signaling complex (DISC) is formed that leads to the activation of caspase 8, which in turn activates a downstream cascade of caspases leading to apoptosis. The activation of the downstream caspases by caspase 8 can be done either directly (type I cells) or indirectly through the help of the mitochondrion (type II cells). In the latter situation, the bcl-2 family of proteins plays an important role as negative regulators of apoptosis. We compared the Fas signaling pathways of ESF tumors and neuroblastomas in vitro and found that the former function as type I cells, whereas the latter function as type II cells. Furthermore, antisense bcl-2 treatment sensitized neuroblastoma but nor ESF tumor cells to Fas-mediated apoptosis. These data suggest that antisense bcl-2 may emerge as an important agent in the treatment of certain groups of neuroblastoma. We are currently studying further the various steps that are involved in the transmission of the apoptotic signal in ESF tumors and neuroblastomas and plan to extend these studies to rhabdomyosarcoma.

Collaborating in this work is Carol Thiele, NIH.

Recent Publications:

Kumar S, et al. *Mod Pathol* 2000;13:988–93.

Mitsiades N, et al. *Cancer Res* 2001;61:2704–12.

Poulaki V, et al. *Cancer Res* 2001;61:4864–72.

Mitsiades N, et al. *Cancer Res* 2001;61:577–81.

Clinical Trials:

Andrea Abati

95–C–0092: 2CDA for gliomas

David E. Kleiner

91–CC–0117: Epidemiology, infectivity, and natural history of hepatitis C virus infection in a blood donor program

94–C–0159: A phase I protocol for the evaluation of the safety and immunogenicity of vaccination with synthetic HIV envelope peptides in patients with early human immunodeficiency virus infection. Open for accrual

95–C–0003: GW/ROB TBI collaborative efforts

95–C–0069: HU/PTX for GBM

95–C–0075: Gemcitabine for pancreas

97–N–0066: Alpha-galactosidase, a replacement therapy in Fabry disease

99–C–0011: Stereotactic radiosurgery

99–C–0116: Treatment planning

Elise C. Kohn

CPB334: A phase I study of the combination of CAI and paclitaxel in adult patients with refractory cancers or lymphoma: This clinical trial has shown safety of combining pulse CAI with 3 weekly paclitaxel and is now testing daily CAI with 3 weekly paclitaxel

MB349: A multi-institutional phase II study of cyclophosphamide, paclitaxel, cisplatin with G–CSF for patients with newly diagnosed advanced stage ovarian cancer: Molecular evidence of the supra-additive effects of the combination of cisplatin, cyclophosphamide, and paclitaxel has led to this phase II study for the treatment of newly diagnosed epithelial ovarian cancer. Molecular diagnostic questions related to ovarian cancer progression are posed in the phase II study and are under way

MB392: A phase II time-to-progression study of orally administered CAI to patients with persistent epithelial ovarian cancer: This protocol follows our evaluation of CAI and asks whether the agent might provide disease stabilization to patients with advanced ovarian cancer. Translational endpoints include assessment of a CAI resistance-associated gene and markers of angiogenesis

MB415: A pilot study of proteomic evaluation of epithelial ovarian cancer patients in first clinical remission. Development of a protein fingerprint profile associated with relapse: This protocol enrolls women in first clinical response to treatment for ovarian cancer and follows them serially until relapse is diagnosed. Advanced proteomic technologies are being applied to serum samples to identify proteomic patterns that are predictive of recurrent disease. This is in concert with similar work ongoing in study sets of sera from newly diagnosed women

Clinical Trials (continued):

Collaborative Clinical Trials:

CoPI GOG–175: A randomized phase III trial of IV carboplatin (AUC 6) and paclitaxel 175 mg/m2 q 21 days x 3 courses plus low-dose paclitaxel 40 mg/m2/wk versus IV carboplatin (AUC 6) and paclitaxel 175mg/m2 q 21 days x 3 courses plus observation in patients with early stage ovarian carcinoma

Scientific PI GOG–0170C: A phase II trial of ZD1839 (Iressa™) (NSC 715055) in the treatment of persistent or recurrent epithelial ovarian or primary peritoneal carcinoma

91–C–0232: A phase I study of Taxol, cisplatin, cyclophosphamide, and granulocyte colony-stimulating factor (G–CSF) in previously nontreated ovarian cancer patients

95–C–0015: A phase I study of the combination of CAI and paclitaxel in adult patients with refractory cancers or lymphoma

95–C–0055: A multi-institutional phase II study of cyclophosphamide, paclitaxel, cisplatin with G–CSF for patients with newly diagnosed advanced stage ovarian cancer

95–C–0056B: A phase II study of continuous-infusion aminocamptothecin for advanced epithelial ovarian cancer recurrent after paclitaxel and either cisplatin or carboplatin

97–C–0138: Tissue acquisition for molecular diagnostics

98–C–0012: A phase II clinical trial of orally administered CAI for patients with advanced, relapsed, or refractory epithelial ovarian cancer

00–C–0018: A pilot study of proteomic evaluation of epithelial ovarian cancer patients in first clinical remission: development of a protein fingerprint profile associated with relapse

Maria Tsokos

97–C–0050: A pilot study of tumor-specific peptide vaccination and IL–2 with or without autologous T cell transplantation in recurrent pediatric sarcomas

97–C–0052: A pilot study of autologous T cell transplantation with vaccine-driven expansion of antitumor effectors after cytoreductive therapy in metastatic pediatric sarcomas

Laboratory of Population Genetics

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The Laboratory of Population Genetics (LPG) utilizes genetic analysis to gain insight into human biologic processes. Until recently, genetic dissection of phenotypes had been largely limited to investigations in experimental organisms. The dawn of the postgenome era presents the opportunity to extend these investigations to humans. It is the major goal of this laboratory to exploit emerging resources and technology in order to understand the genetic basis of the complex phenotypes related to human cancer. The program focuses on both the development of the resources and methods necessary to achieve these goals as well as the application of the methods in characterizing the molecular genetic epidemiology of disease. The LPG is organized around three interrelated areas: human genetic mapping, genetic phenotype dissection, and experimental organism genetics.

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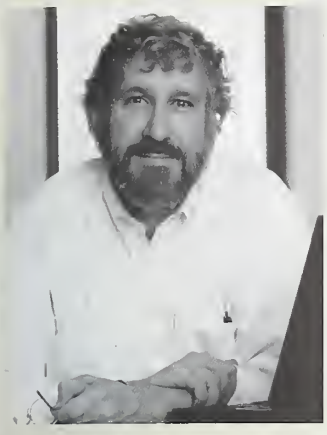

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Biography: *Dr. Buetow received a B.A. in biology from Indiana University in 1980 and a Ph.D. in human genetics from the University of Pittsburgh in 1985. From 1986 to 1998, Dr. Buetow was at the Fox Chase Cancer Center in Philadelphia, where his group generated and electronically distributed the human genetic map. Dr. Buetow serves a dual role for the National Cancer Institute. He is the director of the NCI Center for Bioinformatics (NCICB) and chief of the Laboratory of Population Genetics (LPG). His research interests include the application of genetics and genomics tools to understanding the genetic basis of complex traits.*

Laboratory of Population Genetics
The Genetic Basis of Complex Disease— An Integrated Genomic and Bioinformatic Approach to Understanding the Cancer Phenotype

Keywords:
genetics
genomics
informatics
liver cancer
ovarian cancer
prostate cancer

Research: The Laboratory of Population Genetics (LPG) conducts human genetic and genomics research, both at the bench and using informatics tool. The major goal of this research program is to apply and extend human genetic analysis methods and resources to better understand the genetics of complex phenotypes, specifically human cancer. The program focuses on both the development of the resources and methods necessary to achieve these goals as well as the application of the methods in characterizing the molecular genetic epidemiology of disease.

Dr. Buetow has spearheaded efforts of the Genetic Annotation Initiative (GAI), an attempt to identify variant forms of the cancer genes identified through the NCI Cancer Genome Anatomy Project (CGAP). His laboratory is particularly interested in genetic variations that make individuals more susceptible to liver, lung, prostate, breast, and ovarian cancer. His group combines computational tools with bench-top laboratory findings to understand how genes and environment interact to increase cancer risk.

As director of the NCICB, Dr. Buetow coordinates and deploys informatics in support of NCI research initiatives. The goal of the NCICB is to maximize interoperability and integration of NCI research and its related information. The center participates in the evaluation and prioritization of the NCI's bioinformatics research portfolio, conducts or facilitates research that is required to address the NCICB's mission, serves as the locus for strategic planning to address the NCI's expanding research initiative's informatics needs, establishes information technology standards (both within and outside of NCI), and communicates, coordinates, or establishes information exchange standards.

Collaborators on this research include Carmen Allegra, Stephen Chanock, Lynette Grouse, Katherine McGlynn, Stephen Sherry, Robert Strausberg, and Shelia Zahm, NIH; Charles Cantor, Hubert Koester, Daniel Little, and Richard

MacDonald, Sequenom, Inc.; Paul Engstrom, Warren Kruger, W. Thomas London, and Joseph Testa, Fox Chase Cancer Center; and Fu-Min Shen, First Shanghai Medical University.

Recent Publications:

Buetow KH, et al. *Proc Natl Acad Sci USA* 2001;98(2):581–4.

Emmert-Buck MR, et al. *J Mol Diagn* 2000;2(2):60–6.

Strausberg RL, et al. *Trends Cell Biol* 2001;11(11):S66–71.

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Biography: *Dr. Hunter received a B.S. in biochemistry from the Pennsylvania State University in 1985. He received a Ph.D. in biology from the Massachusetts Institute of Technology in 1991. He was an associate member at the Fox Chase Cancer Center from 1996 to 1999. In 1999, he joined the Laboratory of Population Genetics as an investigator.*

Laboratory of Population Genetics
Modeling Human Cancer in Mouse Models

Keywords:
low penetrance genes
susceptibility genes

Research: Cancer is a complex disease involving the interaction of many genetic pathways and environmental factors. Although huge strides have been made by the identification of oncogene and tumor suppressor genes, comprehensive understanding of human cancer has been hampered by the genetic heterogeneity of human populations and the complex environments in which we live. To attempt to gain better understanding of the genetic components of human cancer, we use the mouse as a model system. The availability of homozygously inbred mice and a controlled environment enable the identification of low penetrance or susceptibility genes and genetic pathways that are important in the initiation and progression of malignant disease.

Among our collaborators are Diana Cozma, Luanne Lukes, Jessica Rouse, and Phillip Wise, NIH; and Danny Welch, Pennsylvania State University College of Medicine.

Recent Publications:

Lifsted T, et al. *Int J Cancer* 1998;77:640–4.

Yang Y–L, et al. *Nat Genet* 1999;21:216–9.

Le Voyer T, et al. *Mamm Genome* 2000;11:883–9.

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Biography: *Dr. Jen received her M.D. from Beijing Medical College in 1984 and her Ph.D. from the University of California–San Francisco in 1991. She carried out postdoctoral work at the Johns Hopkins Oncology Center from 1992 to 1996 and served as an assistant professor there until 1999. She became an investigator in the NCI's Laboratory of Population Genetics in September 2000.*

Laboratory of Population Genetics
The Biology and Genetics of Lung Cancer

Keywords:
chromosome loss
gene expression profiling
gene mutation
genetics
loss of heterozygosity (LOH)
lung cancer
SAGE
tissue microarray

Research: We used hierarchical clustering to examine gene expression profiles generated by serial analysis of gene expression (SAGE) in a total of nine normal lung epithelial cells and nonsmall cell lung cancer (NSCLC). We observed that 115 highly differentially expressed genes were able to distinguish the neoplastic state and the cell type of lung cancer. Furthermore, these 115 transcript tags clustered into groups suggestive of the unique biological and pathological features of the different tissues examined. We are currently characterizing some of these highly differentially expressed genes for their potential in lung cancer. We have also used the oligo-based cDNA array containing 12,000 elements of full-length genes to survey more than 50 primary lung cancer samples. We observed that gene expression profiles generated by cDNA array could produce similar distinctions between the tumor and the normal tissues. However, there were substantial differences among the tumors tested. We are conducting experiments to determine whether genetic changes associated with these tumors contributed to the gene expression differences observed. We have previously identified PGP9.5, a ubiquitin hydrolase, as being frequently overexpressed in lung cancer and may participate in lung tumorigenesis. We used a yeast two-hybrid system and showed that JAB1 was one of the proteins that interacts with PGP9.5. Furthermore, PGP9.5 appeared to facilitate nuclear transportation of JAB1, which in turn led to downregulation of p27 from the cell nucleus. We are using site-directed mutagenesis approaches and cellular models to determine the precise role and consequences of PGP9.5 interaction with JAB1 and other proteins. Finally, we are continuing our efforts to identify genes that could potentially be used as tumor markers or therapeutic targets. To this end, we have developed in situ hybridization assays that allow us to sensitively and rapidly identify genes that are associated with lung cancer. We are also in the process of developing lung tumor microarrays so that we will be able to screen a large number of well-characterized tumors on a single tissue slide.

Our collaborators include Kenneth Buetow, Michael Emmert-Buck, and Robert Strausberg, NIH; Franco DeMayo, Baylor College of Medicine; David Sidransky, Johns Hopkins Medical School; William Travis, Armed Forces Institute of Pathology, Washington, DC; and Ping Yang, the Mayo Clinic.

Recent Publications:

Doug SM, et al. *J Natl Cancer Inst* 2001;93:858–65.

Caballero OL, et al. *Genes Chromosomes Cancer* 2001;32:119–25.

Nacht M, et al. *Proc Natl Acad Sci* 2001; in press.

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Biography: *Dr. Lee received his B.S. in biology from the University of Science and Technology of China in 1982 and his Ph.D. in biochemistry from Duke University in 1989. He carried out postdoctoral work at Duke from 1990 to 1994 and joined the National Institute on Aging (NIA) as a senior staff fellow in 1994. Dr. Lee became a junior faculty member at Johns Hopkins Medical School between 1995 and 1999, then joined IBM as a software engineer in 1999. He came to the NCI in 2000 as a tenure track investigator.*

Laboratory of Population Genetics
Genetics and Epigenetics of Cancer and Informatics Solutions to Biomedical Research

Keywords:
bioinformatics
epigenetics
gene chip
gene discovery
genetics
genomic imprinting
genomics
loss of heterozygosity (LOH)
positional cloning

Research: Both genetic and epigenetic mutations contribute to human cancers. Mutations in oncogenes, tumor suppressor genes, and DNA repair genes have been identified in human cancers. Epigenetic mutations such as methylation of tumor suppressor genes and DNA repair genes and loss of imprinting of the *IGF–II* gene are also associated with cancers. The major goal of our research is to identify cancer genes and epigenetic markers for human cancers. Rapid progress in the human genome project has significantly speeded up discovery in genetic research and disease research. Sequence analysis and bioinformatics play a vital role in understanding the genetic basis of human diseases. One of Dr. Lee's major interests is to apply bioinformatics to genetic research.

Positional cloning and candidate cloning of cancer genes. We have generated transcript maps for regions showing loss of heterozygosity (LOH) at 13q11 in esophageal cancer. Mutational analysis of candidate genes in the human chromosome 13q11 have been carried out to identify tumor suppressor genes. We are also taking a candidate gene cloning approach to analyze several hundreds of genes involved in cancer such as oncogenes, tumor suppressor genes, and DNA repair genes. We are performing mutational analysis as well as methylation of CpG islands in the promoter region of these cancer genes. In addition, altered gene expression in tumors will be analyzed by methods such as Affymetrix expression chips.

Genome-wide search of imprinted genes. We have used transcribed single nucleotide polymorphisms (SNPs) to isolate several imprinted genes. We are using Affymetrix chips to genotype DNA and to quantitatively analyze the

allele-specific gene expression. Similarly, we will systematically isolate epigenetic markers. We will examine both the imprinted genes and epigenetic markers in tumors and their matched normal tissues, in populations with high and low risk of cancer. The alteration in imprinting will be analyzed for its association with other genetic changes such as genomic instability, mutations in cancer genes, and genotype.

Bioinformatics approach to cancer gene discovery. We have undertaken a computational approach to systematically search for all human imprinted genes. We have decided to search for imprinting genes from a single nucleotide polymorphism database containing all SNPs in expressed sequence tags (ESTs). The Bayesian statistics were used to estimate the genotype frequency. Significant reduction in the frequency of these libraries expressing both alleles suggests that the SNP is located in an imprinted gene. We have identified about 100 candidate imprinted genes and are validating these results by experiments. A similar approach is also used to identify both mutations in cancer cells and SNPs associated with cancers. We are interested in identifying genetic elements important for genomic imprinting and genomic instability. We are taking a number of approaches including comparative genome analysis and motif analysis to search for genetic elements.

Informatics solutions for research. We are also developing several tools for automating positional cloning and mutational analysis. We have developed programs to parse genes into exons and generated database for exons and their flanking intron sequences and mapped SNPs to these genetic elements. We are also developing tools for analyzing gene expression and constructing comprehensive genetic networks.

Our collaborators include Ken Buetow, Nan Hu, Ying Hu, Myung-soo Lyu, and Philip Taylor, NIH.

Recent Publications:

Lee M, et al. *Cancer Res* 1998;58:4155–9.

Lee M, et al. *J Clin Invest* 2000;106:1447–55.

Lee M, et al. *Proc Natl Acad Sci* 1999;96:5203–8.

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Biography: *Dr. Struewing received his M.D. from Indiana University School of Medicine in 1985 and his M.S. in preventive medicine from the University of Maryland School of Medicine in 1988, where he also completed his residency in general preventive medicine. Following 3 years as a U.S. Navy epidemiologist, he joined the NCI in 1991, where his studies have focused on inherited breast and ovarian cancers.*

Laboratory of Population Genetics
Inherited Breast and Ovarian Cancer

Keywords:
Ashkenazi
DNA repair
genetics
hereditary cancer
ovarian cancer
p16

Research: Inherited forms of breast and ovarian cancers (those due to germline inheritance of mutations in single genes) account for about 5 percent of all cases of these cancers. Studies of families predisposed to breast, and particularly ovarian, cancer have been an active area of research for several decades through the NCI's Familial Cancer Registry. Since the cloning of the *BRCA1* gene in 1994 and the *BRCA2* gene in 1995, the two genes that account for about two-thirds of inherited breast and ovarian cancers (those occurring in families with five or more breast cancers diagnosed before age 50 or ovarian cancer), mutational analysis has revealed a very large number of mutations, most leading to truncated proteins. The technical difficulty of detecting relatively rare mutations in these two large genes has hampered large-scale population-based studies of them. However, the presence of three relatively common "founder" mutations in *BRCA1* and *BRCA2* within the Ashkenazi Jewish population has allowed more broad-based studies, including one in which over 5,000 Ashkenazi Jewish volunteers from the Washington, DC, area participated. We estimated that the risk of breast cancer for carriers of a *BRCA1* or *BRCA2* mutation was 56 percent by age 70 and the risk of ovarian cancer was 16 percent. These estimates contrast with higher risk estimates of 85 percent and 45 percent, respectively, which were derived earlier from the study of selected high-risk families.

Efforts are now underway to identify the genetic and environmental factors that may modify, or trigger, cancer risk in those who are carriers of *BRCA1/2* mutations. Of course, these factors may also be susceptibility factors among the majority of women who do not carry these mutations. Since both the BRCA1 and BRCA2 proteins seem to be involved in the repair of damaged DNA, we have focused on other genes involved in DNA repair processes in our search for modifying factors. We are planning a comprehensive set of laboratory assays to determine which genetic variants in DNA repair genes are biologically important and risk factors for cancer. These will be utilized both in family-based settings and in epidemiologic case-control and cohort studies. Because of the large number of subjects and genotypes required, we are also evaluating methods for pooling DNA samples prior to laboratory analysis.

An important potential environmental modifying factor for the risk of ovarian cancer is the use of oral contraceptives, which confer rather strong protection against this disease in the general population Whether oral contraceptives are similarly protective among women who carry *BRCA1/2* mutations is the focus of a collaborative study being conducted in Israel.

Our collaborators include William Bonner, Mark Greene, Alice Sigurdson, and Margaret Tucker, NIH; Georgia Chenevix-Trench, Queensland Institute of Medical Research, Australia; Andrew Godwin, Fox Chase Cancer Center; and Kenneth Offit, Memorial Sloan-Kettering Cancer Center.

Recent Publications:

Wang WW, et al. *Cancer Epidemiol Biomarkers Prev* 2001;10:955–60.

Modan B, et al. *N Engl J Med* 2001;345:235–40.

Goldstein AM, et al. *Br J Cancer* 2001;85:527–30.

Laboratory of Receptor Biology and Gene Expression

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The research program in the Laboratory of Receptor Biology and Gene Expression concerns the elucidation of mechanisms involved in the regulation of genetic expression in eukaryotic cells, and the identification of genes and regulatory processes involved in modulated states of expression during oncogenesis. Particular consideration is given to the study of the steroid/thyroid/RAR superfamily of nuclear receptors (Hormone Action and Oncogenesis Section [HAO], Signal Transduction Group). A special emphasis is placed on the function of these hormone-dependent regulators in the context of chromatin and higher-order nuclear structure. The laboratory has made major contributions to the general hypothesis that the modification of chromatin structure is an important component of gene regulation by steroid receptors and other transactivators.

The laboratory is expanding into the general area of nuclear structure and gene function. A major recent advance was the development of fluorescent chimeric receptor proteins that allow study of nuclear receptor subcellular trafficking and gene targeting in living cells (HAO Section). A high-resolution imaging facility is under development (Fluorescence Imaging Group) and a new research group (Cell Biology of Gene Expression Group) has been recruited. The imaging facility will support investigations into subcellular and intranuclear compartmentalization, particularly in living cells. It is anticipated that state-of-the-art technologies, including confocal and digital imaging microscopy, will be available. Also, a new investigator who is an expert in the application of scanning force microscopy to chromatin structure has joined the lab.

The lab also supports efforts in studies on the transcriptional regulation of human retroviruses, including HTLV–1 and HIV (Virus Tumor Biology Section). The interaction of viral regulatory proteins HTLV–1 Tax and HIV Tat with cellular targets is a major focus of these efforts.

Laboratory of Receptor Biology and Gene Expression Staff

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Biography: *Dr. Hager received his Ph.D. in genetics at the University of Washington in the lab of Ben Hall. He pursued postdoctoral studies with Dick Epstein at the Institut de Biologie Moleculaire in Geneva and with Dr. William Rutter at the University of California–San Francisco. He carried out the first molecular cloning of retroviruses at the NIH and also reported the first identification of steroid responsive regulatory elements. Dr. Hager is currently chief of the Laboratory of Receptor Biology and Gene Expression, where his program interests include the role of chromatin structure in gene regulation, the mechanism of steroid receptor function, and the architecture of active genes in the interphase nucleus.*

Laboratory of Receptor Biology and Gene Expression
Steroid Receptors, Chromatin, and Nuclear Structure

Keywords:
Ah receptor
gene regulation

Research:

Mechanism of Nucleoprotein Remodeling by Nuclear Receptors on Reconstituted Chromatin

We have used the mouse mammary tumor virus promoter (MMTV) for several years as a model system to study the impact of chromatin structure on gene activation, particularly by steroid receptors. In the current chromatin program, we are attempting to characterize the wide variety of activities that are recruited to a gene target in response to steroid receptor activation and to understand in molecular detail the nature of the chromatin remodeling events. We originally proposed that the nucleoprotein transition in MMTV involved the reorganization of one nucleosome, Nucl B, in the MMTV phased array of nucleosomes. Two subsequent findings indicated that this view was oversimplified. First, each translational setting in the nucleosome array results not from the presence of a single, uniquely positioned core, but rather from a "family" of closely grouped nucleosomes. Second, the chromatin remodeling event maps at higher resolution not to the B family, but to a more extended region of transition including all of the B family and the 3′ half of the C family. These findings indicated that the receptor-induced transition must be understood in terms of a more complex model, perhaps invoking higher order chromatin fiber structure.

We have recently succeeded in reconstructing the receptor-induced chromatin remodeling event with MMTV nucleosome arrays assembled in vitro. Surprisingly, the receptor-dependent nucleoprotein transition can be precisely duplicated with these assembled arrays using highly purified glucocorticoid receptor (GR) and a HeLa extract as a source of remodeling activity. The chromatin remodeling event in vitro is dependent on ATP and maps precisely to the B and 3′ half of C nucleosomes, as is found in vivo. These results indicate that the in vitro system accurately recapitulates the in vivo transition and places us in position to study the molecular details of the transition with the much more powerful tools that are available in reconstituted systems.

Steroid/Nuclear Receptor Trafficking in Living Cells

In 1996 we demonstrated that intracellular distribution and trafficking by the glucocorticoid receptor could be effectively studied in living cells with fusions to the green fluorescent protein. We have now tagged essentially all of the classic steroid/nuclear receptors, including GR, PR, ER, TR, AhR, and the PPAR family, and find that the technology is generally applicable to all of the receptor groups. These advances have led to several unexpected and important findings for members of the nuclear receptor superfamily. In particular, we find that behavior with regard to subcellular localization and ligand dependent redistribution for several members of the family is quite different from the classic view. Our results indicate that the currently accepted description of steroid/nuclear receptor trafficking is seriously deficient.

Trafficking by the progesterone receptor. The progesterone receptor occurs naturally in two forms, A and B. Potential roles for these forms have been the subject of much investigation, but little is understood concerning the functional significance of alternate forms. Using GFP labeled derivatives, we discovered that the A and B forms traffic differentially in living cells despite identical nuclear localization signals. These findings indicate that as yet undescribed interactions between the two receptor forms and either nuclear or cytoplasmic interacting proteins (or both) are determinative in compartmentalization, and that these interactions play a large role in distribution of the forms. This view contrasts with the view that nuclear localization signals (NLS) are the primary determinant of distribution.

Trafficking and distribution by the estrogen receptor, alpha form. The estrogen receptor also occurs naturally in two forms, alpha, and the recently described beta form. With GFP labeled variants, we have shown that ER alpha is constitutively located in the nucleus in living cells, but moves within intranuclear compartments in response to ligand activation. The receptor is also differentially distributed in the nuclei of ER negative and ER positive human breast cancer cells, indicating that states of intranuclear organization change in the progression from ER(–) to ER(+) stages of the disease. These findings indicate that states of subcellular architectural organization may be important in development of hormone-independent disease.

Trafficking and distribution by the thyroid receptor. The thyroid receptor has been described as a "nuclear receptor" since its original discovery and characterization; that is, it is found constitutively present in the nucleus in the presence or absence of ligand. Using the GFP technology, we examined TR distribution in living cells for the first time, and discovered the classic dogma to be incorrect. A significant portion of the receptor is found cytoplasmically localized in living cells, and moves to the nucleus with ligand treatment. Following on this observation, we have characterized in very recent work mutations of TR that disrupt interactions with corepressors and find that these mutant receptors are located completely in the cytoplasm in untreated cells, but translocate to the nucleus in a ligand-dependent fashion. These are quite unexpected results, and indicate that the classic literature with regard to TR trafficking is incorrect.

Mechanism of Transcriptional Repression by the Glucocorticoid Receptor
The glucocorticoid receptor, as well as other members of the nuclear receptor superfamily, will both activate and repress gene expression. Using the GnRH promoter as a model, we discovered a novel mechanism for GR function in transcriptional repression. GR does not bind to a DNA recognition site in this promoter, but rather binds indirectly to the promoter via ligand-dependent protein-protein interactions with Oct 1. Oct 1 has a noncanonical binding site in the promoter, and GR repression is dependent on this site. We showed by in vitro DNA binding experiments that purified GR will interact directly with Oct 1 when it is bound to the GnRH *Oct 1* site, but will not bind to Oct 1 on a wild-type Oct 1 binding site. Furthermore, we showed that the Oct 1 protein is in a different conformation when bound to the *Oct 1* site; that is, the DNA binding site for Oct 1 serves as an allosteric effector for the Oct 1 interaction with GR. This provides direct evidence for a tethering mechanism for GR function at this promoter and is the first demonstration in vitro of differential GR protein-protein interactions determined by the architecture of an interacting protein DNA binding site.

Recent Publications:

Lim CS, et al. *Mol Endocrinol* 1999;13:366–75.

Htun H, et al. *Mol Biol Cell* 1999;10:471–86.

Sheldon LA, et al. *Mol Cell Biol* 1999;19:8146–57.

McNally JG, et al. *Science* 2000;287:1262–5.

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Biography: *Dr. Leuba received his Ph.D. in biochemistry and biophysics at Oregon State University where he studied chromatin fiber structure with Dr. Ken van Holde. He received an NIH postdoctoral fellowship to study chromatin fiber structure by atomic force microscopy in the laboratory of Dr. Carlos Bustamante in the Institute of Molecular Biology at the University of Oregon. This training was followed by a postdoctoral fellowship with Dr. Stuart M. Lindsay at Arizona State University to study chromatin using dynamic force microscopy. Dr. Leuba joined the NCI as an NCI scholar in 1998. His interests are in applying single molecule methods to elucidating basic questions on chromatin structure and dynamics.*

Laboratory of Receptor Biology and Gene Expression
Chromatin Fiber Structure and Dynamics as Revealed by Single Molecule Approaches

Keywords:
chromatin structure
histones
single molecule detection and manipulation

Research: Our research is investigating chromatin structure and dynamics as revealed by single molecule approaches. Single molecule approaches (e.g., atomic force microscopy AFM, optical tweezers) can answer particular questions that are difficult (if not impossible) to answer by population-ensemble experiments (e.g., gel electrophoresis, etc.). Our interests in

chromatin are (1) linker and core histones (variants, stoichiometry) and their contributions to fiber structure, (2) posttranslational modifications of histones (acetylation, phosphorylation, ADP-ribosylation, etc.) affecting structure, (3) effect of DNA methylation on fiber structure, and (4) interactions of chromatin fibers or single nucleosomes reconstituted on specific DNA sequences with other macromolecular complexes involved in DNA functioning (e.g., polymerases, chromatin remodeling factors, etc.).

AFM Imaging and Manipulations

With AFM our approach is two-fold: on one side, high-resolution imaging of protein/DNA complexes (chromatin fibers of various composition, stoichiometry, and posttranslational modifications) and, on the other side, direct manipulation of single chromatin fibers with the AFM tip to probe the forces holding the structure of the chromatin fiber together. Force is a factor in many processes involving chromatin and chromosome structural reorganizations during the life of any eukaryotic cell. Force may be needed to clear histones from the DNA for biological processes such as transcription, replication, and repair. Force generation and application to biological structure is a major component of dynamic biological processes, but forces governing chromatin structure and function have not been experimentally approached up to now. Our studies of pulling short reconstituted chromatin fibers nonspecifically attached to the tip and surface lead us to conclude that we were measuring the force of adhesion of the nucleosomes to the glass substrate. To circumvent this experimental artifact, we are now tethering the DNA ends between the AFM tip and the surface, with the idea of directly reconstituting chromatin onto a single DNA molecule (similar to the optical tweezers experiments, below).

Force Measurements with Optical Tweezers

Our interests in the effect of force applied to chromatin fibers has led us to collaborate with researchers at the University of Twente (The Netherlands) on applying optical tweezers to chromatin fibers. With the optical tweezers we can probe the lower region of forces 1 to 150 picoNewtons (pN), whereas with the AFM we can probe forces above 100 pN. In these optical tweezers experiments, we first attached a piece of DNA between two beads, demonstrated it was an intact single molecule of DNA that can undergo the well known B–DNA to S–DNA transition, and then assembled histones onto this single DNA molecule by injecting a *Xenopus laevis* egg nucleosome assembly extract into the liquid cell of the instrument. These kinds of experiments open a whole new approach because with the nuclear extract it is possible to assemble chromatin with various complements of histones (i.e., only core histone H3/H4 tetramers, fluorescently modified histones, with or without linker histone subtypes, etc.). We have found that our optical tweezers setup is sensitive enough to detect the disruption of single nucleosomes among the ~240 nucleosomes assembled on the 48,502 bp of lambda DNA.

We have determined that a range of forces of 15 pN to 40 pN is sufficient to unravel a single nucleosome. The unraveling of an individual nucleosome results in a length increase of the chromatin fiber of ~65 nm. This length increase is on the order of the length of two wraps of DNA around the nucleosome core particle. We believe that our measurements are fundamental to understanding the dynamic changes in nucleosome structure as

nucleosomes are formed and then disassembled to allow access of transcription, replication, and repair machineries to the underlying DNA template.

DNA Methylation and Chromatin Structure
Methylation of certain bases is the sole postsynthetic modification in DNA. Methylation of cytosine takes place in CpG dinucleotides. Concentrations of methylatable CpGs form the so-called CpG islands; the methylation status of CpG islands in enhancers/promoters of genes determine the transcriptional activity of the gene (CpG islands downstream of initiation sites do not affect transcription). Since methylation of CpG islands in promoters blocks transcription, we have been investigating a possible methylation-dependent chromatin compaction. We are using both AFM imaging and biochemical approaches to study this issue. We found that chromatin fibers hypermethylated in vivo were more compact than fibers isolated from control fibers. Modeling studies suggest that more DNA is wrapped around nucleosomal particles in the methylated chromatin fibers than in the control fibers. In vitro studies point to a cooperation between CpG methylation and linker histone binding in the formation of more compacted fibers; DNA methylation or linker histone binding alone do not cause fiber compaction.

Archael Protein HMf (Histone from *Methanothermus fervidus*)
HMf has the same histone-fold structure as the eukaryal core histones although it lacks completely the posttranslationally modified histone tails. We have investigated the ability of this protein to form chromatin fiber structure to gain insights into possible similarities and differences between eukaryal chromatin and its Archael counterpart. We have found that this protein can indeed by reconstituted onto DNA to form bona fide chromatin fibers as revealed by AFM and biochemical studies. Interestingly, the lessor stability of Archael mononucleosomes and short oligonucleosomes suggests that the posttranslationally modifiable tails of the eukaryal histones lead to greater stability of eukaryal chromatin as well as serve to regulate accessibility to the underlying DNA template for DNA functioning.

Collaborators include Martin Bennink, University of Twente, The Netherlands; David Brown, University of Mississippi Medical Center; Paola Caiafa, University "La Sapienza," Rome, Italy; and Jordanka Zlatanova, Polytechnic University, Brooklyn, NY.

Recent Publications:

Bennink ML, et al. *Nat Struct Biol* 2001;8:606–10.

Leuba SH, et al. *Single Molecules* 2000;1:185–92.

Zlatanova J, et al. *Prog Biophys Mol Biol* 2000;74:37–61.

Zlatanova J, et al. *Crit Rev Eukaryot Gene Expr* 1999;9:245–55.

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Biography: *Dr. McNally received his Ph.D. in biophysics at the University of Chicago in the lab of Hewson Swift and Jack Cowan, and he pursued postdoctoral studies with Ted Cox at Princeton University. He has developed image processing techniques for quantitative analysis of 3D cell locomotion within a living tissue and applied these methods to identify roles for myosin II in amoeboid cell motion. Dr. McNally is imaging director in the Laboratory of Receptor Biology and Gene Expression, where his program interests currently include transcriptional control mechanisms of steroid hormone receptors in living cells and large-scale chromatin structure.*

Laboratory of Receptor Biology and Gene Expression
In Vivo Imaging Analysis of Transcriptional Regulation

Keywords:
chromatin structure, function, and dynamics
receptor functions

Research: Transcription is a fundamental life process, but it has not been easy to study in live cells. We are developing techniques to accomplish this, using the mouse mammary tumor virus promoter (MMTV) as a model system for studying transcriptional control by steroid hormones. To analyze transcriptional regulation at the MMTV, we are using a cell line containing a GFP-tagged glucocorticoid receptor (GR) and an ~200-fold tandem array of the MMTV promoter, which provides a target site large enough to be visualized by fluorescence light microscopy. The target appears in the nucleus as a single fluorescent spot approximately 0.5 μm in diameter, or as a more extended string-like structure. These structures are the bona fide MMTV target, as they consistently colocalize with transcripts for the genes known to be downstream of the MMTV promoter in these cells. To assess the dynamics of GR binding to the promoter, we have used fluorescence recovery after photobleaching (FRAP). After complete bleaching of the target site, fluorescence at the target recovers in a matter of seconds, demonstrating rapid exchange of GFP–GR molecules at this site. The rapid exchange is not a consequence of damage to the receptor by the bleach, as demonstrated by an alternative bleaching strategy—namely, FLIP—or fluorescence loss in photobleaching. In FLIP studies, the nucleoplasmic fluorescence is bleached at a site far from the MMTV target. Fluorescence at the MMTV target still decays rapidly, implying that nonbleached GFP–GR molecules are rapidly leaving the template. Future work is directed at understanding the mechanism of rapid GR exchange by investigating FRAP recovery in the presence of drugs and different GR mutants.

In a second project, we are using the same GFP–GR MMTV array cell line to study large-scale chromatin structure. We find that upon hormone addition, the array undergoes dramatic morphological changes that correlate with transcriptional activity. Within 3 hrs after hormone addition, arrays visualized by GFP–GR or DNA FISH decondense to varying degrees, in the most pronounced cases from a ~0.5 μm spot to form a fiber 1 to 10 μm long. Arrays later recondense by 3 to 8 hrs of hormone treatment, consistent with earlier studies that demonstrate a transient activation of the MTMV

promoter in these cells. The degree of decondensation is proportional to the amount of transcript produced by the array, as detected by RNA FISH. Decondensation is blocked by two different drugs that inhibit polymerase II, DRB, and α-amanitin. These observations demonstrate a role for polymerase in producing and maintaining decondensed chromatin. They also support fiber-packing models of higher order chromatin structure. Finally, the data suggest that transcription from a natural promoter may occur at much higher DNA packing densities than previously reported, since the most decondensed state observed corresponds to a packing density of at least 50-fold, significantly higher than the 6-fold packing of a 10 nm nucleosome fiber. Future work aims to understand the mechanism of this decondensation and its relationship to the structure of other large, active stretches of chromatin.

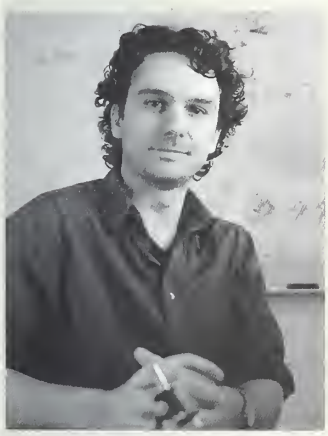
Recent Publications:

Clow P, et al. *Mol Biol Cell* 1999;10:1309–23.

Müller W, et al. *J Cell Biol* 2001;154:33–48.

McNally JG, et al. *Science* 2000;287:1262–5.

Clow P, et al. *Development* 2000;127:2715–28.



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Biography: *Tom Misteli received his Ph.D. in 1995 from the University of London and the Imperial Cancer Research Fund, London, UK. He was a postdoctoral fellow at the Cold Spring Harbor Laboratory, NY, where he initiated the use of in vivo imaging techniques to visualize gene expression processes in living cells. He joined the NCI in 1999 and heads the Cell Biology of Gene Expression Group.*

Laboratory of Receptor Biology and Gene Expression
Nuclear Architecture and Gene Expression

Keywords:
chromosome organization
nuclear architecture
pre-mRNA splicing
transcription

Research: My laboratory studies cell biological aspects of gene expression. We use molecular techniques in conjunction with live-cell microscopy to study how genes are expressed in the context of the intact cell nucleus of living cells. These studies provide insights into basic biological mechanisms, and are also relevant to the successful development of gene therapy approaches.

We have developed photobleaching techniques, which allow us for the first time to visualize the mobility of proteins in the nucleus of living cells and more importantly provide a means to develop quantitative assays to probe the functioning of proteins at the molecular level in living cells. To this end we are using kinetic modeling and computer simulations to determine

characteristic kinetic properties of nuclear proteins such as diffusion coefficients, on/off rates with nuclear compartments, fluxes and residence times in nuclear compartments. These measurements will provide the framework for the detailed investigation of how nuclear proteins function in living cells.

We are also interested in the organization of genomes in the interphase cell nucleus. To this end we have initiated studies to map in 3 and 4 dimensions the positioning of chromosomes within the nucleus and we are testing whether positioning of chromosomes affects gene expression and regulation. These studies are considered first steps towards rigorous interphase cytogenetic methods.

We have also initiated a project to study gene alternative splicing of the microtubule-binding protein tau. Tau has been implicated in the formation of senile plaques and fibrillar tangles in numerous neurological diseases such as Alzheimer's disease and FTDP–17. Specifically, inclusion of exon 10 encoding a microtubule-binding domain has been linked to the disease phenotype. We are currently investigating the mechanisms involved in alternative splice site selection in the tau gene in vivo and are using this knowledge to correct the splicing defect in disease tissue.

Recent Publications:

Eils R, et al. *Mol Biol Cell* 2000;11:413–8.

Misteli T, et al. *Mol Cell* 1999;3:697–705.

Misteli T, et al. *Nature* 1997;387:523–7.

Phair R, et al. *Nature* 2000;404:604–9.

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Biography: *Dr. Robertson received his Ph.D. in pharmacology from the Johns Hopkins University School of Medicine where he studied the role of DNA methylation in viral latency in Richard Aminder's lab. He then pursued postdoctoral studies with Dr. Peter Jones at the University of Southern California examining epigenetic mechanisms of tumor suppressor gene inactivation, then with Dr. Alan Wolffe at the National Institute of Child Health and Human Development examining the control components of the methylation machinery. Since 2000, he has headed the Epigenetic Gene Regulation and Cancer Group as an NCI cancer scholar.*

Laboratory of Receptor Biology and Gene Expression
DNA Methylation, Gene Regulation, and Cancer

Keywords:
DNA methylation
gene expression
methyltransferase
tumor suppressor

Research: Our objective is to understand the factors regulating de novo DNA methylation in normal cells and during development and determine how these control pathways go awry in tumor cells and contribute to tumorigenesis. DNA methylation, or the covalent addition of a methyl group to the 5-position of cytosine in the context of the CpG dinucleotide, is required for mammalian development, has profound effects on chromatin structure, and has roles in both DNA repair and genome stability. It has become clear that while essential for development, DNA methylation patterns become dysregulated in cancer, with a generalized genome-wide hypomethylation (loss) in combination with region-specific hypermethylation (gain), primarily at CpG islands. In addition, DNA hypermethylation has been shown to efficiently and heritably inactivate genes. When this occurs in the promoter region of a growth-regulatory gene, it can give that cell a growth advantage and ultimately lead to cancer.

We have focused our studies on the regulation of the cellular enzymatic methylation machinery, the DNA methyltransferases (DNMTs). There are currently three known functional DNMTs in mammalian cells. In one area of research we have examined the mRNA expression of each DNMT during the cell cycle in normal and transformed cells. We find that the three enzymes are differentially regulated during the cell cycle and that tumor cells have subtle defects in this regulation that could lead to the expression of an inappropriately high level of methyltransferase activity at an incorrect time. This in turn could lead to aberrant de novo methylation events that would accumulate each time the cell divides and eventually lead to improper gene silencing.

Mechanisms for the establishment or targeting of methylation patterns in normal cells are almost completely unknown. Methylation clearly can be targeted since certain genomic regions are always heavily methylated, like pericentromeric heterochromatin, or the regions of DNA adjacent to the centromere, while regulatory regions for many essential genes are always unmethylated. We have recently taken a biochemical approach to identify

factors that interact with the DNMTs that may alter their nuclear localization and enhance or inhibit their enzymatic activities on particular DNA substrates. Our studies revealed that the most abundant methyltransferase, DNMT1, interacts with the retinoblastoma gene product Rb. Rb is a critical regulator of cell division and helps a cell decide whether to divide or to go into a semipermanent resting state. Rb, or other components of the Rb pathway, are mutated in almost all tumor cells. We have determined that cancer-associated mutations in Rb abolish the ability of Rb to interact with DNMT1. Thus we may have identified one mechanism whereby methylation patterns become disrupted in tumors due to an inability of Rb to interact with DNMT1. The identification of factors that interact with and direct methylation in normal cells opens up exciting new possibilities for understanding tumor development and may ultimately lead to novel therapies designed to restore normal methylation patterns and growth control.

This research was carried out in collaboration with Peter Jones, University of Southern California–Los Angeles; and Alan Wolffe, Sangamo Biosciences, Richmond, CA.

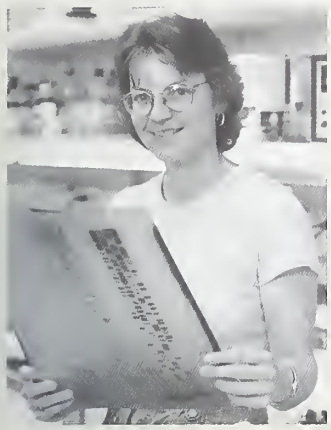
Recent Publications:

Robertson KD, et al. *Mol Cell Biol* 1998;18:6457–73.

Robertson KD, et al. *Nucleic Acids Res* 1999;27:2291–8.

Robertson KD, et al. *Nucleic Acids Res* 2000;28:2108–13.

Robertson KD, et al. *Nat Genet* 2000;25:338–42.



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Biography: *Dr. Smith received her Ph.D. in biochemistry from the University of Vermont where she studied regulation of the alpha-fetoprotein gene during liver differentiation. She then moved to the NIH for postdoctoral studies focusing first on vitamin D receptor function with Dr. Stephen Marx in the National Institute of Diabetes and Digestive and Kidney Diseases and later on regulation of the mouse mammary tumor virus promoter by glucocorticoids with Dr. Gordon Hager. Since 1997, she has headed the Signal Transduction Group as a tenure track investigator.*

Laboratory of Receptor Biology and Gene Expression
Signal Transduction and Chromatin Structure in Mammary Cells

Keywords:
breast cancer
cAMP signaling
chromatin
gene transcription
progesterone receptor

Research: Our goal is to define mechanisms by which signaling pathways modify chromatin structure to influence the expression of target genes. In vivo, cells use various signal transduction pathways to respond to their extracellular environment. These signals can modulate the expression of cellular genes, all of which are packaged into a complex nucleoprotein structure referred to as chromatin. These structures can be modified to either accommodate or repress transcription. We use the mouse mammary tumor virus (MMTV) promoter as a model to study the role of chromatin structure in its transcriptional regulation by progesterone and by cAMP signaling. Our studies have definitively shown that the nucleoprotein structure of the promoter determines its response to the two signaling pathways. Our work underlines the importance of studying transcriptional regulatory mechanisms in the context of chromatin. In addition, it provides a basis for explaining how the same promoter may respond differently to the same extracellular signal in different tissues or cell types in vivo.

Our study of progesterone receptor function has shown that the chromatin structure of the MMTV promoter can be a barrier to activation by the progesterone receptor (PR). However, this barrier can be overcome through some mechanism of intracellular receptor processing. We have determined that the progesterone receptor can exist in two functional states in mammary-derived cell cultures depending on how it is processed. These states are characterized by differences in intracellular localization and the ability of the receptor to remodel chromatin at target promoters and to be activated by other signaling pathways. The nature of the processing events which determine the functional state of the progesterone receptor is currently under investigation. These differences in function would have profound effects on receptor action in vivo and may represent a previously unknown mechanism by which PR function is regulated in the mammary gland.

A wide variety of hormones use cAMP signaling to elicit cellular responses. However, with the exception of target promoters containing a binding site for CREB (cAMP response element binding protein), very little is known about how cAMP signaling leads to changes in transcription at other target promoters. We determined previously that cAMP signaling can regulate the MMTV promoter in a manner influenced by its nucleoprotein structure. When the promoter is packaged into an ordered and repressive chromatin structure, it is strongly repressed by cAMP signaling independent of glucocorticoids. However, when the promoter is in a derepressed, accessible conformation, cAMP signaling synergizes with glucocorticoids to activate transcription. Our recent studies have shown that cAMP regulation of both forms of the MMTV promoter are dependent on the function of protein kinase A, but independent of the function of CREB. However, the two promoter forms are differentially dependent on the coactivator and histone acetyltransferase, p300. This work has led us to conclude that cAMP regulation of the two promoters is mediated by separate cAMP-induced signaling pathways. Thus the nucleoprotein structure of a given promoter may determine which signals target it. Such a mechanism may account for some differences in cell type-specific regulation of a particular gene promoter.

Additional work on cAMP regulation of the MMTV promoter has led to the discovery of a novel mechanism of cAMP signaling and transcriptional repression. We have recently determined that cAMP-induced repression of the MMTV promoter in ordered, replicating chromatin is mediated in part through histone modifications. We have shown that cAMP signaling leads to dramatic increases in acetylation and phosphorylation of histone H3 across the MMTV promoter. In addition, protein kinase A is highly capable of phosphorylating histone H3 in a nucleosomal context in vitro. Thus we conclude that histones can be direct targets of signaling pathways and that histone modifications can lead to repression of transcription. This stands in contrast to an increasingly large volume of literature showing a link between increased histone acetylation and transcriptional activation. Our study shows that promoter context may influence the response to histone modification. Current work is focused on understanding the mechanism by which these histone modifications lead to repression, as well as identifying the kinase and acetyltransferase which carry out these modifications at the MMTV promoter in vivo.

Recent Publications:

Smith CL, et al. *J Biol Chem* 1997;272(22):14227–35.

Lim CL, et al. *Mol Endocrinol* 1999;13:366–75.

Sheldon LA, et al. *Mol Cell Biol* 1999;19:8146–57.

Smith CL, et al. *Mol Endocrinol* 2000;14:956–71.

Regulation of Cell Growth Laboratory

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The Regulation of Cell Growth Laboratory studies the mechanisms that regulate cell growth and death, using a variety of animal models. Normal cell growth is a tightly regulated process that is subject to numerous positive and negative signals. These signals are perturbed during cancer development, resulting in abnormally proliferating cells that are resistant to apoptosis, which would usually eliminate such aberrant cells. The general interest of the laboratory is to understand signal transduction pathways that activate cell division, cell death or survival, and cell differentiation to determine how these responses are altered in cancers. The ultimate goal is to develop therapeutics based on the inhibition of positive growth signals or reactivation of pathways that promote cell death or differentiation.

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Carol Shawver	Support Staff
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Biography: *Dr. Vousden received her Ph.D. in genetics from the University of London. She carried out postdoctoral fellowships with Professor Chris Marshall at the Institute of Cancer Research in London and Dr. Douglas Lowy at the NCI before becoming head of the Human Papillomavirus Group at the Ludwig Institute for Cancer Research in London in 1987. After joining the ABL–Basic Research Program as head of the Molecular Carcinogenesis Section in 1995, she was appointed director of the Molecular Virology and Carcinogenesis Laboratory in 1997 and interim director of the ABL–Basic Research Program in 1998. She is now chief of the Regulation of Cell Growth Laboratory.*

Regulation of Cell Growth Laboratory
Regulation of Cell Growth and Survival

Keywords:
apoptosis
ARF
cell cycle
E2F–1
Mdm2
p53

Research: Our research is aimed at understanding the mechanisms that regulate cell growth and survival, both in normal and tumor cells. In particular, we are interested in the tumor suppressor gene product p53. The p53 protein plays an important role in response to stress such as DNA damage or oncogene activation, and loss of p53 is associated with the development of many human cancers. p53 functions to mediate growth suppression by inducing cell cycle arrest or apoptosis, thereby preventing tumor development. Since p53 can also suppress the growth of normal cells, the p53 protein is maintained at low levels in unstressed cells through rapid proteolytic degradation. We showed that the stability of p53 is regulated through interaction with the Mdm2 protein, which targets p53 for proteasome-dependent degradation. Interestingly, *Mdm2* is transcriptionally activated by p53, establishing a negative feedback mechanism by which p53 can control its own activity. We have found that a region of Mdm2 that forms a RING finger is essential for Mdm2 to degrade p53, although this region does not participate in the binding of the two proteins. This region of Mdm2 is important for the ability of the protein to function as an E3 ubiquitin ligase, a component of the system through which p53 is degraded. Indeed, expression of Mdm2 mutants that have lost the ability to target degradation but retain the ability to bind p53 show dominant-negative activity, stabilizing p53 by protecting it from endogenous wild-type Mdm2.

Activation of p53 in response to stress occurs largely by stabilization of the p53 protein, following inhibition of Mdm2 activity. One important signal for the activation of p53 is abnormal proliferation, and we showed that this response involves transcriptional activation of the $p14^{ARF}$ protein by E2F1, a transcription factor that is deregulated in almost all cancers. $p14^{ARF}$ stabilizes p53 by binding to, and inhibiting the ability of MDM2 to target p53 for degradation.

p53 induction can result in either cell cycle arrest or apoptosis, and a number of p53-inducible genes that contribute to these responses have been identified. We have recently described PUMA, a BH3 domain containing protein, as a target for p53-mediated transcriptional activation. PUMA expression results in rapid apoptosis in many cell types, and is likely to contribute to the p53-mediated apoptotic response. Other genetic alterations in tumor cells can also sensitize cells to respond to p53 by undergoing programmed cell death. We have been particularly interested in the family of E2F transcription factors, which plays an essential role in mediating cell cycle progression. One member of the E2F protein family, E2F1, has been shown to participate in the induction of apoptosis, and E2F1 can function as a tumor suppressor, presumably by participating in the activation of apoptosis in abnormally proliferating cells. Although deregulated E2F1 can result in the stabilization of p53 through $p14^{ARF}$, as described above, we have shown that E2F1 can also induce significant apoptosis in the absence of p53, and that this apoptotic function of E2F1 is separable from the ability to accelerate entry into DNA synthesis. Recently, we have been able to demonstrate that this activity of E2F1 results from the inhibition of survival signals, such as NFκB, following activation of death receptors. Because deregulation of E2F1 occurs in the majority of human tumors, this ability of E2F1 to inhibit antiapoptotic signaling may contribute to the enhanced sensitivity of transformed cells to chemotherapeutic agents.

Our recent studies have uncovered a new complexity in the role of NFκB in the regulation of apoptosis, however. We have shown that, unlike E2F–1-mediated cell death, p53-induced apoptosis requires NFκB activity. Interestingly, p53 can activate NFκB DNA binding activity and it is possible that apoptotic target gene activation requires both p53 and NFκB function. Our findings indicate that inhibition of NFκB in tumors that retain wild-type p53 may diminish, rather than augment, a therapeutic response, and we are currently investigating the contribution of NFκB to the chemotherapeutic response using in vivo models.

Collaborators on this study include Bill Kaelin, Dana-Farber Cancer Institute; Moshe Oren, Weizmann Institute of Science; Gordon Peter, Imperial Cancer Research Fund, London; and Allan Weissman, NIH.

Recent Publications:

Lohrum MA, et al. *Nat Cell Biol* 2000;2:179–81.

Ashcroft M, et al. *Mol Cell Biol* 2000;20:3224–33.

Ryan KM, et al. *Nature* 2000;404:892–7.

Nakano K, et al. *Mol Cell* 2001;7:645–8.

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Biography: *Dr. Jairaj Acharya obtained his M.B.B.S. from the University of Gulbarga, India, in 1985 and received his Ph.D. from the Indian Institute of Science, Bangalore, in 1993. He then joined Dr. Charles Zuker at the University of California–San Diego as an associate of the Howard Hughes Medical Institute. He was recruited by the NCI in 1999.*

Regulation of Cell Growth Laboratory
Phospholipid Signaling

Keywords:
Drosophila
genetics
membrane proteins
phospholipids

Research: Our long-term objective is to understand the complex interrelationship between phospholipid and sphingolipid metabolism and metabolic signaling in vivo. Intermediates of phospholipid (PL) and sphingolipid (SL) metabolism serve as second messengers for a number of signaling cascades including activation of G-protein-coupled receptors such as adrenaline and thrombin as well as receptor tyrosine kinases by growth factors. They mediate a number of processes ranging from protein secretion to activation of apoptosis. We have initiated studies to understand several aspects of lipid signaling in *Drosophila*.

Lipid Reservoirs and Signaling

Sphingomyelin (phosphorylethanolamine ceramide) could serve as a reservoir for several lipid messengers such as ceramide, ceramide 1-phosphate, sphingosine, and sphingosine 1-phosphate. We have identified several putative homologous genes of this cascade in *Drosophila*. We are attempting to delineate the importance of this pathway in vivo by transgenic and mutagenic studies

Lipid Distribution and Signaling

PL and SL at the plasma membrane play an important role in stimulus-response coupling, cell differentiation, movement, and exo- and endocytosis. They are asymmetrically distributed in biological membranes and different proteins catalyzing uni- and bidirectional movements of lipids perpetuate asymmetry. Our current efforts focus on scramblase, a protein proposed to be involved in bidirectional transbilayer movement of phospholipids. We anticipate that a combination of genetic, molecular, and biochemical approaches in *Drosophila* will define the important players involved in PL and SL signaling in their normal cellular environment.

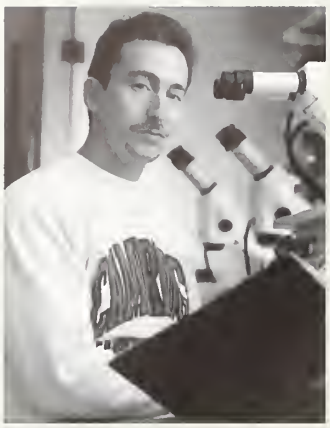
Recent Publications:

Acharya JK, et al. *Neuron* 1997;18:881–7.

Acharya JK, et al. *Neuron* 1998;20:1219–29.

Acharya U, et al. *Cell* 1998;92:183–92.

Gossen M, et al. *Science* 1995;270:1674–7.



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Biography: *Dr. Daar obtained his Ph.D. degree from the State University of New York at Buffalo where he studied the molecular defects underlying common glycolytic enzyme deficiencies. He then obtained his postdoctoral training with Dr. George Vande Woude at NCI, investigating signal transduction events and cell cycle control points influenced by oncogene function.*

Regulation of Cell Growth Laboratory
Developmental Signal Transduction

Keywords:
cell signaling
developmental biology
receptor tyrosine kinases

Research: Our current research interests are aimed toward examining the mechanism by which receptor tyrosine kinases and protein tyrosine phosphatases signal differentiation events and morphogenetic movements. An understanding of these signal transduction pathways may improve our understanding of oncogenesis, since most neoplasias appear to result from failure of proliferating cells to respond to differentiation signals. The *Xenopus* embryo is well suited for investigations of these processes because the frog has a well characterized and invariant cell fate map and cell lineage can be easily traced during experiments. Mutant receptors, ligands, and other proteins can be ectopically expressed in embryos. Thus, their effects on signal transduction and differentiation can be assessed morphologically and histologically as well as biochemically in a developing vertebrate.

Our laboratory is currently investigating the role of the *Xenopus* EphA4 receptor tyrosine kinase and X–EphrinB1 (a *Xenopus* ligand for Eph receptors) in cell signaling and function using the *Xenopus* oocyte and embryo systems. At present, our emphasis is placed upon the mechanism by which these Eph family members exhibit crosstalk with the fibroblast growth factor (FGF) signaling pathway.

Members of the Eph family have been implicated in regulating numerous developmental processes such as axon outgrowth, neural crest cell migration, hindbrain segmentation, and angiogenesis. Recently, studies from our laboratory identified a member of another tyrosine kinase family—the fibroblast growth factor (FGF) receptor—that can interact with and regulate the Ephrin signaling molecule. FGF, like the Ephrins, has been implicated in axon guidance, neural cell migration, and angiogenesis. Our research demonstrates that crosstalk between the FGF receptor and an Eph ligand can exist in both neural and nonneural tissues, and that when tyrosine phosphorylated in response to FGF receptor activation, Eph ligand signals that reduce adhesion can be inhibited. This study is the first evidence for direct interaction between an Eph transmembrane ligand and members of the FGF receptor tyrosine kinase family.

XephA4 is the only A subtype Eph receptor that has significant binding activity in response to EphrinB molecules. Both EphA4 and EphrinB1 are expressed during late gastrulation and are found in the presumptive rhombomeres and neural crest. Additional suggestive evidence for a common cellular function between EphrinB1 and EphA4 comes from a constitutively activated XephA4 receptor that has been shown to cause cell dissociation (much like XephrinB1) when overexpressed in early *Xenopus* development. In collaboration with Tony Pawson's laboratory, we are conducting structural and functional studies on EphA4. We have ectopically expressed an EphA4 mutant where the tyrosine at residue 928, believed to be responsible for the binding of LMW–PTP, is changed to phenylalanine. Morphologically, the Y928F mutant causes a striking phenotype where animal blastomeres lose cell adhesion in early development but revert to normal adherence at late gastrulation. Of particular interest is the induction of ectopic tails that is a consequence of mutant expression. Further work in our laboratory has revealed that the ectopic tail induction results from activation of the FGF signaling pathway. Collectively, our data shows that EphrinB1 and the EphA4 receptor both induce similar phenotypes and both cross talk with the FGF-signal transduction pathway.

Collaborators include Robert Friesel, Maine Medical Research Institute; Tony Pawson, Samuel Lunenfeld Research Institute, Toronto, Canada; Mitsugu Maeno, Niigata University, Niigata, Japan; and Colin Stewart, NCI–Frederick.

Recent Publications:

Chong L, et al. *Mol Cell Biol* 2000;20:724–34.

Chong L, et al. *Methods Mol Biol* 2000;124:21–37.

Ishimura A, et al. *Diff Growth & Dev* 2000;42:307–16.

Maeda R, et al. *Oncogene* 2001; 20:1329–42.

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Regulation of Cell Growth Laboratory
Oncogene-Dependent Apoptosis

Keyword:
apoptosis

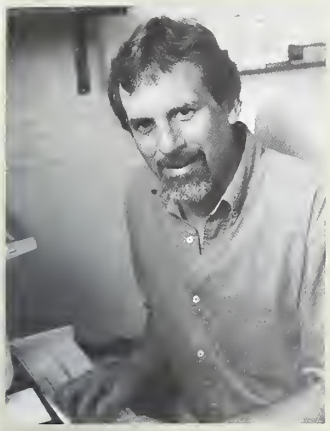
Research: Cancer arises through mutations that trigger uncontrolled cell proliferation. Unexpectedly, oncogenes that deregulate cell proliferation often also induce apoptosis. Thus it appears that apoptosis functions as a defense, protecting an organism from potentially tumorigenic cells. It follows that a critical step in tumorigenesis is the acquisition of mutations that compromise the apoptotic response induced by oncogenes. Indeed, many mutations found in tumors inhibit apoptosis. The observation that some oncogenes are proapoptotic suggests that, in principle, reestablishing the link between oncogene expression and the apoptotic machinery would selectively kill cancer cells. To accomplish this, a biochemical understanding of what the apoptotic machinery is and how oncogenes activate it is needed. To investigate how the apoptotic machinery is linked to proapoptotic oncogenes, this lab uses a cell-free system that mimics E1A-dependent apoptosis. This system is based on the observation that extracts from cells transformed with E1A activate the apoptotic machinery, whereas extracts from untransformed cells do not. The basis of this difference appears to be a protein called Apoptotic Protease Activating Factor-1 (Apaf-1). Apaf-1 acts with cytochrome c to trigger activation of caspases, a family of cysteine proteases that drive the apoptotic process. However, Apaf-1 is present in extracts from untransformed cells, although caspase activation does not occur. A major focus of the laboratory is understanding how E1A expression modulates Apaf-1's ability to trigger caspase activation. In addition, the mechanism by which other proapoptotic oncogenes activate caspases is being examined using cell-free systems.

Recent Publications:

Faleiro L, et al. *EMBO J* 1997;16:2271–81.

Fearnhead HO, et al. *Genes Dev* 1997;11:1266–76.

Fearnhead HO, et al. *Proc Natl Acad Sci USA* 1998;95:13664–9.



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Biography: *Dr. Johnson received his Ph.D. from the Department of Biology, University of California–San Diego, in 1983 and was a postdoctoral fellow at the Fred Hutchinson Cancer Research Center and the Carnegie Institution from 1984 to 1988 with Dr. Steven McKnight. He was corecipient of the 1988 to 1989 AAAS Newcomb-Cleveland Prize for a paper describing the leucine zipper motif in bZIP DNA-binding proteins. In 1989, he joined the ABL–Basic Research Program as leader of the Eukaryotic Transcriptional Regulation Group and was appointed section head in 1998. In 1999, Dr. Johnson joined the Center for Cancer Research, NCI.*

Regulation of Cell Growth Laboratory
Function and Regulation of the C/EBP Family of Transcriptional Activators

Keywords:
B–ZIP protein
DNA binding proteins
gene knockout
TNF gene regulation
transcriptional regulation

Research: Changes in gene expression underlie many fundamental biological processes, including cellular differentiation and development. Our work focuses on the CCAAT/enhancer-binding protein (C/EBP) family of transcription factors, which are involved in establishing the terminally differentiated phenotypes of many cells. The C/EBP proteins (C/EBPα, C/EBPβ, C/EBPδ, C/EBPε, and C/EBPγ [Ig/EBP]) belong to the basic-leucine zipper (bZIP) class of DNA-binding proteins. The C/EBPs recognize a common DNA sequence, exhibit similar leucine zipper dimerization specificities, and, with the exception of C/EBPγ, function primarily as transcriptional activators. Our research is aimed at elucidating the functions of C/EBP proteins in regulating the growth, differentiation, and survival of mammalian cells and understanding the mechanisms that control the expression and activity of C/EBP proteins.

Analysis of C/EBP–Deficient Mice

To identify the specific roles of C/EBP family members in vivo, we have generated mice carrying targeted deletions of *c/ebp* genes. Analysis of C/EBPβ–/– mice showed that mutant females are sterile. Ovary transfers between wild-type and mutant mice demonstrated that the reproductive defect in *c/ebpβ–/–* animals is intrinsic to the ovary. We found that C/EBPβ expression is induced by luteinizing hormone (LH) specifically in ovarian granulosa cells. The granulosa layer surrounds the developing oocyte and, following ovulation, matures to form the corpus luteum. Corpora lutea were completely absent from the ovaries of mutant mice, thereby accounting for the sterile phenotype of these animals. Thus, our studies indicate that C/EBPβ is essential for differentiation of granulosa cells into luteal cells following the ovulatory surge of LH.

C/EBPδ has been implicated in the regulation of acute-phase response genes in hepatocytes and differentiation of adipocytes and myeloid cells of the hematopoietic system. We found that C/EBPδ transcripts are also widely

expressed in the peripheral and central nervous systems of the mouse, including neurons of the hippocampus. Mice lacking C/EBPδ appear to undergo normal embryonic and postnatal development. Since a C/EBP homolog in the sea snail *Aplysia* is essential for certain forms of synaptic plasticity associated with neuronal learning and memory functions, we undertook behavioral studies of C/EBPδ–/– mice. Compared to their wild-type littermates, mutant animals displayed enhanced context-dependent fear conditioning, a learning/memory response that requires hippocampal functions. However, the mutant mice performed normally in the auditory-cued fear-conditioning task, which is hippocampus independent. Thus, C/EBPδ appears to function as an inhibitor of context-dependent learning and memory processes in hippocampal neurons.

Differential Activation of Target Genes
The identical DNA-binding specificities of the C/EBP family members raise the question of whether these proteins are functionally redundant. Our analysis of target promoters has shown that C/EBP proteins exhibit differential abilities to transactivate target genes and therefore possess distinct functional identities. For example, the liver-specific *CYP2D5* gene is activated by C/EBPβ but not C/EBPα, while the promoter for the nerve growth factor (NGF) gene is activated by C/EBPδ but not C/EBPβ or α. In each case, transactivation involves recognition of a low-affinity C/EBP site. Binding to these cryptic elements appears to be facilitated by interactions with an Sp1-related protein bound to an adjacent site. We postulate that differential cooperative interactions with other transcription factors govern the unique promoter specificities of the C/EBP family members.

Negative Regulation of TNFα Gene Expression in Macrophages
Macrophages are a major source of proinflammatory cytokines, such as tumor necrosis factor-α (TNFα), which mediate inflammatory and immune responses. We have identified an activity, termed "TNFα-inhibiting factor" (TIF), that is secreted by macrophages in response to lipopolysaccharide (LPS) stimulation and negatively regulates LPS-induced transcription of the TNFα gene. Inhibition of TNFα expression by TIF was accompanied by selective induction of the p50 subunit of NFκB. Overexpression of p50 repressed LPS-dependent activation of a TNFα promoter-reporter construct. We postulate that TIF functions as an autocrine signal that attenuates TNFα expression in activated macrophages and that the inhibitory mechanism involves increased expression of NFκB p50. This mechanism may serve to limit production of TNFα, whose deregulated expression is associated with diseases such as rheumatoid arthritis and septic shock. TIF appears to be distinct from several known TNFα-inhibiting factors (IL–4, IL–10, and TGFβ) and thus may represent a novel anti-inflammatory cytokine.

Our collaborators include Jacqueline Crawley, Frank Gonzalez, Lothar Hennighausen, Richard Paylor, and Lino Tessarollo, NIH; Jonathan Keller, SAIC–Frederick; Italo Mocchetti, Georgetown University; Richard Schwartz, Michigan State University; and Robert Smart, North Carolina State University.

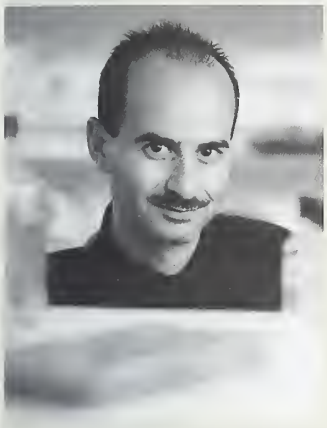
Recent Publications:

Sterneck E, et al. *Genes Dev* 1997;11:2153–62.

Baer M, et al. *Mol Cell Biol* 1998;18:5678–89.

Sterneck E, et al. *Proc Natl Acad Sci USA* 1998;95:10908–13.

Baer M, et al. *J Biol Chem* 2000;26582–90.



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Biography: *Dr. Philipp Kaldis received his Ph.D. from the Institute for Cell Biology, Swiss Federal Institute of Technology (ETH), Zürich, Switzerland, in 1994 where he worked on the mitochondrial creatine kinase with Dr. Theo Wallimann and Dr. Hans Eppenberger. In 1995, he joined Dr. Mark Solomon's laboratory at Yale University, Department of Molecular Biophysics and Biochemistry, New Haven, Connecticut, as a postdoctoral fellow/associate research scientist where he worked on the activation of cyclin-dependent kinases (cdks). In 2000, Dr. Kaldis became head of the Cell Cycle Section in this laboratory.*

Regulation of Cell Growth Laboratory
Regulation of the Cell Cycle by Cyclin-Dependent Kinases

Keywords:
cell cycle regulation
cyclin dependent kinase

Research: The cell cycle is precisely regulated to insure that the DNA content of a cell is duplicated error free and only once and is divided into two virtually identical cells. In many of these processes cyclin-dependent kinases (cdks) are involved. In mammals, at least 11 cdks have been identified. CDC2 (a.k.a. CDK1), CDK2, CDK4, and CDK6 are driving the cell cycle. The other cdks are involved in other processes (like transcription) or have unknown functions. The cdks are regulated on several levels, including binding to subunits (cyclins, inhibitors, assembly factors), protein degradation, transcriptional control, localization, and multiple phosphorylation. Cdks differ in their interaction with various cyclins and their substrate specificity. Nevertheless, there may be substantial overlap between cdks and many of their downstream targets are unknown. Our laboratory is using biochemical, molecular, and transgenic/knock-out mice techniques to determine the functions of different cdks.

Recent Publications:

Kaldis P, et al. *Mol Biol Cell* 1998;9:2545–60.

Kaldis P. *Cell Mol Life Sci* 1999;55:284–96.

Ross KE, et al. *Mol Biol Cell* 2000;11:1597–609.

Kaldis P, et al. *J Biol Chem* 2000;275:32578–84.

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Biography: *Dr. Morrison obtained her Ph.D. from Vanderbilt University School of Medicine. She then began studying signal transduction as a postdoctoral fellow in the laboratories of Dr. Thomas Roberts at Harvard Medical School and Dr. Lewis Williams at the University of California–San Francisco. Dr. Morrison joined the ABL–Basic Research Program in 1990 and became head of the Cellular Growth Mechanisms Section in 1995. From 1996 to 1997, she was on sabbatical in the laboratory of Dr. Gerald Rubin at the University of California–Berkeley. In 1999, Dr. Morrison joined the Center for Cancer Research, NCI.*

Regulation of Cell Growth Laboratory
Regulation of Signal Transduction Pathways

Keywords:
cancer
kinase
phosphatase
signal transduction

Research: The proliferative state of normal cells is precisely regulated by signals received from the external environment. Understanding how these signals are relayed from the cell surface to the nucleus is the major objective of the Cellular Growth Mechanisms Section. Studies in this area are particularly relevant to cancer research because release from regulatory controls at any point along the signaling cascade results in uncontrolled cell growth or oncogenesis. The approach of our laboratory has been to use a combination of biochemical, molecular, and genetic techniques to examine the regulation of key protein kinases involved in signal transduction. Ultimately, we hope that these studies will provide insight into the signaling events that occur during normal growth and development and how these processes are perturbed during oncogenesis.

The transmission of growth and developmental signals from the membrane to the nucleus requires the coordinated action of a diverse set of proteins. Previous work from our laboratory and others has demonstrated that the Raf-1 serine/threonine kinase serves as a central intermediate in many signaling pathways, functioning downstream of activated tyrosine kinases and Ras and upstream of mitogen-activated protein kinase (MAPK) and MAPK kinase (MKK or MEK). While Raf-1 is a critical component in signal transduction, the precise way in which Raf-1 becomes activated and inactivated during growth, development, and oncogenesis is not completely understood. Therefore, a major focus of our research has been to investigate the mechanisms regulating Raf-1 activity and to determine the role that phosphorylation and protein interaction play in the Raf-1 activation/inactivation process. Recently, our research efforts have also focused on another protein kinase, kinase suppressor of Ras (KSR). KSR was originally identified in *Drosophila* and *C. elegans* and is an evolutionarily conserved component of Ras-dependent signaling pathways. Our studies of the mammalian KSR protein are directed toward determining how this kinase functions to transduce Ras-dependent signals. In addition, our laboratory has had an ongoing interest in using the fruit fly, *Drosophila*, as a model system

for studying signaling events. Our goal in this project is to use the powerful genetic techniques amenable to this system to further elucidate the pathways and proteins involved in the transmission of cellular signals.

Collaborators on this research include Marc Therrien, Clinical Research Institute of Montreal.

Recent Publications:

Cutler RE Jr, et al. *Proc Natl Acad Sci USA* 1998;95:9214–9.

Muller J, et al. *Mol Cell Biol* 2000;20:5529–39.

Morrison DK, et al. *J Cell Biol* 2000;150:57–62.

Morrison DK. *J Cell Sci* 2001;114:1609–12.

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Biography: *Dr. Sterneck received her Ph.D. in 1992 from the University of Heidelberg following training with Drs. Thomas Graf and Achim Leutz at the European Molecular Biology Laboratory and the ZMBH Center for Molecular Biology, Heidelberg, Germany. She was then recruited as a postdoctoral fellow by Dr. Peter F. Johnson at the ABL–Basic Research Program in Frederick. Dr. Sterneck began her appointment as a principal investigator at the NCI–Frederick in September 1998 upon successful competition for an NCI scholarship.*

Regulation of Cell Growth Laboratory
Cell Growth Control In Vivo by C/EBPß and C/EBPδ

Keywords:
animal model
C/EBP
cell growth regulation
cell signaling
differentiation
DNA binding protein
gene expression
gene knockout
gene regulation
granulosa cell
hormone
involution
mammary gland
mouse embryo fibroblast
ovary
Sertoli cell
transcription factor

Research: The C/EBP family of transcription factors consists of 6 members with similar DNA-binding specificity that can homo- and heterodimerize with each other, giving rise to potentially 21 different transcriptional regulators. Many tissue culture model systems suggest a role for C/EBP proteins in the regulation of cell proliferation, growth arrest, or programmed cell death. However, limited information exists about their function in primary cells and in vivo. The long-term goal of this project is to elucidate the specific developmental and molecular functions of individual C/EBP proteins in vivo. Specifically, we are using mouse models that lack either the C/EBPβ or the C/EBPδ gene in a number of approaches:

- C/EBPβ-deficient ovarian granulosa cells are unable to differentiate in response to luteinizing hormone. Thus, we are using hormone-stimulated ovarian tissue as a tool to identify the target genes of C/EBPβ by representational difference analysis and microarray hybridization technology.
- We found that, under specific experimental conditions, C/EBPβ-deficiency renders granulosa cells permissive to transdifferentiation into Sertoli-like

cells, a cell type that gives rise to about 0.3 percent of human ovarian tumors. We are now addressing the molecular mechanism underlying this phenotype by testing the regulation of candidate genes, such as inhibin, in mutant tissue.

- C/EBPβ-null mice display multiple pathologies, including immune system dysfunctions, which preclude proper analysis of specific phenotypes. In order to improve our mouse model, we are developing a mutation that allows for regulated conditional deletion of the C/EBPβ gene in mice.
- We found that C/EBPδ-deficient mice develop mild mammary gland hyperplasia. We are now characterizing mammary epithelial cell growth regulation in these mice in more detail, with particular emphasis on programmed cell death during postlactational involution, since this is the stage at which C/EBPδ is normally expressed. We are also using primary mouse embryo fibroblasts as an in vitro model to characterize the role of C/EBPδ in cell growth control. In summary, these approaches shall contribute to our understanding of the role and mechanisms of C/EBP control and function in vivo. This knowledge should enable us to better understand growth regulation in different cell types and the pathological aberrations thereof.

Recent Publications:

Sterneck E, et al. *Proc Natl Acad Sci USA* 1998;95:10908–13.

Cantwell C, et al. *Mol Cell Biol* 1998;18:2108–17.

Robinson G, et al. *Genes Dev* 1998;12:1907–16.

Zhu S, et al. *Mol Cell Biol* 1999;19:7181–90.

Regulation of Protein Function Laboratory

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Covalent modifications of cellular proteins play crucial roles in influencing their fate and function and in regulating numerous cellular processes. A primary focus of the newly-formed Regulation of Protein Function Laboratory is to enhance our understanding of the regulatory roles played by polypeptide protein modifiers such as ubiquitin and ubiquitin-like molecules and by N- and O-linked glycosylation. Current areas of investigation are focused on the role of ubiquitin and other polypeptide modifiers on regulated proteins such as p53, BRCA1, growth factor receptors, proteins that are targeted for degradation from the endoplasmic reticulum, and proteins that are regulated during embryonic development. An additional focus of the laboratory is on establishing structure-function relationships for enzymes involved in ubiquitination and in development of tools that might specifically inhibit ubiquitin protein ligases that play roles in the development of cancer and other diseases.

Regulation of Protein Function Laboratory Staff

Office of the Chief

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Biography: *Dr. Weissman received his M.D. from Albert Einstein College of Medicine in 1981. After residency in internal medicine at Washington University, he came to the NIH where he received his research training with Richard Klausner. In 1989, he joined the NCI as an independent investigator. He was appointed chief of the Regulation of Protein Function Laboratory in 2001.*

Regulation of Protein Function Laboratory
Regulated Protein Degradation: Transmembrane Receptors and the Ubiquitin Conjugating System

Keywords:
endoplasmic reticulum
membrane proteins
protein degradation
T cell receptor
ubiquitin

Research: Regulated protein degradation is integral to myriad cellular processes and its dysregulation is associated with an increasing number of pathological processes. The discovery in 1992 that T cell antigen receptor (TCR) subunits are ubiquitinated in response to receptor engagement at the cell surface led to a long-term focus on the identification and characterization of enzymes involved in ubiquitination and their cognate substrates and the role of the ubiquitin conjugating system in cellular processes.

Enzymes of the Ubiquitin Conjugating System

As a followup to the identification by the laboratory of a family of closely related core E2s, a yeast two-hybrid screen was carried out to identify E2-interacting proteins. This led to the isolation of a novel RING finger protein and the determination that a large number of otherwise unrelated RING finger proteins have the capacity to mediate E2-dependent ubiquitination. With these findings, RING finger proteins now join the HECT family proteins as families of proteins that function as ubiquitin protein ligases (E3s). These observation have led the laboratory in new and exciting directions including studies on the IAPs as mediators of ubiquitination, studies on Mdm2 as an E3 (see below), and collaborative structural studies aimed at understanding the function of RING fingers and the role of metal coordination in their function.

Regulation of Cellular p53 Levels

Cellular levels of p53 are tightly regulated by proteolysis. The work of Peter Howley and coworkers has established that in cells expressing oncogenic strains of HPV, a HECT domain E3, E6–AP, is responsible for regulating p53. This occurs through the formation of a ternary complex of E6–AP with HPV E6 and p53. Under normal circumstances, however, levels of p53 are regulated by Mdm2, not by E6–AP. We have established that Mdm2 ubiquitinates p53 through its atypical C terminal RING finger. Additionally, we have determined that substitution of a heterologous RING finger for that of Mdm2 targets the chimeric protein for degradation but fails to degrade p53. This suggests specificity at the level of the RING finger. Studies are oriented towards further characterization of Mdm2 including the means by

which its activity is regulated and the development of small molecule inhibitors of its function.

Determining the Fate of Transmembrane Proteins in the Secretory Pathway
For a number of years the molecular basis for degradation of proteins from the endoplasmic reticulum (ER) was elusive. It is now apparent that ER-associated degradation (ERAD) is a tightly coupled process that involves retrograde translocation from the ER to the cytosol, ubiquitination, and proteasomal degradation. The mechanisms responsible for what appears to be a thermodynamically unfavorable process of retrograde movement through the ER membrane are unknown. Studies from our lab have focused on understanding this process in mammalian systems. In the course of these studies we have established a novel link between the activity of ER and Golgi mannosidases and degradation of the CD3-δ subunit from the TCR.

We have recently identified two mammalian E2s that associate with the ER. One of these, MmUBC6, is the homolog of the transmembrane yeast E2 (*Sc*Ubc6), while the other, MmUBC7, is homologous to *Sc*Ubc7. We have recently determined that MmUBC7 plays a role in the degradation of TCR subunits from the ER and appears to be involved at the initiation of retrograde movement from the ER. Ubiquitination of proteins usually involves both E2 and E3s; however, E3s implicated in degradation from the ER in mammalian systems have been elusive. We have now identified, and are characterizing, a mammalian protein that binds MmUBC7 and mediates ubiquitination. Evidence suggests that this polytopic membrane protein is implicated in degradation of TCR subunits from the ER. The roles of these mammalian E2s and E3s in targeting of other proteins for degradation and in stress responses are being actively investigated.

Ubiquitination and Degradation of Cell Surface Receptors
Our interest in ubiquitination was kindled by the observation that cell surface TCRs are substrates for ubiquitination in response to receptor occupancy. We have determined that multiple subunits of the TCR are ubiquitinated in response to receptor ligation, and that multiple intracytoplasmic lysines are targeted. We have also established that this modification requires the activation of protein tyrosine kinases. There is now increasing evidence from both yeast and mammalian systems that ubiquitination of cell surface receptors is involved in their endocytosis and lysosomal trafficking. Fundamental questions regarding ubiquitination of cell surface receptors that are being addressed include the molecular basis by which receptor ubiquitination is regulated and the means by which ubiquitination affects intracellular trafficking. To address these, we are using both the TCR and the epidermal growth factor receptor as model systems.

Ubiquitination and Embryonic Development
The use of Nedd4 as bait in a yeast two-hybrid screen has led to the identification of a number of substrates that are expressed during embryogenesis. We are engaged in studies aimed at evaluating these substrates and the molecular basis for their recognition and ubiquitination by Nedd4.

We collaborated in these studies with R. Andrew Byrd, Michael Kuehn, Stanley Lipkowitz, and Karen Vousden, NIH.

Recent Publications:

Lorick KL, et al. *Proc Natl Acad Sci USA* 1999;96:11364–9.

Fang S, et al. *J Biol Chem* 2000;275:8945–51.

Tiwari S, et al. *J Biol Chem* 2001;276:16193–200.

Weissman AM. *Nature Reviews Mol Cell Biol* 2001;2:169–78.

Structural Biophysics Laboratory

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The Structural Biophysics Laboratory (SBL) was officially created in October 1999, when the former Macromolecular Structure Laboratory (MSL) was divided into two laboratories con-comitant with the transition of the laboratory into the Center for Cancer Research, NCI. The SBL has established itself with the focus on solution structural biology and biophysics aimed at understanding and regulating the mechanism of action of the systems under study. The major structural tool for the SBL is nuclear magnetic resonance (NMR), and the laboratory has a very strong interdisciplinary drug design effort, encompassing synthetic and mechanistic chemistry, structure-based design and modeling, confocal microscopy, and biochemical and biological mechanisms. A new section focuses on nucleic acid structure and protein-nucleic acid complexes. There is also an anticipated expansion to include a section whose research will focus on the biophysics of macromolecules and their assemblies.

The SBL has been responsible for the enhancement of NMR facilities and introduction of automated parallel synthesis to the NCI. The NMR facilities have been enhanced with the installation of new instrument consoles at 500 and 600 MHz, installation of a new 800 MHz instrument, and future addition of cryogenic triple-resonance probes to all three instruments. The SBL also operates the Biophysics Resource (BR), which is a shared-use facility that provides access to biophysical instrumentation for characterization and study of biomacromolecular systems. The BR houses circular dichroism and steady-state fluorescence spectrophotometers, isothermal titration and differential scanning calorimeters, an hplc-electrospray mass spectrometer, and an hplc-light scattering system.

The SBL currently consists of three principal investigators, each heading a section. The sections are scientifically independent; however, there is considerable interaction between them. The recent addition of a second NMR group and the anticipated future inclusion of a biophysics group will lead to a very complementary and interactive group of researchers.

The main goal of the Macromolecular NMR Section (MNMR), directed by Dr. R. Andrew Byrd, is the elucidation of the three-dimensional (3D) solution structure and dynamics of proteins and complexes, involving protein-protein and protein-nucleic acid interactions, as a means of understanding the mechanism of action for these systems. The primary targets of our efforts are proteins involved in the transmission of signals between and within cells and

proteins controlling gene expression. Our studies provide insight into the complex regulation of cell replication, which is crucial to the development and proliferation of cancer. Our principal tool is NMR, which is unique among biophysical methods in its ability to provide atomic resolution information on structure and dynamics in solution, and our research includes development and refinement of NMR techniques for structural biology. Two areas where our results are providing important new information are in the structural biology of hepatocyte growth factor (including interactions with heparin), which is implicated in cancer metastasis, and studies on the anti-terminator protein NusB, which is involved in the process of transcription elongation.

The goal of the Molecular Aspects of Drug Design Section, directed by Dr. Christopher J. Michejda, is to discover new approaches to the design of drugs against cancer and AIDS. The general strategy is to integrate information obtained from biological and molecular studies of disease development with structural data on potential drug targets to design new drugs with greater effectiveness and lower systemic toxicity. Our specific approach is to combine mechanistic, computational, and synthetic chemistry with biochemical and biological studies of the candidate drugs. Recent accomplishments of the section include the discovery of new, potently cytotoxic compounds that are highly selective against colon and pancreatic cancers. The latter is especially encouraging because of the lack of effective chemotherapeutic agents against this form of cancer. We have also discovered a general method for the design of antagonists of G-protein-coupled receptors (GPCRs) and other membrane-spanning proteins. We have found that properly oriented, synthetic helical peptides that correspond to individual transmembrane domains of GPCRs are able to disrupt the function of the receptors in a highly specific manner.

The goal of the newly established Protein-Nucleic Acid Interactions Section, directed by Dr. Yun-Xing Wang, is to study nucleic acids, principally RNA, and complexes utilizing NMR. The majority of structural information for RNA and its complexes has been derived from NMR due to the diversity and nonregularity of RNA structure and its complexes with proteins. This new section will broaden the research program of the SBL and establish the first experimental RNA structural effort within the NCI.

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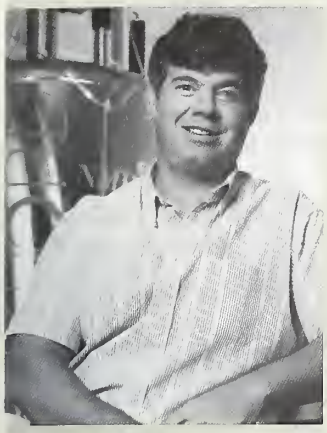

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Biography: *Dr. Byrd received his Ph.D. from the University of South Carolina in 1977, specializing in high-resolution biomolecular NMR. He was a postdoctoral fellow and subsequently a research officer in the Molecular Biophysics Laboratory of the National Research Council of Canada, where he investigated biological membranes by solid-state NMR. Following a period as a senior investigator at the Center for Drugs and Biologics/FDA, he established the Macromolecular NMR Section of the ABL–Basic Research Program at the NCI–Frederick in 1992. Dr. Byrd is the chief of the Structural Biophysics Laboratory in the Center for Cancer Research, NCI. He chaired the Experimental NMR Conference in 1992 and cochaired the International Conference on Magnetic Resonance in Biological Systems in 1996.*

Structural Biophysics Laboratory
Structural Biology of Proteins Involved in Signal Transduction and Regulation of Gene Expression

Research: Our research focuses on the elucidation of three-dimensional (3D) solution structure and dynamics of proteins and complexes, involving protein-protein and protein-nucleic interactions, as a means of understanding the mechanism of action for these systems. The nuclear magnetic resonance (NMR) technique is unique among biophysical methods in its ability to provide atomic resolution information on such systems in solution. The primary targets of our efforts are proteins involved in the transmission of signals between and within cells and proteins controlling gene expression. By investigating the structural biology of these systems, our studies can provide insight into the complex regulation of cell replication, which is crucial to the development and proliferation of cancer.

Determining 3D solution structures requires state-of-the-art capabilities in multidimensional, triple- and quadruple-resonance NMR spectroscopy and isotopic labeling of proteins and nucleic acids. We devote a part of our effort to the development of improved NMR techniques and hardware, as well as protein engineering. Recent work in our laboratory includes the establishment of procedures for the preparation of triply labeled proteins (e.g., N15, C13, and H2) both in uniform patterns and with selective methyl protonation in an otherwise N/C/D background. These procedures are permitting us to explore larger proteins with NMR techniques. Current efforts include both 30- and 40-kDa systems.

Among our current structural projects, several efforts involve proteins associated with signal transduction (e.g., the SH3 domain in the Hck cytoplasmic protein tyrosine kinase, hepatocyte growth factor (HGF), and interleukin 13), and the processes of termination and antitermination of gene expression in bacteriophage lambda. The Hck SH3 domain has been shown to bind to the Ras GTPase-activating protein (GAP) and form part of the signaling cascade. We determined the solution structure of the Hck SH3

domain and its complex with a target peptide from GAP. We have also examined the backbone dynamics of both the free protein and the protein complexed with the GAP-peptide. These studies revealed the expected fold for the free protein and only small structural perturbations upon binding to the target peptide. Dynamics studies are consistent with the minimal structural perturbation determined from chemical shifts and nuclear Overhauser effects but indicate some as yet unresolved effects on hydrogen bonding within the protein structure. Future efforts, in conjunction with our collaborators, will address interactions between the SH2 and SH3 domains of Hck. These studies should assist in understanding the roles of these domains, including some unexpected processes such as the binding of the Hck SH3 domain to the HIV–1 Nef protein.

We have recently determined the solution structure for human interleukin 13 (IL–13) and are presently investigating the receptor interaction surfaces. Our work is continuing in collaboration with receptor biologists and clinical oncologists who are interested in the surprising and recent discovery of the overexpression of IL–13 receptors in high-grade gliomas.

The control of lambda development encompasses the fundamental processes of gene expression in prokaryotes. Expression of the lambda early genes entails a series of termination and antitermination events. Transcription antitermination requires the lambda N protein and a collection of host proteins called Nus (N-*u*tilization substances) binding to specific RNA sites called NUT (N-*u*tilization). Although it is known that the N and Nus proteins form complexes at the NUT sites, there is very little information about these interactions, which are functionally related to the regulatory processes employed by the mammalian elongin class of proteins. We are currently studying the solution structure and complex formation of two Nus proteins, NusB and NusE. Our data indicate that these proteins form stable complexes in solution. Structural studies to date have determined that the secondary structure of the NusB protein is largely alpha-helical. Since the NusB protein is not homologous to any other known protein, there are no models of its tertiary fold. Our ongoing research is utilizing NMR spectroscopy, circular dichroism spectropolarimetry, and fluorescence spectroscopy to determine the solution structure of NusB, NusE, and the complexes of these proteins with single-stranded RNA.

Another project involves the structural biology of hepatocyte growth factor/scatter factor (HGF/SF), which is continuing in collaboration with Drs. Donald Bottaro and Robert Linhardt. We are involved in the solution structure determination of several domains of this critical protein, which binds to the c-Met receptor, mediates crucial aspects of development, maintenance, and regeneration of various tissues and organs, and is also implicated in the growth, invasion, and metastasis of tumor cells. Our work is producing high-resolution structures of single- and multidomain fragments of the HGF alpha-chain and we are investigating the critical interactions of these fragments with heparin.

Our collaborators include Donald Bottaro, EntreMed, Inc.; Donald Court, Debbie Morrison, Karen Vousden, and Allan Weissman, NIH; and Robert Linhardt, University of Iowa.

Recent Publications:

Altieri AS, et al. *Nat Struct Biol* 2000;7:470–4.

Horita DA, et al. *Protein Sci* 2000;9:95–103.

Horita DA, et al. *J Mol Biol* 2001;307:861–70.

Eisenmesser EZ, et al. *J Mol Biol* 2001;310:231–41.

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Biography: *Dr. Michejda was trained as a physical-organic chemist at the University of Rochester, where he received his Ph.D., and at Harvard University as a postdoctoral fellow. He was a professor of chemistry at the University of Nebraska in Lincoln before coming to the NCI–Frederick in 1978. His initial studies at the NCI–Frederick were on chemical carcinogenesis, but this work eventually evolved to his present interest, which concerns fundamental problems in the development of drugs against cancer and viral diseases.*

Structural Biophysics Laboratory
Design of Drugs Against Cancer and Viral Diseases

Research: The goal of the Molecular Aspects of Drug Design Section is to discover new approaches to the design of drugs against cancer and viral diseases, especially AIDS. The general strategy is to integrate information obtained from biological and molecular studies of disease development with structural data on potential drug targets to design new drugs with greater effectiveness and lower systemic toxicity. Our specific approach is to combine mechanistic, computational, and synthetic chemistry with biochemical and biological studies of the candidate drugs.

The ability to repair DNA damage is critical to the survival of a cell, whether normal or cancerous. Thus, in principle, cellular responses to damage, which trigger a cascade of events that lead to upregulation or downregulation of transcription of a number of genes, may offer novel and highly selective targets for drugs. We have found that bisimidazoacridones and other related bifunctional agents (collectively referred to as BIAs), which were discovered in our laboratory, appear to target various checkpoint controls in the cell cycle of selected tumors. Several members of the series are very toxic to human colon cancer and pancreatic cancer cells in tissue culture and in nude mice; however, they have low systemic toxicity. Other members of the series induce cell cycle arrest of tumor cells without inducing apoptosis. These compounds are cystostatic. Consideration of the mechanism by which BIAs interact with DNA led us to propose that some members of the series may be inhibitors of HIV integrase. Some BIAs, in fact, were found to be potent inhibitors of integrase in vitro and of the cytopathic effects of HIV in infected cells. Together with our collaborators, we found that the antiviral BIAs target a late stage of the viral life cycle in live cells rather than inhibiting integrase. The

present data suggest that the molecular target for these antiviral agents is a component of transcriptional initiation.

The targeting of drugs to specific receptors on tumor cells is a conceptually attractive method to enhance specificity and to decrease systemic toxicity. We have chosen the gastrin receptor (GR) as a prototype to test this hypothesis. Our work has shown that GR, a member of the G-protein-coupled receptor superfamily, undergoes endocytosis via clathrin-coated pits, is transported to the lysosomes, and recycles to the cell surface. Gastrin, the ligand for GR, is a peptide hormone that can be readily modified, because only the C terminal tetrapeptide is required for recognition by the receptor. We have attached several cytotoxic moieties to various gastrin-derived peptides. Some of these conjugates have been shown to be very cytotoxic in GR+ cells but relatively benign in GR– cells. The immediate goal of this project is to further define the characteristics of an effective receptor-targeted drug by using the GR model. The results of these studies should be applicable to many other receptors. The techniques used for measuring trafficking of GR have been applied successfully to study the trafficking of other G-protein-coupled receptors, such as the cholecystokinin receptor type A and the chemokine receptors required for HIV entry into target cells. Moreover, we have found that it is possible to disrupt the function of any G-protein-coupled receptor, and indeed the function of other proteins that have multiple membrane-spanning domains, by peptides that mimic the structure of one of the transmembrane (TM) domains. We found that this is a highly specific phenomenon, which suggests a new drug design paradigm that depends only knowing the primary structure of the target protein. We have shown that peptides that target the TM domains of the chemokine receptors CXCR4 and CCR5 are potent blockers of HIV entry into cells. Similarly, we found that the function of the p-glycoprotein, an ABC transporter protein that is an important contributor to drug resistance, was blocked in a highly specific manner by a peptide that corresponded to one of the 12 TM domains of the protein.

Reverse transcription of the HIV RNA genome by reverse transcriptase (RT) is a step in viral replication that is absolutely required. Thus, RT has been an attractive target for drug design. Unfortunately, effective inhibitors of wild-type HIV RT are usually rapidly rendered ineffective by mutations in the enzyme. In collaboration with Dr. Edward Arnold and his coworkers and with Drs. Stephen Hughes, Paul Boyer, and John Julias, we have been searching for a new generation of inhibitors that will be active against mutant forms of RT. Our approach utilizes a combination of structural, molecular modeling, and synthetic methodologies to gain a fundamental understanding of how this very complex enzyme carries out its function, and how the function may be disrupted. Some new, potent inhibitors of RT based on the 1,2-disubstituted benzimidazole nucleus have been designed and synthesized. Some of them also inhibit most of the variant forms of RT and are potent inhibitors of the virus in infected cells.

Our collaborators are Edward Arnold, Rutgers University; Susan Bates, Paul Boyer, Michael Gottesman, Melinda Hollingshead, Stephen Hughes, Xinhua Ji, John Julias, Terry Moody, Dwight Nissley, Yves Pommier, Jeffrey Strathern, and Stephen Wank, NIH; Robert Buckheit, Jr., and James Turpin, Southern Research Institute; and William Jorgensen, Yale University.

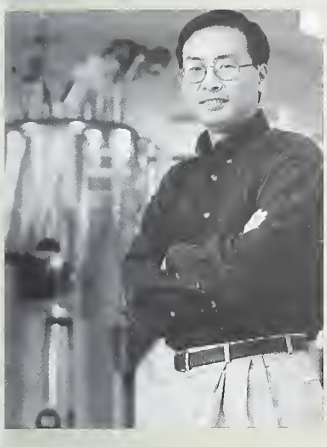
Recent Publications:

Kroeger Smith MB, et al. *Protein Eng* 2000;13:413–21.

Cholody WM, et al. *Cancer Chemother Pharmacol* 2001;47:241–9.

Tarasov SG, et al. *Photochem Photobiol* 1999;70:568–78.

Tarasova NI, et al. *J Biol Chem* 1999;274:34911–5.



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Biography: *Dr. Wang received his Ph.D. from the Johns Hopkins University in 1994. He was a postdoctoral fellow in Dr. Dennis Torchia's laboratory, where he studied the hydration dynamics of HIV–1 protease in complex with inhibitors and elucidated the 3D structure and a new function of the antitumor/anti-HIV protein MAP30. He joined the Structural Biophysics Laboratory in 2000. Using NMR spectroscopy, his group studies the structure and functions of protein-nucleic acid complexes as well as proteins associated with tumors and HIV.*

Structural Biophysics Laboratory
Search for the Structural Basis of Biomacromolecular Function and Activity

Keywords:
anti-HIV
antitumor
NMR
structure

Research: The general research interests of our group are to determine the structure of biological molecules, including nucleic acids, enzymes, RNA(DNA)-protein complexes, and clinically important enzyme-drug complexes, using NMR and various other biophysical and biochemical methods. The knowledge of these biological structures will help us to understand the structural basis for the function of biological molecules and complexes.

Included among our collaborators are Ad Bax, Marius Clore, Angela Gronenborn, and Dennis Torchia, NIH; Sylvia Lee-Huang, University of New York; and Nouri Neamati, University of Southern California.

Recent Publications:

Ishima R, et al. *Structure Fold Des* 1999;7:1047–55.

Wang Y–X, et al. *Cell* 1999;99:433–42.

Wang Y–X, et al. *J Biomol NMR* 1999;14:181–4.

Wang Y–X, et al. *Protein Sci* 2000;9:138–44.

Laboratory of Tumor Immunology and Biology

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The Laboratory of Tumor Immunology and Biology conducts research in the areas of tumor immunology, mechanisms of tumor cell-immune cell interactions, and immune mechanisms. The laboratory functions as an integrated translational research program with the goal of designing and developing new immunotherapies and immunologic strategies for cancer treatment and prevention. Ongoing studies involve (1) the analysis of basic immunologic mechanisms and principles, (2) the design and development of novel recombinant vaccines, (3) analysis of tumor cell-immune cell interactions, (4) the discovery of antigenic determinants expressed in human cancers as vaccine or monoclonal antibody targets, (5) the design and development of new recombinant immunoglobulin molecules of monoclonal antibodies that can be employed in cancer therapy and diagnosis, (6) the development of new animal models that can be employed in preclinical studies to most reflect human vaccine trials, (7) cytokine biology as it integrates with cancer immunotherapy, (8) the development of clinical grade vaccines and monoclonal antibodies for use in immunotherapy trials for a range of human cancers, (9) collaborative clinical trials involving recombinant vaccines and vaccine strategies in patients with a range of human cancers, and (10) the development of new immunoassays to analyze immune responses to vaccines from patients enrolled in vaccine trials.

Recombinant Vaccine Group
Designs and develops novel recombinant vaccines and vaccine strategies for cancer immunotherapy. Employs experimental models to analyze the role of novel vectors, costimulatory molecules, and novel vaccine strategies to optimize host immune responses and antitumor effects.

Cytokine Group
Studies the interactions of cytokines on the different arms of the immune system to optimize antitumor responses to recombinant vaccines and monoclonal antibody therapy. Defines novel strategies for the delivery and use of cytokines as biologic adjuvants and immunotherapeutics. Develops new animal models for the analysis of new vaccines and vaccine strategies.

Cellular Immunology Group
Studies the role of the human immune response to tumor-associated antigens. Defines and develops immunodominant determinants and modifications of those determinants toward the optimal activation of human immune responses to tumor-associated antigens. Defines mechanisms to enhance the potency of antigen-presenting cells for specific T cell activation. Develops immunologic methods and immunoassays to better define the efficacy of vaccine therapies for a range of human cancers.

Molecular Immunology Group
Studies the mechanism of T cell activation as it relates to vaccine therapy. Studies the role of various gene products in both naive and memory T cell populations concerning such functions as cell cycle, apoptosis, and cytokine expression. Defines mechanisms that relate to immune activation, immune regulation, and/or signaling pathways.

Immunoregulation Group
Studies the mechanism of the immune response mediated by various immunogens, including recombinant vector-based vaccines, and studies the role of distinct antigen-presenting cells in the activation and regulation of the immune response.

Molecular Biology Group
Designs and develops novel recombinant forms of monoclonal antibodies. Designs recombinant immunoglobulin molecules to improve affinity, reduce potential host immune responses, and optimize pharmacokinetic and metabolic properties toward the development of reagents for use in cancer therapy and diagnosis.

Immunotherapeutics Group
Studies combinatorial therapy approach to cancer. Studies the integration of various immunotherapies, such as recombinant vaccines and monoclonal antibodies, and their combinatorial role with other therapeutic modalities. Develops strategies to better translate approaches in experimental systems toward novel immunotherapies for a range of human cancers.

Clinical Trials Group
Conducts clinical trials, based on hypothesis-driven preclinical findings, involving novel recombinant vaccines and vaccine strategies for the therapy of a range of human cancers.

Tumor Immunobiology Group
Studies the interaction of immune cells and tumor cells to better define the mechanisms of tumor killing and tumor escape.

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Biography: *Dr. Schlom obtained his Ph.D. from Rutgers University. At the NIH, he has been involved in translational research involving the immunotherapy of a range of carcinomas. His most recent studies involve the design and characterization of recombinant vaccines for cancer therapy.*

Laboratory of Tumor Immunology and Biology
Design and Development of Novel Recombinant Immunotherapeutics and Strategies for Cancer Immunotherapy

Keywords:
antigen presentation
cancer immunotherapy
cancer vaccines
carcinoembryonic antigen
colorectal cancer
costimulatory molecules
CTL
cytokines
immunotherapy
monoclonal antibodies
poxvirus vectors
tumor antigens
vaccines

Research: An integrated translational research program has been developed with the goal of designing and developing new immunotherapies and immunologic strategies for cancer treatment and prevention with emphasis on immune intervention for a range of human carcinomas.

Vector-based delivery of tumor-associated antigens, T cell costimulatory molecules, and cytokines in the induction of immune responses and antitumor immunity—experimental studies: Tumor-associated antigens (TAAs) are by definition either weakly immunogenic or functionally nonimmunogenic in the immune competent host. Thus, if cancer vaccines are to be effective in either preventing or eliminating tumors, vaccines and vaccine strategies must be developed to present antigens to the immune system in a way that makes them more immunogenic. Efforts have focused on the development of strategies that convert poorly immunogenic antigens into more potent immunogens in the induction of host T cell responses. Studies have now shown that two classes of recombinant poxvirus vectors (vaccinia and avipox) that contain transgenes for tumor antigens can render those gene products more immunogenic. Recombinant vaccinia vectors have been shown to be potent in the induction of the immune response, while recombinant avipox vectors, which are replication defective, have been shown to be ideal for multiple boosting in the absence of host-limiting immunity to the vector. The need for T cell costimulation has been shown to be extremely important in the generation of potent T cell responses, especially when the antigen in question is weakly immunogenic. Recombinant poxvirus vectors have been constructed that contain one, two, or three transgenes for T cell costimulatory molecules. Recombinant vectors containing a triad of costimulatory molecules (B7–1, ICAM–1, and LFA–3, designated TRICOM) have been shown to be extremely effective in enhancing host T cell responses. Peptide-pulsed dendritic cells or B cells that have been infected with avipox-TRICOM vectors have been shown to be far more effective in the induction of both naive and memory T cell responses (both in vitro and in vivo) than the use of control peptide-pulsed dendritic cells or B cells, respectively. The mechanism by which T cell costimulation enhances T cell activation has also

been studied. It has now been demonstrated that T cells actually acquire T cell costimulatory molecules from antigen-presenting cells upon activation. Depending on the level of signal 1 and the level of expression of costimulatory molecules, this phenomenon can have either immune stimulatory or immune regulatory consequences. Recombinant avipox vectors have now been constructed which contain the cytokine GM–CSF. Studies have now demonstrated that the use of this vector along with recombinant vaccines greatly enhances the number of dendritic cells in nodes proximal to the vaccination site, with consequent increases in both host immune responses and antitumor activity.

Development of more valid animal models for the analysis of new vaccine strategies: The availability of valid animal models that mirror the human cancer patient is extremely important in the analysis of new vaccines and vaccine strategies. Carcinoembryonic antigen (CEA) is overexpressed on a wide range of human carcinomas, including colorectal, pancreatic, breast, nonsmall cell lung, and head and neck tumors, and to a lesser extent on normal colonic epithelium. A CEA transgenic mouse, which expresses CEA in a manner similar to that found in humans, has been utilized for the analysis of induction of immunity and antitumor effects using recombinant CEA-based vaccines. These studies have now demonstrated that poxvirus-based CEA/TRICOM vectors can eliminate established tumors that could not be eliminated with less potent vaccines and vaccine strategies. Recent studies have also demonstrated that TRICOM-based vaccines can also prevent the induction of spontaneous tumors in other mouse models.

Design and development of recombinant immunoglobulin molecules: Efforts have focused on the development of novel recombinant immunoglobulin forms that can be used for the detection and/or therapy of human carcinomas. MAbCC49, when used as a radioimmunoconjugate, has previously been shown to efficiently target human ovarian, prostate, and colorectal cancers in clinical trials. A CDR-grafted "humanized" version of CC49, devoid of the CH2 domain, has now been developed (HuCC49ΔCH2). This molecule has the property of rapid clearance from the body and reduced immunogenicity, properties that make it more suitable as a radioimmunotherapeutic. Recombinant variants of this molecule have also been developed that have enhanced affinity for the target antigen.

Activation of human T cells by carcinoma-associated antigens: The primary focus of these studies is to identify those epitopes of tumor-associated antigens expressed on human carcinomas that have the ability to activate human T cells. Several different epitopes of human CEA have been identified that have the ability to activate human T cells, which in turn can lyse human carcinoma cells. An agonist epitope of one of these determinants has now been identified that has the ability to activate T cells to far greater levels than the native epitope and has the ability to kill tumor cells expressing native CEA. Similar studies have also been conducted with human prostate-specific antigen (PSA) and the MUC–1 antigen expressed on a wide range of human carcinomas. Human TRICOM vectors have now been developed that simultaneously express three human costimulatory molecule transgenes. Peptide-pulsed human dendritic cells, infected with TRICOM vectors, are far more effective in the activation of human T cells than the use of peptide-

pulsed dendritic cells. These findings along with those of preclinical murine models using TRICOM vectors have led to the translation of these studies to ongoing clinical trials.

Development of vaccines for clinical trials/clinical trials program: As a result of hypothesis-driven preclinical studies, a series of recombinant vaccines has been designed and developed for use in clinical trials. These are both peptide-based and vector-based vaccines, which are directed against three different carcinoma-associated antigens: CEA, PSA, and MUC–1. Vector-based vaccines not only contain transgenes for tumor-associated antigens, but also transgenes for multiple costimulatory molecules (i.e., TRICOM vectors) as well as cytokine genes. A series of immunoassays has now been developed to analyze patients' immune responses both pre- and postvaccination. Collaborative vaccine clinical trials are currently ongoing with the NCI CCR Medical Oncology Research Unit and eight other cancer centers throughout the country. These trials have demonstrated the ability of advanced carcinoma patients to mount T cell responses to "self" tumor-associated antigens as a consequence of vaccination. Phase I/II clinical trials have now demonstrated the advantage of (1) the use of recombinant poxvirus-based vaccines, (2) diversified prime and boost vaccine regimens, and (3) the use of agonist epitopes in enhancing the generation of T cell responses and in achieving objective clinical responses and/or increased survival in advanced carcinoma patients.

Our collaborators include J.C. Barrett, J. Berzofsky, M. Brechbiel, W. Dahut, M. Hamilton, S. Hursting, E. Kass, S. Steinberg, Y. Tagaya, and C. Van Waes, NIH; J.P. Eder and D. Kufe, Dana-Farber Cancer Center; F. Guadagni, Regina Elena Cancer Institute, Rome; H. Kaufman, Albert Einstein Cancer Center; A. LoBuglio, University of Alabama Comprehensive Cancer Center; K. Lyerly, Duke University Medical Center; J. Marshall, Georgetown University Medical Center; M. von Mehren and L. Weiner, Fox Chase Cancer Center; D. Panicali, Therion Biologics; S. Pestka, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey; M. Roselli, Department of Surgery, University of Rome "Tor Vergata"; and W. Weiss, Walter Reed Army/Navy Malaria Program.

Recent Publications:

Hodge JW, et al. *Cancer Res* 1999;59:5800–7.

Hodge JW, et al. *J Natl Cancer Inst* 2000;92:1228–39.

Salazar E, et al. *Int J Cancer* 2000;85:829–38.

Zhu MZ, et al. *Cancer Res* 2001;61:3725–34.

CEA-Based Vaccines

T97–0084: H. Kaufman, principal investigator, A. Einstein Cancer Center

T97–0044: M. von Mehren, principal investigator, Fox Chase Cancer Center: Phase I studies of the use of avipox-CEA/B7–1 dual gene recombinants in patients with advanced CEA-expressing carcinomas. These trials are designed to evaluate the safety and efficacy of a dual transgene vaccine to induce CEA-specific T cell responses in patients with advanced carcinoma. Immune responses of all patients are evaluated both pre- and postvaccination.

Phase I studies of peptide-based vaccines in patients with advanced CEA-expressing carcinomas:

T96–0058: M. Hamilton and P. Arlen, principal investigators, NCI Medicine Branch: CAP–1 peptide in Ribi adjuvant.

T95–0044: D. Cole, principal investigator, Medical College of South Carolina: rV–CEA prime followed by CAP–1 peptide boost.

T96–0087: K. Lyerly, principal investigator, Duke Comprehensive Cancer Center: CAP–1 peptide-pulsed autologous dendritic cells. These studies are designed to determine the safety and immunogenicity of an immunodominant CEA peptide, delivered via different vaccine modalities, in patients with advanced carcinoma. Immune responses of all patients are evaluated both pre- and postvaccination.

T97–0033: J. Marshall, principal investigator, Georgetown University Cancer Center: The role of diversified vaccination and cytokines in patients with advanced CEA-expressing carcinomas. This is a randomized phase I/II trial to determine the safety and validity of the use of diversified vaccination protocols in patients with advanced cancer. It is designed to compare immune responses of patients randomized to one of two different vaccination protocols in which patients receive either recombinant vaccinia CEA (V) followed by boosts with avipox CEA (A) (i.e., VAAA) or the reciprocal AAAV regimen. Following evaluation of those results, additional cohorts of patients will receive either GM–CSF along with vaccinations or GM–CSF and low-dose IL–2. Immune responses of all patients are evaluated both pre- and postvaccination.

P833: J. Marshall, principal investigator, Georgetown University Cancer Center: Phase I study of CEA/TRICOM vaccines in patients with advanced CEA-expressing carcinomas. This trial incorporates several principles learned from previous clinical trials and preclinical studies and is the first clinical trial to evaluate the safety and efficacy of vectors containing CEA along with three different costimulatory molecules (B7–1, ICAM–1, and LFA–3—i.e., TRICOM). Moreover, the vector contains the entire CEA gene containing an agonist (6D) epitope. The first cohorts will receive different doses of avipox-CEA(6D)/TRICOM (designated A). The second cohorts will receive a primary vaccination with different doses of rV–CEA(6D)/TRICOM (designated V) followed by boosts with avipox-CEA(6D)/TRICOM. The third cohorts will receive the VAAA regimen accompanied with local GM–CSF administration. Immune responses of all patients are evaluated both pre- and postvaccination.

Clinical Trials (continued):

MUC–1 Vaccine Studies

T98–0057: J.P. Eder and D. Kufe, principal investigators, Dana-Farber Cancer Center: Phase I study of rV–MUC–1 vaccine in patients with metastatic breast carcinoma. This is a phase I dose escalation trial in which patients with metastatic breast cancer are being administered rV–MUC–1 vaccine, which will be evaluated for safety and the ability to mount T cell responses to MUC–1. Immune responses of all patients are evaluated both pre- and postvaccination.

Prostate Vaccine Program

T95–0086: J.P. Eder and D. Kufe, principal investigators, Dana-Farber Cancer Center; M. Sanda, principal investigator, University of Michigan: Phase I studies of the vaccination of prostate cancer patients with rV–PSA vaccine. These studies were designed to evaluate the safety of PSA-based vaccines and the ability of patients with prostate cancer to mount PSA-specific immune responses. Immune responses of all patients are evaluated both pre- and postvaccination.

T98–0004: J.P. Eder and D. Kufe, principal investigators, Dana-Farber Cancer Center: A phase II trial of diversified prime and boost vaccination using rV–PSA and avipox-PSA vaccines. This is a phase II trial in patients who had a previously resected or irradiated prostate cancer and now have rising serum PSA. The two vaccine regimens consist of patients receiving either two vaccinations of vaccinia PSA(V) followed by three vaccinations with avipox-PSA(A) (VVAAA) or the reciprocal AAAVV protocol. Immune responses of all patients are evaluated both pre- and postvaccination.

E7897: H. Kaufman, principal investigator, A. Einstein Cancer Center: A multicenter (six centers) randomized ECOG trial employing diversified vaccination of rV–PSA and avipox-PSA. This is a phase II trial in patients who had a previously resected or irradiated prostate cancer and now have rising serum PSA. Patients are randomized into one of two cohorts to receive either rV–PSA(V) followed by three vaccinations with avipox-PSA(A) (VAAA) or the reciprocal AAAV regimen. Immune responses of all patients are evaluated both pre- and postvaccination.

T99–0097: P. Arlen, principal investigator; W. Dahut and J. Gulley, associate investigators, NCI Navy Oncology Clinic: Vaccine versus second line hormonal therapy: A randomized phase II study of either immunotherapy with a regimen of recombinant poxviruses that express PSA/B7–1 plus adjuvant GM–CSF and IL–2, or hormone treatment with nilutamide, in patients with hormone refractory prostate cancer and no radiographic evidence of disease. Prostate cancer patients are being randomized into two arms: arm 1 receives vaccine; arm 2 receives hormonal therapy (nilutamide). Patients can cross over if their serum PSA is rising at 6 months. The vaccine employed is a priming vaccination with rV–PSA admixed with rV–B7–1 followed by booster vaccinations with avipox-PSA. All vaccinations will be given with rGM–CSF and followed by low-dose IL–2. This (and the trial described below) are the first vaccine trials to employ the concept of admixing pox vectors, and the first clinical trials to use costimulation in a prostate cancer vaccine. Immune responses of all patients are evaluated both pre- and postvaccination.

Clinical Trials (continued):

P894: W. Dahut, principal investigator; J. Gulley, protocol chairperson; P. Arlen, associate investigator, NCI Navy Oncology Clinic: Vaccine with local radiotherapy: A randomized phase II study of a PSA-based vaccine in patients with localized prostate cancer receiving standard radiotherapy. This is a randomized trial for patients with localized prostate cancer. In arm 1, patients will receive radiotherapy only; in arm 2, patients will receive radiotherapy plus vaccine. The vaccine regimen is identical to that described above (i.e., primary vaccination with rV–PSA admixed with rV–B7–1 followed by booster vaccinations with avipox-PSA; all vaccinations will be given with rGM–CSF and followed by low-dose IL–2). Immune responses of all patients are evaluated both pre- and postvaccination.

Intratumoral Vaccine Studies (Vector-Based Costimulation and Cytokine Studies)

T99–0006: H. Kaufman, principal investigator, A. Einstein Cancer Center: A phase I study to determine the maximum tolerated dose for intralesional injections of rV–B7–1 in the treatment of malignant melanoma. This is a phase I trial in which increasing doses of rV–B7–1 are being injected directly into melanoma lesions. Immune responses of all patients are evaluated both pre- and postvaccination.

T96–0106: R. Freedman, principal investigator, M.D. Anderson: A phase I study of intraperitoneal therapeutic tumor vaccine consisting of autologous tumor cells infected with avipox-B7–1, plus interferon. Autologous ovarian cancer cells are infected with avipox-B7–1 and x-irradiated. Prior to x-irradiation, tumor cells are also treated with interferon to upregulate MHC Class I and putative TAAs. The vaccine is administered intraperitoneally to patients with advanced ovarian cancer. Immune responses of all patients are evaluated both pre- and postvaccination.

P3210: E. Kass/C. Van Waes, principal investigators, National Institutes of Deafness and Communicative Disorders (NIDCD), NIH: This is a phase I dose escalation study in which avipox TRICOM will be administered intratumorally to patients with advanced head and neck carcinoma. Immune responses of all patients are evaluated both pre- and postvaccination.

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Biography: *Dr. Abrams received his Ph.D. in microbiology and immunology at Indiana University in Indianapolis, then completed a postdoctoral fellowship at Washington University in St. Louis. He then joined the Laboratory of Tumor Immunology and Biology, NCI, as a senior staff fellow and, in 1998, was appointed as a principal investigator. He received Federal Technology Transfer Awards in 1996 and 1998 for discovery of human T lymphocyte peptide epitopes reflecting* ras *codon 12 mutations.*

Laboratory of Tumor Immunology and Biology
Host Immune Responses to Tumor-Specific Antigens Induced by Peptide Vaccines

Keywords:
cancer vaccines
CD4+ and CD8+ T lymphocytes
cell-mediated immunity
Fas
immunotherapy
ras oncogenes
tumor-specific peptides

Research: Understanding the molecular and cellular events underlying the apoptosis of human cancer cells may have important implications for the control of metastatic disease not only by conventional anticancer strategies such as chemotherapy but also by immune effector mechanisms. CD8+ cytotoxic T lymphocytes (CTLs) and CD4+ T cells have been found to play crucial roles in host defense against human malignancies. However, as with the onset of chemoresistance, it is also becoming clearer that neoplastic cells may evade cell-mediated immunity at multiple levels of the effector/target interaction which, consequently, may impact the metastatic process. We have been investigating immune effector mechanisms involved in T cell-mediated cytotoxicity of human carcinoma, with emphasis on the potential role of Fas/Fas ligand (FasL) interactions. Although the Fas/FasL system has been proposed as a potentially important component of T cell-mediated cytotoxicity in a number of animal models and experimental systems, its role in human carcinoma remains largely uncharacterized. If it is a relevant process, it also raises the possibility that failure of such solid tumor types to adequately undergo apoptotic death via the Fas pathway may confer a selective survival advantage which may contribute to tumor escape and metastatic development.

We have developed an in vitro model to compare the lytic sensitivity of a human primary colon carcinoma cell line (SW480) to its metastatic-derived isolate (SW620) to antiras Val12 oncogene-specific CTLs produced from both normal and carcinoma-bearing individuals. Previously, we have identified mutated ras peptides reflecting the Gly to Val substitution at position 12 as novel HLA–A2-restricted, CD8+ CTL neoepitopes. We found that these CTLs lysed the HLA–A2+ Ag-bearing SW480 and SW620 cell lines in vitro, although IFN-γ treatment of the targets was necessary for regulation of the lytic phenotype. Moreover, we found that the CTL mechanisms employed in lysis of the primary and metastatic cell lines were distinct. IFN-γ pretreatment rendered SW480 cells sensitive to both Fas-dependent and Fas-independent (perforin) pathways, whereas SW620 cells displayed lytic susceptibility to Fas-independent mechanisms only. IFN-γ

treatment of SW480 cells, but not SW620 cells, was also associated with an enhancement in pro-caspase-3 protein expression, and its subsequent activation in response to Ag-specific CTL attack, as well as inhibition of lysis by peptide-based caspase inhibitors. In addition to cytokine modulation of the tumor-cell death phenotype, we found that treatment of SW480 cells with the anticolon cancer agent, 5-fluorouracil (5–FU), led to enhanced Fas and ICAM–1 expression and triggered Ag-specific CTL-mediated lysis via Fas- and perforin-based pathways. In contrast, these phenotypic and functional responses were not observed with SW620 cells. These results may have implications for fresh tumor isolates, which may provide insights into understanding processes of both tumor immunity and tumor escape. For example, (1) downregulation of the Fas pathway, including elements of Fas-mediated signaling, may represent unique tumor escape mechanisms in response to Ag-specific T cell attack; (2) IFN-γ and certain antineoplastic agents may help to enhance or restore a Fas-sensitive phenotype in certain (colon) carcinoma cells, supporting a potentially important role and use of that cytokine (or those that induce endogenous IFN-γ production) or chemotherapeutics in the immunotherapy of certain solid malignancies; (3) the inability of IFN-γ or 5–FU to sensitize metastatic (colon) carcinoma cells, such as SW620, to Fas-mediated apoptosis or CTL attack suggests that such loss of function may be an important adaptation in the evolution of malignant potential; and (4) IFN-γ may promote efficient CTL/target interactions leading to enhanced T cell activation and triggering of perforin-mediated lysis of metastatic carcinoma cells that might otherwise remain resistant or refractory to Fas-mediated apoptosis.

Another area of investigation dedicated to study fundamental aspects of the host-tumor relationship involves the development of murine models based on immune recognition of tumor cells intrinsically expressing tumor-associated or tumor-specific antigens. The use of such animal models may thus allow us to conceptually translate information gained from in vitro human studies to an in vivo environment.

Recent Publications:

Bergmann-Leitner ES, et al. *J Immunol* 2000;164:4941–54.

Bristol JA, et al. *Cell Immunol* 2000;205:73–83.

Bergmann-Leitner ES, et al. *Cell Immunol* 2001;209:49–62.

Ryan MH, et al. *Cancer Immunol Immunother* 2001;49:603–12.

AIDS Vaccine Program

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The AIDS Vaccine Program (AVP) consists of both investigator-initiated research and service laboratories. Research efforts primarily involve structure/function studies of proteins associated with retroviruses and pathogenic mechanisms of immunodeficiency virus infection. The structure/function studies have identified a highly conserved retroviral motif—the retroviral zinc finger of the nucleocapsid protein—as a potential target for development of antiviral drugs, as well as for preparation of immunogens in vaccine development.

The Retroviral Mutagenesis Laboratory, headed by Dr. Gorelick, has identified the CCHC motif, one of the most highly conserved sequences in retroviruses, and by site-directed mutagenesis has shown that the retroviral zinc fingers are not only necessary for packaging of viral genomic RNA but are required for early infection events as well. Studies with mutants show those that maintain Zn^{2+} coordination (mutants with CCCC or CCHH fingers) are able to package wild-type levels of genomic RNA, yet remain noninfectious. This finding indicates that the evolutionary pressure to maintain the retroviral CCHC finger is apparently related to its involvement in early infection events and recognition and packaging of the viral genome. We have utilized the fact that cells transfected with NC mutant viral DNA express noninfectious virus particles containing the full complement of viral proteins; these proteins are deficient in the genomic RNA that can bind to and enter susceptible cells. Duplication of this process in an animal should expose the immune system to all the steps in virus replication and could mimic infection with an attenuated virus vaccine. To test this strategy, five macaques were immunized with NC mutant DNA and four controls were immunized with control DNA. All animals were challenged with infectious SIV and three of the four controls developed AIDS within 1 year following infection. In contrast, four of the five NC mutant virus DNA-vaccinated monkeys showed normal CD4 cells counts at more than 1 year postinfection.

The Retroviral Assembly Laboratory, led by Dr. Ott, is involved in an analysis of cellular proteins associated with immunodeficiency viruses. By development of a procedure of selective proteolytic digestion of purified viruses, contaminating microvesicles can be removed. This allows internal proteins to be identified, and we have found that cyclophilin A, actin, cofilin, ezrin, moesin, and ubiquitin are found in the virus interior. Ubiquitin is covalently

bound to one of the viral proteins, p6, and mutations to prevent this binding result in noninfectious virions. Cellular proteins incorporated into the virus may provide a function for infectivity and may also provide insight into the assembly and budding processes of retroviruses.

The Protein Chemistry Laboratory, directed by Dr. Henderson, is devoted to the study of proteins that compose infectious retroviruses. This research includes identification of viral and cellular proteins that comprise the mature virus and extend to biophysical and biochemical analyses to explore the function of these proteins. Recent work has focused on the biochemistry and biophysics of these proteins and their roles in viral replication cycles.

The Retroviral Pathogenesis Laboratory, headed by Dr. Lifson, has developed sensitive and quantitative methods of measuring immunodeficiency viruses in humans and primates. Using these techniques to quantitate the plasma virus levels of SIV-infected macaques has revealed that the amount of virus in the plasma of infected monkeys, within the first week of infection, predicts the clinical course and outcome of the disease. Also, the rate and extent of virus infection in vivo correlates with susceptibility of the macaques' peripheral blood mononuclear cells to SIV infection in vitro. This unexpected finding suggests that intrinsic host factors, in addition to specific immune responses, profoundly affect the natural history of AIDS and may account, in part, for the broad spectrum of different clinical courses seen in infected individuals.

Projects currently under investigation by the Retroviral Vaccines Laboratory, directed by Dr. Arthur, include the following: (1) determine whether alloimmunization can protect monkeys from SIV propagated in monkey cells, since we have previously shown that xenoimmunization with HLA–DR and microvesicles from human cells could protect monkeys from challenge with SIV propagated in human cells; (2) characterize the protein and lipid composition of microvesicles to determine whether research opportunities that address virus budding can be identified; and (3) determine whether aldrithiol retroviral inactivation procedures are adaptable to production modes and characterize the inactivated viruses, including their immunologic character, as well as development of other methods to inactivate immunodeficiency viruses.

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Biography: *Dr. Arthur obtained his Ph.D. in 1970 from Louisiana State University in Baton Rouge. He has been involved in research on retroviruses since 1973 and has been conducting research on AIDS and HIV–1 since 1984. Before becoming director of the AIDS Vaccine Program, SAIC–Frederick, he was head of the Biological Products Laboratory at NCI–Frederick.*

AIDS Vaccine Program
Retroviral Vaccines

Keywords:
β2 microglobulin
cellular proteins
class I
HIV
HLA–DR
SIV

Research: The Retroviral Vaccines Laboratory has purified major histocompatibility complex (MHC) class I and II proteins and used these proteins to immunize macaques that were subsequently challenged with simian immunodeficiency virus (SIV). The only animals that were protected from challenge with SIV propagated in human cells were those with demonstrable immune responses to MHC class II (i.e., those immunized with HLA–DR or microvesicles). These animals were not protected from challenge with SIV propagated in monkey cells. This was the first demonstration that immunization with a purified cellular protein could protect animals from virus infection. Because of the finding that xenoimmunization is protective from challenge, detailed analyses of the protective nature of alloimmunization are warranted to assist in the design of AIDS vaccines. We currently have an immunization and challenge experiment in progress in the SIV/macaque model to determine if immunization with alloantigens can provide protection from SIV challenge.

Microvesicles are found in sucrose gradients in a range of densities that includes the same density as retroviruses; consequently, they contaminate sucrose-gradient purified virus. We recently published a characterization of these microvesicles that contain various proteins, including HLA–DR and β2 microglobulin, and a substantial amount of RNA and DNA. A detailed analysis of microvesicles may provide clues as to how to prepare purified retroviruses without the contaminating microvesicles. More importantly, a comparison of the proteins and lipids of viruses and microvesicles may be informative as to mechanisms of glycoprotein transport and viral budding.

Even though most viral vaccines used in humans have been composed of live attenuated viruses or whole killed viral particles, the latter approach is receiving little attention in research on experimental primate immunodeficiency viral vaccines. We have recently shown that virus inactivated with an agent that covalently modifies the nucleocapsid zinc finger motifs could inactivate HIV–1 infectivity while allowing the inactivated virus to retain the conformational and functional integrity of the virion and viral-associated cellular proteins on the virus membrane. These studies have been extended to SIV and to the evaluation of the possibility of utilizing the inactivation

procedure in preparation of large quantities of inactivated viruses. Inactivated virus produced and purified in this manner will be evaluated as a prophylactic vaccine in the SIV monkey model. These preparations are also being used as laboratory reagents in studying coreceptor usage, pathogenesis experiments, CD4 stimulation studies, and in other ways as well.

Our collaborators include Raoul Benveniste, NIH; Mary Carrington and Robert Fisher, SAIC–Frederick; and James Hildreth, Johns Hopkins University.

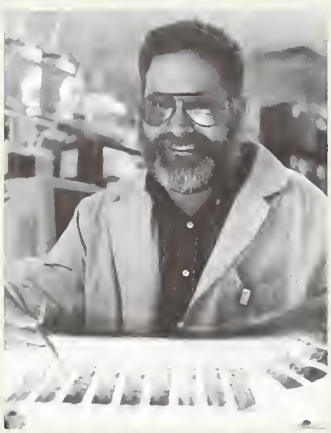
Recent Publications:

Bess JW Jr, et al. *Virology* 1997;230:134–44.

Arthur LO, et al. *AIDS Res Hum Retroviruses* 1998;14 Suppl 3:S311–9.

Gao F, et al. *Nature* 1999;397:436–41.

Lifson JD, et al. *J Virol* 2000;74:2584–93.



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Biography: *Dr. Robert Gorelick received his Ph.D. in 1985 from the University of Delaware in enzymology. In 1985, he began his postdoctoral research with the ABL–Basic Research Program, NCI–Frederick, to determine the function of the retroviral nucleocapsid protein Zn^{2+}-finger. He was awarded an NIH National Research Service Award in 1988. In 1990, Dr. Gorelick joined the AIDS Vaccine Program and extended these studies to HIV–1 and simian immunodeficiency virus (SIV). Currently, he is involved in the development of retroviral vaccines, making use of the replication-defective nucleocapsid protein Zn^{2+}-finger mutants.*

AIDS Vaccine Program
Determination of the Function of Retroviral Nucleocapsid Zn^{2+}-Fingers in Viral Replication

Keywords:
DNA immunization
HIV–1
nucleocapsid protein
zinc fingers

Research: Structure/function studies in the Moloney murine leukemia virus (Mo-MuLV), HIV–1, and SIV systems have been established in our laboratory using site-directed mutagenesis. Our goal is to determine the involvement of conserved Gag protein regions in the viral life cycle.

Investigations of the functions that are disrupted upon alteration of conserved regions in these retroviruses are currently being performed. Previous studies have shown that striking defects in viral properties result from various point mutations in the gene coding for the nucleocapsid (NC) Zn^{2+}-finger. In many cases, these defects include a reduction in the level of packaged genomic RNA. Although a number of these mutants package measurable levels of genomic RNA, they are much more defective in

replication, with some of the mutant viruses being as much as 10^6-fold less infectious than wild-type virus. This is very compelling evidence demonstrating that, not only are the retroviral NC Zn^{2+}-fingers involved in assembly processes, but they also perform other functions in the viral infection events. To further explore the role of the NC Zn^{2+}-finger in early infection processes, we developed a number of Mo-MuLV, SIV, and HIV–1 NC mutant viruses that package wild-type levels of full-length genomic RNA, but are nevertheless replication defective. In these mutants, the assembly function of NC in RNA recognition and packaging (at the level of the Gag precursor) has effectively been separated from other roles that the NC Zn^{2+}-fingers perform in early infection events. These new mutants are providing us with excellent tools to ascertain the functions performed by retroviral NC Zn^{2+}-fingers in early infection processes. In vitro and in vivo assays are being developed to identify defects in infectivity caused by mutations introduced into the retroviral NC Zn^{2+}-finger. It appears that these defects are manifesting themselves in reverse transcription and, possibly, integration processes.

NC mutant SIV(Mne) particles have been characterized by tissue culture methods, and the proviral DNA construct used for expression of these particles is being employed in direct DNA immunization studies in the SIV/*Macaca nemestrina* model. To demonstrate the safety of such immunogens, a pigtailed macaque was injected with replication-defective NC mutant SIV particles (the equivalent of 1.4×10^7 animal infectious doses, had these particles been replication competent), and no signs of infection were observed in this animal throughout the 2-year postinoculation period. Because mutating NC Zn^{2+}-fingers appear to be so detrimental to viral replication, we hypothesized that NC mutant virions could be used as safe and effective immunogens against primate lentiviral infections. Therefore, studies to examine the efficacy of NC mutant particles (produced by DNA immunizations) in the prevention of disease have also been performed. Plasmid DNA containing an NC mutant SIV(Mne) provirus was injected into five pigtailed macaques for the purpose of producing replication-defective virion particles in vivo. This study will allow us to obtain additional information on safety and possible protection from AIDS.

The expression of SIV NC mutant particles via a DNA clone is similar to that found in an attenuated viral vaccine, which is one of the most effective methods of immunization. In common with attenuated virus vaccines, NC mutant virions expressed from a proviral DNA clone expose the immune system to most of the steps in a viral replication cycle: assembly, budding, receptor binding, and cell membrane fusion. These immunogens are safer than attenuated virus vaccines because NC mutant virions are infectious for only a single replication cycle; the particles are replication defective.

The NC mutant DNA plasmid that was injected into the pigtailed macaques produced mutant virus in these animals without ill effects, again demonstrating the safety of such an immunogen. The macaques were challenged with pathogenic SIV(Mne) and four of five immunized animals were protected from disease, whereas three of four control animals showed progressive SIV disease within 1 year postchallenge. Studies are continuing, as we attempt to increase both the protective response as well as the margin of safety of the SIV NC mutant DNA plasmids.

Key collaborators include Raoul E. Benveniste, Judith Levin, and Alan Rein, NIH; Frederic D. Bushman, Salk Institute; and Karin Musier-Forsyth, University of Minnesota.

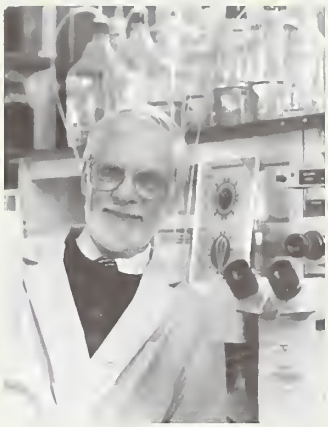
Recent Publications:

Guo J, et al. *J Virol* 2000;74:8980–8.

Gorelick R, et al. *J Virol* 2000;74:11935–49.

Yovandich J, et al. *J Virol* 2001;75:115–24.

Williams M, et al. *Proc Natl Acad Sci USA* 2001;98:6121–6.



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Biography: *After receiving his Ph.D. at the University of Colorado in 1965, Dr. Henderson became an NIH postdoctoral fellow at Harvard University in 1965, a Nobel visiting scientist fellow at the University of Goteborg, Sweden, in 1968, and a research associate at Yale University in 1973. Currently, he is a senior scientist at SAIC–Frederick, where he has carried out retroviral research since 1976.*

AIDS Vaccine Program
Structure and Function of Retroviral Gag Proteins

Keywords:
HIV
nucleocapsid protein
SIV
zinc finger

Research: Retroviruses assemble in the cytoplasm and acquire their envelope components as they bud through the membrane of the infected cell. During and shortly after budding, structural (Gag) and enzymatic (Pol) proteins inside the lipid envelope are acted upon by a viral protease and cleaved at specific bonds to generate the mature proteins of the infectious virus. A maturation proteolytic processing step is common to all retroviruses, but each virus subgroup has a unique proteolytic cleavage pattern giving rise to different protein components in the mature virus.

Over the years, we have determined the Gag maturation proteolytic patterns for many retroviruses and defined the mature structural proteins for each subgroup. The number of mature Gag proteins varies from three for the simplest case (i.e., human T lymphotropic virus type 1, HTLV–1) to six for the more complex viruses (i.e., HIV–1). Many lines of evidence indicate that mature Gag proteins perform vital functions during the viral replication cycle. To better understand structures and functions of mature Gag proteins, we have cloned and expressed several example proteins and are studying their chemical, physical, and structural properties.

One of the mature Gag proteins common to all pathogenic retroviruses is a small basic protein that binds to nucleic acids and is referred to as the nucleocapsid (NC) protein. Retroviral NC proteins have varying amino acid

sequences, but all contain at least one peptide segment with a common sequence (–Cys–X_2–Cys–X_4–His–X_4–Cys–) which binds zinc and is designated as a CCHC zinc finger. HIV–1 and simian immunodeficiency virus (SIV) NC proteins have two CCHC zinc fingers, whereas Moloney murine leukemia virus (M–MuLV) NC protein has only one. NC proteins from HIV–1, SIV, and M–MuLV have been cloned and expressed in bacteria, purified to homogeneity by high performance liquid chromatography (HPLC), and reconstituted with zinc. Their nucleic acid-binding properties have been investigated using a wide variety of physical techniques. The proteins bind to single-stranded nucleic acids covering about six to seven nucleotides each, with varying binding constants depending upon the base composition of the nucleotide segment. In every case, the wild-type protein utilizes an aromatic amino acid side chain to intercalate between bound bases, and the complex is stabilized by ionic interactions between charged groups on the protein and the nucleic acid. The intercalation is dependent upon the structure of the zinc finger and the orientation of the aromatic residue. The integrity of the complex is disrupted by loss of the bound zinc ion, mutations that influence the structure of the zinc finger, or the orientation of side-chain residues. In other studies, we have shown that NC zinc fingers are easily attacked by a wide variety of electrophilic reagents and that such reagents can inactivate live virus by this mechanism.

Our collaborators are Raoul Benveniste, Judith Levin, and Alan Rein, NIH; and Jose Casas-Finet, SAIC–Frederick.

Recent Publications:

Chertova EN, et al. *Biochemistry* 1998;37:17890–7.

Fisher RJ, et al. *J Virol* 1998;72:1902–9.

Gorelick RJ, et al. *Virology* 1999;256:92–104.

Ott DE, et al. *Virology* 2000;266:42–51.

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Biography: *Dr. Lifson received his M.D. from Northwestern University Medical School in 1982, then pursued his residency and research fellowship training in the Department of Pathology at the Stanford University School of Medicine. He became involved in AIDS-related research in 1983, playing a key role in establishing the first program in the United States to try to prevent transfusion-mediated transmission of AIDS through laboratory testing, while also conducting basic in vitro studies of AIDS pathogenesis. After several years of continuing research in AIDS pathogenesis while working in the biotechnology industry, Dr. Lifson moved to the NCI–Frederick in 1995, establishing the Retroviral Pathogenesis Laboratory within the AIDS Vaccine Program. His work there has focused on the continuing development and application of quantitative virological and immunological methods for understanding retroviral pathogenesis and evaluating approaches for the prevention and treatment of retroviral infection and AIDS.*

AIDS Vaccine Program
Determinants of Primate Lentivirus Pathogenesis

Keywords:
AIDS
HIV
primary infection
SIV
vaccine

Research: The basic objective of the Retroviral Pathogenesis Laboratory is to improve our understanding of the viral and host factors that determine the pathogenesis of primate immunodeficiency-associated lentiviruses. This objective is pursued with a methodological emphasis on quantitative molecular and immunological assays, applied to specimens derived from in vivo sources, in the context of understanding natural history pathogenesis and of modifying pathogenesis through vaccination or treatment.

Current studies in the laboratory are focused on trying to understand the viral and host factors that lead to the establishment of persistent productive infection upon inoculation with the primate lentiviruses. We are particularly focusing on the period of early primary infection where key aspects of the dynamic interaction between virus and host appear to be established. In this work, we utilize experimental simian immunodeficiency virus (SIV) infection of macaques as a model system to facilitate study of this critical early interval at defined times following inoculation. Differences in "intrinsic" susceptibility to productive infection on a cellular level, viral replication dynamics, and both innate and adaptive immune responses are studied. Results to date suggest that the underlying natural history of infection of a particular host by a particular viral isolate is established early in the course of infection, and can vary markedly between individuals, even when molecularly cloned virus is used as the inoculum. The immune and nonimmune host variables that may underlie such differences are under intensive investigation. Additional studies with transient postinoculation antiretroviral treatment of SIV-infected macaques indicate that the virus/host balance is subject to modulation during primary infection, and that even transient suppression of virus during this critical interval can have profound and long-lasting beneficial effects. These studies have the potential to provide important new insights into the basic pathogenetic mechanisms operative in primary lentivirus infection. The

extension of these findings from the SIV system to the HIV system could have profound ramifications for understanding basic disease processes, and important implications for optimizing approaches to the treatment and prevention of HIV disease.

In addition, we are conducting in vivo animal model evaluations of several different vaccine approaches, most notably a novel whole inactivated-virion vaccine immunogen that is prepared by preferential covalent modification of free S–H groups on virion internal structural proteins, particularly the viral nucleocapsid protein. This process results in elimination of detectable infectivity, in vitro and in vivo, while preserving the conformational and functional integrity of virion surface proteins. Virions inactivated in this fashion induce cellular and humoral immune responses, and, in initial studies, vaccinated animals resisted infection following intravenous challenge with pathogenic virus. Variations of this promising vaccine immunogen are being evaluated in a number of different vaccine studies, and other reagent applications for the inactivated virions are also being explored.

Key collaborators include Janice Clements, Johns Hopkins University; Ronald Desrosiers and R. Paul Johnson, Harvard University; Vanessa Hirsch and Leonid Margolis, NIH; Chris Miller, University of California–Davis; and Martin Nowak, Institute for Advanced Study.

Recent Publications:

Lifson JD, et al. *J Virol* 1997;75:9508–14.

Rossio JL, et al. *J Virol* 1998;72:7992–8001.

Johnson RP, et al. *J Virol* 1999;73:4952–61.

Lifson JD, et al. *J Virol* 2000;74:2584–93.

Assembly Laboratory in 1999.

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Biography: *Dr. David Ott received his Ph.D. in molecular biology in 1987 from the State University of New York at Stony Brook. He began studying retroviruses as a postdoctoral fellow in the laboratory of Dr. Alan Rein at the NCI–Frederick. After working on retroviral-mediated human gene therapy in private industry, Dr. Ott joined the AIDS Vaccine Program in 1993 and was named the head of the Retroviral*

AIDS Vaccine Program
Retroviral Assembly

Keywords:
AIDS
HIV–1
retrovirology
virus-cell interaction

Research: The Retroviral Assembly Laboratory seeks to understand retroviral assembly from the perspective of both the virus and the cell. Clearly, viral proteins have information that allows them to assemble. However, cellular proteins also play a role in viral assembly and infection. Using molecular biology, protein chemistry, and cell biology methods, we are examining the assembly of both wild-type and mutant viruses to better understand the assembly process and viral replication.

We have been isolating and identifying the host proteins present inside HIV–1 virions to obtain clues about virus-cell interactions. One obstacle to these studies is the presence of proteins outside the virion, either adhered to the particle or present in microvesicles that copurify with HIV–1. We have developed a protease digestion technique that removes the proteins on the viral exterior but spares those inside the particle. This treated material can then be biochemically analyzed. Our results have found several cellular proteins inside the HIV–1: abundant species identified to date are actin cytoskeletal proteins, immunophilins, and ubiquitin. We are currently using molecular biology and cell biology methods to examine the roles of these proteins in the viral life cycle, especially those that affect assembly. In addition to interior proteins, we are examining the mechanism of HLA class II incorporation onto the surface of the virion. The presence of HLA class II might provide a mechanism for viral pathogenicity. We have found that a small region in the cytoplasmic domain of Env (the protein complex that allows the virus to enter the cell) is required for efficient HLA class II incorporation. Other experiments in progress are designed to explore a possible linkage between viral RNA translation, genomic RNA packaging, and virus assembly. Finally, we are examining the role of viral proteins in assembly. The Gag protein is the essential structural protein of the virus and contains all of the information for particle production. Since the $p6^{Gag}$ portion of HIV–1 Gag appears to interact with cellular proteins during assembly and budding, we are also studying the role of $p6^{Gag}$ and cellular proteins in assembly. Potential roles for $p6^{Gag}$ in HIV–1 assembly are being studied.

Together, all of this research on viral assembly should add to our understanding of HIV–1 replication that, in turn, might offer new antiviral therapies.

Key collaborators on these studies are Louis Henderson and Ulrich Schubert, NIH.

Recent Publications:

Ott DE, et al. *J Virol* 1998;72:2962–8.

Ott DE, et al. *J Virol* 1999;73:19–28.

Poon TK, et al. *J Virol* 2000;74:3918–23.

Ott DE, et al. *Virology* 2000;266:42–51.

Computational Applications Program

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The activities of this program, directed by Dr. Burt, fall into several areas of investigation, all with the theme of the application of computational methodology to biological research. The central focus of this program is to describe interactions and reactions in biological environments. Current research areas are: enzyme mechanisms, solvation effects, accurate estimation of binding energies, rapid docking of ligands into receptors, bioinformatics, chemical reactions, and the role of metals in biological processes. All of the staff members of the Computational Applications Program are highly trained in theoretical methodology and use advanced computing in all their work. They not only pursue their own scientific investigations but are actively involved in collaborative research with experimental investigators at the NCI–Frederick and throughout the world. The Computational Applications Program is unique in that it resides in the Advanced Biomedical Computing Center, of which Dr. Burt is also director, and allows the Center to provide both scientific computing support for intramural and extramural researchers as well as scientific expertise to the user community through collaboration and research.

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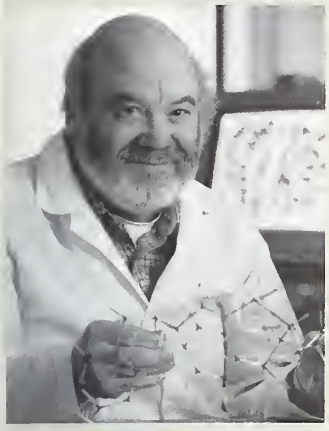

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Biography: *Dr. Burt received his Ph.D. from the University of Mississippi and studied as a postdoctoral fellow at Stanford University. He has held positions at SRI International, Abbott Laboratories, and Sandoz Pharmaceuticals as a computational chemist and is currently the director of the NCI's Advanced Biomedical Computing Center (ABCC).*

Computational Applications Program
Biomedical Computational Chemistry

Keywords:
bioinformatics
catalysis
computational chemistry

Research: Our research activities include both the development of new computational methods and their application to biomedical problems in several areas. Using techniques such as quantum chemistry, molecular dynamics, quantitative structure-activity relationships, homology modeling, and sequence alignment, we are addressing questions related to molecular systems ranging from small molecules to proteins. We have focused our research efforts on HIV- and cancer-related targets.

HIV-Related Targets

In order to elucidate the molecular properties of HIV–1 proteases (PRs), we have calculated the nuclear magnetic resonance (NMR) order parameters of an HIV–1 PR/KNI–272 complex, calculated the pKa values of several HIV–1 inhibitors, calculated the changes in inhibitory potency of several inhibitors due to protein mutations, and developed a methodology for predicting the free energy of binding of inhibitors to HIV–1 PR in solution. We also generated three-dimensional models for the HIV–1 RNA dimer linkage structure as a first step in exploring the possibility of inhibiting dimer formation.

Cancer-Related Targets

The design of effective small molecule drugs against cancer relies on a detailed knowledge of both the macromolecular target and the process that is being disrupted or inhibited. We have focused on developing models of the macromolecular targets of interest and elucidating the complex chemical reaction mechanisms of enzymatically active therapeutic targets.

Thymidine phosphorylase is a potential target for cancer therapy because it appears to play a role in tumor angiogenesis. Understanding its catalytic mechanism would aid in the design of better inhibitors by allowing one to mimic the transition state of the reaction. Unfortunately, the two substrates are too far away for reaction to take place by a direct attack in the crystal structure of the enzyme. We performed molecular dynamics simulations on this system to reveal a different structure of the enzyme in which the two domains have moved in such a way as to place the two substrates in close contact. Quantum mechanical calculations were then performed using this

structure, and we found that the reaction can proceed by a direct nucleophilic attack with a low-energy barrier. These simulations were instrumental in suggesting a solution to the enigma posed by the crystal structure environment and are consistent with the reaction mechanism observed for other N-glycosidic enzymes.

We also performed a density functional study of the catalytic mechanism of DNA polymerase-13 on the active site of this protein to evaluate the possibility of a mechanism that proceeds through a phosphate intermediate, as suggested by recent experimental investigations. Our results suggest that the phosphate mechanism is not likely due to severe energy barriers. Based on these results, we have characterized the contribution of nearby active site residues to the overall reaction energetics and studied their roles in a catalytic mechanism that proceeds through a pentacoordinated intermediate. These results show promise in helping us understand the experimental data on this reaction.

Protein kinases are key components of most intracellular signaling pathways. Cell cycle progression is regulated by the activity of cyclin-dependent kinases (Cdks). The crystal structures of a number of cyclin-dependent kinases-2 complexed with ligands have been published recently, and we have modeled the structure of Cdk4 using the sequence homology between Cdk2 and Cdk4 and the coordinates of the Cdk2 crystal structure. This model is being used to probe the ATP binding site for compounds that could potentially block the function of Cdk4. The superposition was achieved using the program Notesplot, which compares the backbone torsion angles between two structures and identifies the region of the molecules to be superimposed. Notesplot was developed at the ABCC.

A library of compounds specifically designed with the goal of inhibiting cathepsin-D (an aspartyl PR) was created in collaboration with chemists from the Structural Biochemistry Program. Modeling studies on a series of acyclic compounds were performed to select the most promising fragments to be coupled to a statine core in a combinatorial type of synthesis. These compounds are currently being tested and show promise in providing a solution to the solubility problems that have plagued the previous generations of aspartyl PR inhibitors.

Recent Publications:

Tawa GJ, et al. *J Am Chem Soc* 1998;120:8856–63.

Tawa GJ, et al. *J Chem Phys* 1998;109:4852–63.

Topol IA, et al. *J Chem Phys* 1999;111:10998–1014.

Rick SW, et al. *Proteins* 1999;37:242–52.

HIV Drug Resistance Program

The recent application of therapies using combinations of antiviral drugs has shown that virus growth in infected people can be brought to a halt and, in many individuals, provide considerable and long-lasting improvement in their condition. These therapies have helped large numbers of people live relatively normal lives despite their HIV infection. Most important, the therapies prove the concept that antiviral drugs can give long-term relief to patients with HIV infection, but fall far short of providing a long-term solution.

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The problem facing all the strategies is the development of resistance in the virus due to the appearance of specific mutations. In an effort to avoid resistance, drugs have to be given at high—somewhat toxic—doses, in expensive combinations, and on exacting and difficult-to-follow schedules. Even then, the therapy often fails and resistant virus appears. There is, therefore, a desperate need to understand how the virus develops resistance to drugs and to use this understanding to develop more effective strategies for treating HIV infection.

The goal of this program is to establish a focused basic science research effort that addresses this need and builds on the existing strength of HIV and retrovirus research within the National Cancer Institute.

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Biography: *Dr. Coffin received his Ph.D. from the University of Wisconsin in the laboratory of Dr. Howard Temin and was a postdoctoral fellow with Dr. Charles Weissmann at the University of Zürich. In 1975, he was made assistant professor of molecular biology and microbiology at Tufts University in Boston, where he was subsequently promoted to full professor, and in 1994 was the recipient of an American Cancer Society professorship. In 1997, he was named director of the new HIV Drug Resistance Program at the National Cancer Institute, and he presently divides his time between Tufts University and the NCI. He has served on a number of national committees to review and set policy regarding retroviruses and disease. In 1999, he was elected to membership in the National Academy of Sciences.*

HIV Drug Resistance Program
Population Dynamics, Genetics, and Evolution of Retroviruses

Keywords:
human immunodeficiency virus (HIV)
integrase
mutation
retroviruses
reverse transcriptase
virus evolution

Research: Our research interests revolve around many different subjects aimed at obtaining a better understanding of the interaction of retroviruses with their host cells and organisms. In our laboratory at Tufts University, we are engaged in studies using simple retroviruses (avian and murine viruses) to elucidate the nature of the retrovirus-receptor interaction; the mechanism and specificity of integration of viral DNA into host DNA; control of viral gene expression; mechanism of retroviral genetic variation; and evolution of the host-virus relationship, as revealed by the "fossil" record provided by endogenous proviruses found in the normal DNA of all vertebrates and many other species.

We have also been interested in the complex relationship between HIV and the infected host. Modeling of this interaction implies that virus and infected cells turn over very rapidly, at about one generation per day. Thus, large numbers of cells are infected and die every day, and their progeny viruses go on to infect another cell. This turnover has important implications for drug resistance. Together with the high mutation rate shared by all RNA viruses, it creates the means for accumulation of many viral variants, potentially including drug-resistant variants, even before the onset of drug treatment. Such preexisting mutants would be the principal cause of early treatment failure. The manner in which such mutants are expected to arise is strongly dependent on the structure of the HIV population in infected individuals—its effective size and distribution among different sites of replication. Through the HIV Drug Resistance Program, we are initiating studies to follow in great detail the appearance, change in frequency, and linkage of mutations in virus present in plasma and tissue sites in both untreated and drug-treated patients. We expect these studies to provide valuable insights into how resistance arises in patients and possibly to provide clues to averting its appearance.

Recent Publications:

Weidhaas JB, et al. *J Virol* 2000;74:8382–9.

Rouzine IM, et al. *Microbiol Mol Biol Rev* 2001;65:151–85.

Zhou H, et al. *J Virol* 2001;75:1359–70.

Rouzine IM, et al. *Proc Natl Acad Sci USA* 1999;96:10758–63.

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Resistance Mechanisms Laboratory

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Despite a constantly expanding spectrum of highly potent and selective antiviral agents, the rapid acquisition of drug resistance continually confounds therapeutic strategies designed to combat human immunodeficiency virus (HIV) infection. Two properties of the virus-coded reverse transcriptase (RT) can be considered central to this problem—namely, an elevated rate at which inappropriate nucleotides are incorporated into the growing DNA chain, and the lack of an efficient proofreading mechanism. The capacity of the replication machinery to exploit information from both RNA genomes packaged into the virus, i.e., recombination, has the further consequence of increasing genetic diversity by an assortment of mutations. As a result, multiple drug resistance can result from recombination between strains originally resistant to a single drug. The Resistance Mechanisms Laboratory of the HIV Drug Resistance Program combines the disciplines of biochemistry with molecular, cellular, and structural biology to better understand these events at the molecular level, with the ultimate goal of applying the knowledge gained to future antiviral strategies.

Both the DNA polymerase and RNase H functions of RT from HIV–1 and related lentiviruses are under investigation as therapeutic targets in the RT Biochemistry Section. Despite an absolute requirement for virus-coded RNase H for replication, there have been few reports on agents targeted to this function. The development of model systems accurately mimicking specialized RNase H-mediated events (e.g., polypurine tract selection and excision from nascent DNA and tRNA primer release prior to second-strand transfer) and mutants specifically impaired in these steps provides important mechanistic information on this C terminal RT domain. This knowledge is currently being applied to develop high-throughput "smart" screening strategies. Novel mutations in the primer grip of the DNA polymerase domain that lead to increased fidelity have been identified and are under investigation.

The projects of the RT Biochemistry Section are complemented by those of the Viral Recombination Section, which investigates the molecular mechanisms of recombination, RNA packaging and virus assembly, and interactions between distinct retroviruses. Evidence has been obtained demonstrating that recombinant viruses can be generated through packaging of heterologous viral RNA genomes, i.e., of avian and murine origin, into a single virion. The implications and limitations of these events are under investigation. Packaging of the viral genome is dependent on interactions between the Gag polyprotein with a packaging signal in the viral RNA. Experiments are under way to define the cis- and trans-acting elements involved in the specificity of viral RNA packaging.

The theme of recombination is extended to the Viral Mutation Section, which makes use of in vivo systems with recombinant retroviruses to understand the mechanisms that generate variation in retroviral populations. In vitro

assays have identified structural determinants important for fidelity and RT template switching, which include the active-site YXDD motif, the dNTP-binding site and thumb subdomain of the DNA polymerase catalytic center, and, surprisingly, the C terminal RNase H domain. The latter observations indicate important "communication" between the DNA polymerase and RNase H catalytic centers of RT. The in vivo approach of the Viral Mutation Section provides an excellent complement to the structure/function studies conducted in vitro by the RT Biochemistry Section.

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Biography: *Dr. Stuart Le Grice received his Ph.D. in biochemistry from the University of Manchester, United Kingdom, in 1976, studying mechanisms of drug resistance in* Escherichia coli. *After postdoctoral training at the Universities of Edinburgh, Heidelberg, and Tufts University School of Medicine, he was appointed senior scientist at Hoffmann La Roche, Switzerland, evaluating the protease and reverse transcriptase of HIV as therapeutic targets. In 1990, he joined the faculty in the Department of Medicine, Case Western Reserve University (CWRU), Cleveland, OH, and was appointed professor of medicine, biochemistry, and oncology in 1995. From 1994 to 1999, he served as director of the CWRU Center for AIDS Research. Dr. Le Grice serves on the editorial board of the* Journal of Biological Chemistry.

HIV Drug Resistance Program
Resistance Mechanisms Laboratory
Protein-Nucleic Acid Interactions Controlling Retroviral Replication

Keywords:
human immunodeficiency virus (HIV)
retroviruses
reverse transcriptase

Research: The success of antiviral therapies will require a detailed knowledge of proteins involved in the replication, assembly, and infectivity of HIV–1 and HIV–2. Central to these processes is the enzyme reverse transcriptase (RT), which has been a leading target for antiviral drugs for over a decade. Since RT-mediated replication of the HIV RNA genome is a multistep process, dissecting and understanding each event at the molecular level will have the consequence of expanding the repertoire of therapeutic targets. Moreover, current evidence suggests RT may not work alone in replicating the HIV genome, but rather recruits additional viral proteins. A better understanding of the "communication" between these accessory proteins and RT can therefore offer additional avenues for intervention. Research in the RT Biochemistry Section exploits recombinant DNA technology to accurately recapitulate steps in HIV replication and evaluate their sensitivity to antiviral agents, using the enzymes of HIV–1, HIV–2, and their counterparts from closely related viruses. Furthermore, since alterations in RT structure result in the rapid acquisition of drug resistance and failure of antiviral therapies, it is important to understand the exact nature of these subtle alterations. Thus, in addition to a biochemical analysis, the laboratory currently applies bioconjugate and mass spectrometric approaches in an effort to obtain high-resolution structural information on both wild-type and mutant variants of this highly versatile enzyme.

Recent Publications:

Rausch JW, et al. *J Biol Chem* 2000;275:16015–22.

Rausch JW, et al. *J Biol Chem* 2000;275:13879–87.

Miller JT, et al. *J Biol Chem* 2001;276:27721–30.

Miller JT, et al. *Methods Mol Biol* 2001;160:335–53.

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Biography: *Dr. Wei-Shau Hu received her Ph.D. in genetics from the University of California–Davis in 1987. She studied the mechanisms of DNA recombination that lead to human α-thalassemia in Dr. James Shen's laboratory. As a postdoctoral fellow under Dr. Howard Temin's guidance, she studied the mechanisms of retroviral recombination at the University of Wisconsin. In 1991, Dr. Hu joined the faculty of West Virginia University as an assistant professor in the Department of Microbiology and Immunobiology and the Mary Babb Randolph Cancer Center, and was promoted to associate professor with tenure in 1998. She joined the HIV Drug Resistance Program at the National Cancer Institute in 1999. She is also an adjunct professor at West Virginia University.*

HIV Drug Resistance Program
Resistance Mechanisms Laboratory
Recombination and Genetic Interaction Between Distinct and Similar Retroviruses

Keywords:
AIDS
dimerization
drug resistance
evolution
gene therapy
genetic variation
HIV
recombination
retroviruses
reverse transcriptase
reverse transcription
virus-cell interactions
zinc fingers

Research: Our current research interests are focused on the following areas: mechanisms of reverse transcription and recombination; RNA packaging and virus assembly; and interactions between distinct viruses.

Retroviral RNA is reverse transcribed to generate viral DNA that integrates into the host genome to form the provirus. Currently, many of the antiretroviral drugs target the step of reverse transcription in the viral life cycle. Recombination occurs frequently during reverse transcription; this process can reassort mutations and increase the variation in the viral population. It has been demonstrated that viral strains with multiple drug resistance can be generated by recombination from strains that are resistant to a single drug. Using in vivo systems, our laboratory is dissecting the mechanisms of reverse transcription and retroviral recombination to gain a better understanding of these very important processes.

Retroviruses package their RNA into viral particles specifically through interactions between the viral Gag polyprotein and a region in the viral RNA called the packaging signal. Our laboratory observed that murine leukemia virus (MLV) and spleen necrosis virus (SNV), an avian retrovirus, have a nonreciprocal recognition between their Gag polyproteins and packaging signals. SNV proteins can package RNA containing either the SNV or MLV packaging signal, whereas MLV proteins can package only RNA with the MLV packaging signal. Our laboratory is dissecting the cis- and trans-acting elements important for this specific recognition.

Using MLV and SNV as model systems, our laboratory observed that viral RNA from distinct viruses can be packaged into a single virion. Furthermore, recombinant retroviruses can be generated from these two viruses. This is the first experiment demonstrating that genetic interaction can occur between

distinct retroviruses. Our laboratory is currently examining the mechanisms and limitations of these interactions.

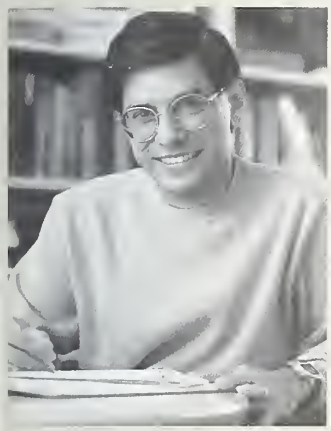
Recent Publications:

Anderson JA, et al. *J Virol* 2000;74:6953–63.

Cheslock SR, et al. *J Virol* 2000;74:9571–9.

Dang Q, et al. *J Virol* 2001;75:809–20.

Hu W–S, et al. *Pharmacol Rev* 2000;52:493–511.



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Biography: *Dr. Vinay K. Pathak received his Ph.D. in genetics in 1988 from the University of California–Davis. He was a postdoctoral fellow under the guidance of Dr. Howard Temin from 1988 to 1991. In 1991, Dr. Pathak became an assistant professor in the Department of Biochemistry and the Mary Babb Randolph Cancer Center at West Virginia University. He was promoted to associate professor with tenure in 1998. He joined the HIV Drug Resistance Program at the National Cancer Institute in 1999. Dr. Pathak is also an adjunct professor at West Virginia University.*

HIV Drug Resistance Program
Resistance Mechanisms Laboratory
Mechanisms of Retroviral Mutation, Reverse Transcription, and Replication

Keywords:
AIDS
antiviral
biochemistry
drug resistance
gene therapy
HIV
integrase
LacZ
mouse leukemia virus
mutagenesis in vivo
nucleocapsid protein
retroviruses
reverse transcriptase
reverse transcription
vectors

Research: Our current research interests are focused on the mechanisms of retroviral reverse transcription, reverse transcriptase (RT) template switching, and fidelity of viral DNA synthesis. Our broad research interests also include retroviral RNA packaging, virion structure and assembly, and development of retroviruses as tools for gene therapy.

Retroviral populations exhibit high levels of genetic variation and evolutionary potential, which can lead to rapid emergence of viral variants resistant to antiretroviral drugs. An important mechanism for generating mutations in retroviral genomes is error-prone DNA synthesis by the virally encoded RT. Frequent template switching by RT between copackaged viral RNAs further increases retroviral variation by assortment of mutations.

Several different aspects of the structure of RT may affect the RT fidelity. Some of these structural features include the YXDD box, amino acids that directly or indirectly define the substrate dNTP-binding site, the primer grip, and the thumb and RNase H domains. Using a rapid in vivo assay that we have developed, we are currently assessing the importance of various structural determinants of RT to the fidelity of DNA synthesis.

Two obligatory template-switching events, minus-strand transfer and plus-strand transfer, are required for completion of viral DNA synthesis. Other internal template-switching events occur frequently during reverse transcription and can lead to the generation of deletions and recombination. To understand the mechanism of RT template switching, we have developed various in vivo assays using retroviral vectors containing directly repeated sequences.

Using the template-switching properties of RTs, we have also developed novel self-activating and self-inactivating retroviral vectors that improve the safety and efficacy of gene therapy. We have shown that the template-switching properties of retroviruses can be used to efficiently delete the viral RNA-packaging signal to further inactivate the retroviral vectors during the process of reverse transcription. RT template switching can also be used to functionally reconstitute genes during reverse transcription. We are currently exploring these approaches to improve suicide gene therapy for the treatment of cancers.

Recent Publications:

Hu W–S, et al. *Pharmacol Rev* 2000;52:493–511.

Halvas EK, et al. *J Virol* 2000;74:10349–58.

Svarovskaia ES, et al. *J Virol* 2000;74:7171–8.

Halvas EK, et al. *J Virol* 2000;74:6669–74.

HIV Drug Resistance Program
Retroviral Replication Laboratory

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The Retroviral Replication Laboratory consists of three sections: the Model Development Section, under the direction of Dr. KewalRamani; the Retrovirus Assembly Section, under the direction of Dr. Rein; and the Vector Design and Replication Section, under the direction of Dr. Hughes.

Dr. Hughes and the members of his section are studying several aspects of retroviruses to provide a better understanding of retroviral replication. There are two major projects. In the first, the goal is to design improved retroviral vectors, which are modified viruses that can be used to deliver foreign genes. The second is intended to better understand the structure and function of the reverse transcriptase (RT) of HIV–1, the virus that causes AIDS. RT is a major target for anti-HIV drugs; mutations in RT can lead to drug resistance. A better understanding of HIV–1 RT and of the mechanisms that underlie drug resistance could lead to the development of better anti-HIV drugs and drug therapies.

Research in the Retrovirus Assembly Section is directed toward a fundamental understanding of the retroviral life cycle, with a special emphasis on the molecular mechanisms involved in retrovirus particle assembly and maturation. The current research of the Rein lab involves protein-protein interactions, protein-nucleic acid interactions, and nucleic acid-nucleic acid interactions. The lab team's experiments combine the power of molecular analysis of defined systems in vitro with the careful study of retroviral replication in living cells. The lab hopes that the insights gained from their basic research will result in the development of new antiviral strategies.

The research focus of the KewalRamani lab begins with obtaining an understanding of the specific interactions between HIV viral components and human proteins. This process will define which host factors are required for a productive infection. An immediate objective of these studies is to provide new targets for the development of novel drug therapies. Using what is learned from these molecular studies, the KewalRamani lab is attempting to develop small animal models to study the interaction of HIV with the host's immune system.

Collectively, the interests of the three sections are relatively broad. We believe that having a broad range of research interests is beneficial; if intellectual or experimental issues arise in one of the sections that are outside the expertise of that section, other members of the laboratory usually can help provide the necessary expertise. We also believe that sharing ideas within the laboratory makes the intellectual environment of the laboratory better for everyone and, in particular, that this approach provides better training for the postdoctoral fellows. All of the postdoctoral fellows should have the opportunity to understand retroviruses in a broad sense and be exposed to a wide range of experimental approaches so that, when confronted with a problem, they will be able to take the most effective experimental approach.

Retroviral Replication Laboratory Staff

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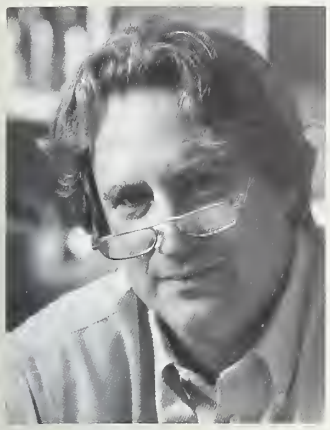

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Biography: *Dr. Hughes received his Ph.D. from Harvard University under the direction of Dr. Mario Capecchi and did postdoctoral research under the direction of Drs. J. Michael Bishop and Harold Varmus at the University of California–San Francisco. He was a senior staff investigator at Cold Spring Harbor Laboratory until 1984, when he established the Gene Expression in Eukaryotes Section (subsequently called the Retroviral Replication and Vector Design Section) in the ABL–Basic Research Program. Dr. Hughes became deputy director of the ABL–Basic Research Program in 1988 and director of the Molecular Basis of Carcinogenesis Laboratory in 1995. In 1999, he joined the HIV Drug Resistance Program at the National Cancer Institute as chief of the Retroviral Replication Laboratory. Dr. Hughes has served as co-organizer of the Retroviruses and Viral Vectors Meetings at Cold Spring Harbor Laboratory, as well as the Annual Meeting on Oncogenes. He was named one of the most frequently cited AIDS researchers by* Science Watch *in 1996.*

HIV Drug Resistance Program
Retroviral Replication Laboratory
HIV–1 Reverse Transcriptase, Retroviral Vector Design, and Molecular Mechanisms of Retroviral Replication

Keywords:
HIV–1
retroviral replication
retroviral vectors
reverse transcriptase

Research: Our work can be divided into three interrelated parts: We are studying the general problem of retroviral replication, developing retroviral vectors that can be used in cells in culture and in intact animals, and focusing on one of the enzymes essential for the replication of HIV–1, reverse transcriptase (RT).

We study the structure and function of HIV–1 RT because it is an important target for anti-AIDS drugs (for example, AZT, 3TC, and ddI are RT inhibitors). We believe that the development of more effective anti-RT drugs will depend on a better understanding of not only wild-type HIV–1 RT but also the drug-resistant variants that arise in response to drug therapy. This project is part of a large collaboration, a critical part of which involves Dr. Edward Arnold and his colleagues, who have used x-ray crystallography to solve a number of different structures of HIV–1 RT (both wild-type and mutant). Our biochemical and genetic experiments on HIV–1 RT are, in many cases, inspired by this structural analysis. In turn, the biochemical analyses have provided guidance for some of the crystallographic experiments. Recent structural and biochemical analyses have shed new light on the mechanisms of HIV–1 RT drug resistance. For example, resistance to the nucleoside analog 3TC appears to involve steric hindrance. Replacement of the methionine normally found at position 184 of RT, which is part of the polymerase active site, with either isoleucine or valine creates a steric gate. A β-branched amino acid at position 184 blocks the appropriate binding of 3TCTP but still permits the incorporation of normal dNTPs.

In contrast, AZT resistance involves an enhanced excision of AZTMP after it has been incorporated into the growing DNA strand. The excision reaction is

essentially the normal polymerization reaction run in reverse, except that the beta and gamma phosphates of ATP serve as the pyrophosphate donor. The mutations that confer resistance to AZT do not interact directly with AZTMP, but instead serve to enhance the ability of the mutant enzyme to bind ATP, which increases the rate of excision. The specificity of the excision mechanism for AZT is inherent in the structure of HIV–1 RT. Steric constraints involving the azido group cause the end of an AZTMP-terminated primer to preferentially reside in the nucleotide-binding site, which favors excision.

We have spent more than 15 years developing a series of retroviral vectors (the RCAS vectors) based on the avian sarcoma/leukosis virus (ASLV) family of retroviruses. Unlike most other retroviral vectors, the RCAS vectors are replication competent. Until recently, however, the RCAS vectors would efficiently infect only avian cells. In order for a retrovirus to infect a cell, a specific protein on the surface of the virus (the envelope or Env protein) must bind to its cognate receptor on the surface of the host cell. Mammalian cells lack the receptors recognized by the RCAS vectors. We solved this problem in two ways: by creating a version of RCAS vectors that uses an Env protein from a mammalian retrovirus and by modifying mammalian cells so they will express a receptor from avian cells. Both of these methods work well; each has particular advantages. One of the advantages of using the cloned avian receptor is that we can create transgenic mice expressing the receptor in a subset of their cells or tissues; in this way, we can control the expression of genes carried by the RCAS vectors in the mouse model.

There is an interesting difference in the behavior of RCAS vectors in avian and mammalian cells: Even though these viruses are replication competent in avian cells, they are replication defective in mammalian cells. Although the version of RCAS vectors that uses an *env* gene from a mammalian virus can infect mammalian cells, these cells do not produce infectious virus. As a result, this vector system represents a substantial improvement over previously developed retroviral vector systems: It is both simple to use (because the virus replicates efficiently in avian cells, a high-titer stock can be obtained quickly and easily) and quite safe (neither complementation nor recombination will allow these viruses to replicate in mammalian cells).

The development of better vectors, and more effective AIDS treatments, rests ultimately on a better understanding of retroviruses and their life cycle. We are engaged in several projects designed to elucidate how retroviruses replicate. To give one example, the inability of avian retroviruses such as the RCAS vectors to replicate in mammalian cells raises an interesting question: How is replication blocked in these viruses?

Although the analyses are not yet complete, there is good evidence that unspliced and partially spliced viral RNAs are not appropriately handled in mammalian cells. By studying this problem, we hope to learn more about the rules (and machinery) that control the splicing and transport of viral RNAs, which could provide useful information about cellular and viral processes. In addition, we hope to obtain information that would be useful in the design and development of new RCAS vectors.

Our collaborators include Edward Arnold, Rutgers University; Mark Federspiel, Mayo Clinic; Amnon Hizi, Tel Aviv University; and Christopher Michejda, NIH.

Recent Publications:

Julias JG, et al. *J Virol* 2001;75:6537–46.

Boyer PL, et al. *J Virol* 2001;75:4832–42.

Sarafianos SG, et al. *EMBO J* 2001;20:14.

Gao HQ, et al. *J Mol Biol* 2000;300:403–18.

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Biography: *A native Wisconsinite, Dr. Vineet N. KewalRamani ventured West to receive his Ph.D. in 1996 from the Department of Microbiology at the University of Washington, completing his graduate research on HIV molecular virology as a Helen R. Whiteley fellow in the laboratory of Dr. Michael Emerman at the Fred Hutchinson Cancer Research Center. Training under the guidance of Dr. Dan Littman in New York as a Damon Runyon-Walter Winchell postdoctoral fellow inspired his lab's current studies of HIV replication blocks in murine cells and using this information to manipulate mouse and virus in developing a murine model for HIV infection.*

HIV Drug Resistance Program
Retroviral Replication Laboratory
Understanding Host-Virus Interactions in HIV Infection; Animal Models for HIV Infection

Keywords:
animal models
chemokine receptors
DC–SIGN
dendritic cells
HIV–1
host-virus interactions
Langerhans cells
mouse model
murine mode
retrovirology
retrovirus
retroviruses
SNV

Research: Our research focus begins with developing a molecular picture of the specific interactions of HIV elements with human target cell proteins to define which host factors are required to sustain a productive viral infection. An immediate objective of these studies is to provide new targets for interventive drug therapies. In addition, using what we learn from these molecular studies, we are endeavoring to develop new animal models to study the interaction of HIV with the immune system of the host. Comprehensive immunological studies of the interaction of HIV with the host immune system have been hindered by the lack of an adequate animal model that sustains a pathogenic HIV infection. To accelerate our understanding of how HIV destroys the host immune system and develop new strategies to bolster the immune system to fend off this insidious attack, we are attempting to genetically manipulate mice to make them permissive to HIV infection.

The use of mice to model human infectious disease is attractive given the level of understanding immune function in mice is unparalleled. Development of a mouse model for HIV infection would additionally be useful in testing the efficacy of various drug protocols in vivo. However, murine cells

are naturally refractory to HIV infection. The recent identification of human cofactors required for HIV entry and gene transcription has enabled circumvention of some restrictions present in murine cells. Mice transgenic for cofactors such as human CD4, CCR5, and Cyclin T1 have been generated. In spite of these advancements, murine cells expressing the human cofactors are still several orders of magnitude less efficient than human cells in the production of infectious HIV.

To enable efficient HIV–1 replication in transgenic mice, we have initiated two complementary sets of studies. The first approach requires further genetic manipulation of the mouse to make it a more suitable host for the virus. Our second, parallel approach relies on manipulation of the HIV–1 genome to make it more suitable for replication in murine cells. This includes protocols to adapt HIV–1 for replication on murine cells through selective pressure in vitro. To develop a basis for our manipulation of mice, we are examining the nature of the additional impediments to HIV replication in murine cells to determine (1) how they affect virus production and (2) whether these blocks are due to a lack of positive factors in the murine cells or the presence of negative factors. Our analyses have so far revealed that the remaining blocks to replication appear to be post gene transcription occurring prior to viral maturation. The dominance or recessive nature of these blocks is currently being ascertained. If murine cells are found to lack positive cofactors typically expressed in human cells, the human cofactors will then be introduced as transgenes into mice already transgenic for human CD4, CCR5, and Cyclin T1. If negative murine factors are identified, they will be interfered with posttranscriptionally, genetically ablated from HIV target cells, or in vitro selected HIV variants will be used that are less susceptible to their prohibitive effects. In fact, we are also performing prospective screenings for positive cofactors that would promote steps of virus replication actively blocked in murine cells by negative factors. These studies on positive and negative cofactors will not only be useful in developing a mouse susceptible to HIV infection but in understanding how HIV interacts with the host cell. Indeed, a better molecular understanding of HIV replication has the potential to provide new targets for disrupting infection of the virus in vivo. Given that lentiviruses have colonized many mammalian species, and early-stage restrictions to HIV replication in murine cells have been obviated once human cofactors were identified, we are confident that continued selective manipulation of mouse and possibly virus will relent a murine model to study HIV infection and immunopathogenesis.

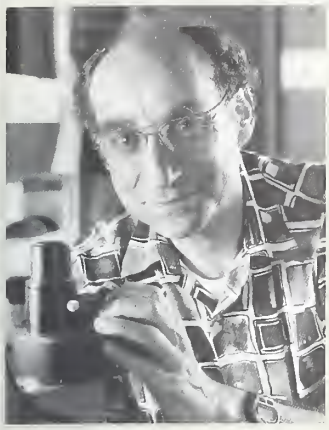
Recent Publications:

Deng H–K, et al. *Nature* 1997;388:296–300.

Unutmaz D, et al. *J Exp Med* 1999;189:1735–46.

Geijtenbeck TB, et al. *Cell* 2000;100:587–97.

Bashirova AA, et al. *J Exp Med* 2001;193:671–8.



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Biography: *Dr. Rein obtained his Ph.D. from the University of California–Berkeley. He has been associated with the NCI since 1976 and served as head of the Retroviral Genetics Section in the ABL–Basic Research Program from 1984 to 1999. His research has dealt with a number of aspects of the biology and molecular biology of murine and human retroviruses, including virus assembly and maturation, viral envelope function, translational suppression, and pathogenesis.*

HIV Drug Resistance Program
Retroviral Replication Laboratory
Mechanisms in Retroviral Replication and Pathogenesis

Keywords:
nucleic acid chaperones
protein targeting
retroviruses
RNA chaperones
RNA-protein interaction
virus assembly

Research: The goal of the research efforts in the Retroviral Assembly Section is to extend our understanding of basic mechanisms in retroviral replication and pathogenesis. This understanding may lead to new methods of combating retrovirus-induced disease, including AIDS.

There appear to be several different modes of interaction between retroviral proteins and nucleic acids, each with important functional consequences for viral replication. First, an exquisitely specific recognition by the Gag polyprotein (the structural protein of the virus particle) selects the viral RNA for packaging during virus assembly. This recognition involves zinc fingers in the protein. We are studying the mechanism by which the Gag protein recognizes and packages the genomic RNA of the virus during assembly in vivo. Our research strongly suggests that the recognition signal involves the three-dimensional structure formed by a dimer of genomic RNA molecules. We are studying the structure of the dimer linkage and its possible role in packaging of viral RNA. Our mutational studies also show that the zinc fingers have other crucial functions in addition to their role in the recognition process. These additional functions are now under investigation.

Second, the Gag polyprotein and its cleavage product, the nucleocapsid (NC) protein, exhibit nucleic acid chaperone activity. That is, they transiently destabilize base pairs, catalyzing conformational transitions to the optimally base-paired structure in a nucleic acid molecule. This sequence-independent activity is used before or during virus assembly, when the Gag polyprotein promotes the annealing of a cellular tRNA molecule to the viral RNA; the tRNA is the primer for reverse transcription when the virus infects a new host cell. The activity is used again during virus maturation (i.e., after Gag is cleaved by the viral protease), when NC induces a conformational rearrangement in the viral RNA dimer within the particle. The activity also appears to be crucial during reverse transcription, facilitating both polymerization and strand-transfer steps during proviral DNA synthesis; recent data suggest that it may be important during the integration of the DNA into the host

chromosome as well. We are studying the molecular mechanism underlying the nucleic acid chaperone activity of these proteins.

Third, the Gag polyprotein interacts with *either* the viral RNA or, alternatively, cellular mRNA molecules, using them as "scaffolding" in the assembly of virus particles. The presence of cellular mRNA molecules in particles lacking the viral RNA was particularly obvious when the virus-producing cells contained an alphaviral vector. This is because alphaviral vectors replicate their RNAs to extraordinary levels, resulting in a nearly monodisperse population of mRNAs in the cell; under these conditions the alphavirus-derived mRNA replacing retroviral RNA was easy to detect in retroviral particles.

We have also found that the HIV–1 Gag polyprotein is able to assemble (in the presence of nucleic acid) into minute spherical virus-like particles in a completely defined system in vitro. The nucleic acid requirement can be fulfilled by oligodeoxynucleotides as short as 10 to 15 nucleotides. The virus-like particles are only 25 to 30 nm in diameter, whereas the cores of authentic virions formed in mammalian cells are ~100 nm in diameter. We found that if assembly reactions are performed in the presence of reticulocyte lysates, particles of 100 nm, rather than 25 to 30 nm, are formed. Therefore, mammalian cells contain a factor that alters the radius of curvature with which Gag polyprotein molecules interact with each other during the assembly process. We have now identified this factor as inositol pentakisphosphate (IP5). We are now analyzing the molecular mechanism of this effect of IP5 on the interactions between HIV–1 Gag molecules. We are also attempting to demonstrate that IP5 or related compounds contribute to the correct assembly of HIV–1 virus particles in vivo.

In all retroviruses, the Gag polyprotein is targeted to the plasma membrane of the virus-producing cell. While we have some understanding of how these proteins bind to membranes, the reason for the specificity for the plasma membrane is completely unknown. We are also exploring the possibility that phosphatidylinositides are involved in targeting these proteins to the plasma membrane. Many of these experiments are using confocal microscopy.

In addition, we have used surface plasmon resonance technology to analyze the binding of HIV–1 NC protein to very short oligonucleotides. Although NC is probably capable of binding to any single-stranded DNA or RNA, these studies showed that it exhibits profound sequence preferences. We are engaged in a detailed investigation of the binding of NC and Gag to short, well-defined oligonucleotides; this information should help us understand the various interactions with nucleic acids discussed above—i.e., chaperone activity of both NC and Gag; assembly of virus-like particles by Gag; and the exquisitely specific encapsidation of genomic RNA by Gag during virus assembly in vivo.

The existence of convenient in vitro assays for specific nucleic acid binding by NC and for virus assembly by Gag has made it possible to screen large libraries of compounds for inhibitors of these functions. Compounds capable of interfering with these functions have been identified and are now being assayed for their ability to prevent HIV–1 replication in cultured cells.

Our collaborators include Larry Arthur, Robert Fisher, Robert Gorelick, Louis Henderson, Stephen Lockett, David Ott, Andrew Stephen, and Eric Towler, SAIC–Frederick; Kelly Dryden and Mark Yeager, The Scripps Research Institute; Bernard Ehresmann, Chantal Ehresmann, and Gerard Keith, Centre Nationale de la Recherche Scientifique, Strasbourg; and Judith Levin and Robert Shoemaker, NIH.

Recent Publications:

Rein A, et al. *Trends Biochem Sci* 1998;23:297–301.

Feng Y–X, et al. *J Virol* 1999;73:4251–6.

Campbell S, et al. *J Virol* 1999;73:2270–9.

Muriaux D, et al. *Proc Natl Acad Sci USA* 2001;98:5246–51.

Molecular Targets Drug Discovery Program

The Molecular Targets Drug Discovery Program (MTDDP) is charged with planning, conducting, and providing leadership and infrastructure for applied, interdisciplinary, inter- and intralaboratory/branch, and collaborative intramural research supporting the translation of basic science advances into new tools, reagents, and leads for molecular target evaluation, drug discovery, and development. In addition, it collectively exploits chemical and biodiversity repositories, including the NCI-designated, high-priority intramural drug discovery, development, preclinical, and clinical research initiatives focused on specific molecular targets, pathways, or processes.

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The three initial MTDDP research groups and functions are as follows:

- **Bioassay Development, Screening, and Dereplication Group:** Develops, adapts, evaluates, and applies novel screening assays, protocols, and emerging technologies for molecularly targeted lead discovery and molecular target validation research; provides screening support for bioassay-guided lead isolation and dereplication of natural products; and provides lead elucidation and deconvolution of synthetic, semisynthetic, and biosynthetic chemical diversity libraries.

- **Natural Products Chemistry Group:** Provides chemistry support for bioassay-guided fractionation, isolation, purification, and structural characterization of novel, molecularly targeted, nonproteinaceous leads from natural products; reisolates bioactive compounds of interest for followup investigations.

- **Protein Chemistry and Molecular Biology Group:** Provides protein chemistry and molecular biology support for (1) bioassay-guided fractionation, isolation, purification, structural characterization, recombinant production, and modification of novel, molecularly targeted proteins and peptides from natural products and other chemical diversity libraries; (2) investigates mechanisms of action, molecular target interactions, and bioassay development for bioactive proteins and peptides; and (3) reisolates bioactive proteins and peptides of interest for followup investigations.

Our collaborators include Laura Barrientos, Robert Blumenthal, Angela Gronenborn, Louis Henderson, Philipp Kaldis, Lewis Pannell, Ray Sowder, and Alexander Wlodawer, NIH.

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Members of the Mouse Cancer Genetics Program (MCGP) make use of molecular mouse genetics as a primary tool to better understand the fundamental processes underlying mammalian development and/or human disease. The Molecular Genetics of Oncogenesis Section is headed by Dr. Copeland while the Molecular Genetics of Development Section is headed by Dr. Jenkins. Both are tenured scientists in the MCGP. Drs. Copeland and Jenkins direct a joint research program and have coauthored all publications since moving to Frederick in 1985. Part of their research involves the use of inbred mouse strains that have high spontaneous incidences of leukemia as a model system to identify new human leukemia disease genes. Once identified, gene knockouts, transgenic mice, genetic criteria, and cell biological approaches are used to begin to understand how these genes function in disease induction, to place these genes into biochemical pathways, and to develop new models of human disease. Members of these two sections are also developing a dense gene-rich genetic linkage map of the mouse genome. In addition, homologous recombination in yeast and bacteria is being exploited to develop a better means of generating targeting vectors for embryonic stem (ES) cell knockouts and for modifying bacterial artificial chromosomes (BACs) for transgenesis. The feasibility of developing a mitotic recombination system for the mouse is also being determined. This recombination system has enormous potential for mouse genetics research, including genomic imprinting studies and chemical mutagenesis screens designed to uncover new recessive mutations in the mouse. Classical mouse coat-color mutations (*d*, *ash*, *ln*, *ru*, and *ru2*), in combination with reverse genetics and gene knockouts, are also used to molecularly dissect the biochemical pathways important for pigment granule transport. Studies are also continuing to genetically dissect, at the molecular level, the Mitf-Tfe family of bHLH–Zip transcription factors and to initiate a genetic modifier screen to identify other proteins in the *Mitf* signal transduction pathway. Finally, classical mouse neurological mutations (*tg*, *nr*, and *mea*) that affect the cerebellum, combined with reverse genetics, are used as model systems for understanding the cellular and molecular mechanisms that guide development of the mammalian brain.

The Neural Development Section is headed by Dr. Tessarollo, who joined the MCGP in May 1998 as a tenure track investigator. Members of this section study the role of trkC receptor isoforms in mouse development. They are also using genomic sequencing to determine how the different trkC receptor isoforms are generated and in situ hybridization analysis, RNase protection,

and RT–PCR to define the expression pattern of trkC isoforms in embryonic and adult tissues. In addition, they are attempting to create conditional deletions of the different trkC isoforms by using gene targeting in ES cells. The goal is to use these different deletions to characterize the function of the trkC isoforms in mouse development. Finally, members of Dr. Tessarollo's section have made a brain-derived neurotrophic factor (BDNF) knock-in mutation at the mouse neurotrophin-3 (NT–3) locus in order to study the specificity of neurotrophin activities in mouse development. They are characterizing the potential role of BDNF in development of the serotonergic system to determine whether alterations in this system cause the abnormal feeding and aggressive behavior observed in heterozygous BDNF knock-out mice.

The Genetics of Cancer Susceptibility Section is headed by Dr. Sharan, a tenure track investigator who joined the MCGP in March 1998. Members of Dr. Sharan's section are functionally analyzing the murine homolog of the human breast cancer susceptibility gene *BRCA2*. Knock-in strategies are used to introduce *BRCA2* mutations identified in human breast cancer patients into the mouse *Brca2* gene in order to study the effects of these mutations on *Brca2* stability/function. This section is also attempting to identify the enhancers and suppressors of *Brca2* tumorigenesis by breeding the *Brca2* knock-out mutation onto different genetic backgrounds and by using retroviral insertional mutagenesis to identify those genes that cooperate with *Brca2* loss in the progression to acute disease. They are also using yeast two-hybrid analysis to identify proteins that interact with *Brca2*.

During the next 4 years, the MCGP will undergo a major expansion in mouse cancer genetics to make use of the new genomic tools provided by the Human Genome Project in conjunction with mouse genetics to develop new and better cancer models and to identify the genes, genetic interactions, and pathways important for cancer induction. We believe this integrated genetic approach will provide a better understanding of human cancer and, ultimately, aid in the design of novel therapeutic approaches for cancer treatment. Over the next 4 years, we plan to recruit approximately 10 tenured or tenure track scientists into the MCGP to create a critical mass of scientists with varied but related expertise in the field and provide an environment that will be highly conducive to cancer genetics research. The NCI–Frederick is an excellent setting for this expansion because a sizeable mouse cancer genetics effort already exists at this site, and the mouse facilities here are exceptional. The NCI Intramural Research Program also provides an exceptional environment for this effort because, although the research is high pay-off, it requires the type of long-term funding that is difficult to obtain from extramural grants.

Space for this expansion has been identified in Building 560 and renovations to two-thirds of this space should be complete in March 2001. Renovations to the other one-third of the space will begin in March 2001 and should be completed in early 2002. Recruitment for these new positions began in the fall of 2000. Space for an additional 11,000 mouse cages has also been identified to support this expansion. When combined with the ~7,000 cages that already are dedicated to the MCGP, this will bring the number of mouse cages dedicated to the program to ~18,000.

A new Molecular Technology Laboratory, headed by Dr. Monroe, is also being established to provide core support to the program in the areas of high-throughput genomic sequencing, genotyping, and expression arrays. Bioinformatics will also be greatly expanded in support of this new effort.

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Biography: *Dr. Copeland received his Ph.D. in biochemistry from the University of Utah and was a postdoctoral fellow at the Dana-Farber Cancer Center and Harvard University Medical School. He later worked at The Jackson Laboratory as an associate staff scientist and at the University of Cincinnati College of Medicine as an associate professor of microbiology and molecular genetics. In 1985, Dr. Copeland joined the ABL–Basic Research Program as director of the Mammalian Genetics Laboratory and head of the Molecular Genetics of Oncogenesis Section. In 1999, his laboratory was incorporated into the NCI's Center for Cancer Research. Concurrent with this incorporation, the laboratory was renamed the Mouse Cancer Genetics Program (MCGP) and has received increased resources to expand in the area of mouse cancer genetics.*

Mouse Cancer Genetics Program
Genetics of Cancer Susceptibility

Keywords:
cancer genetics
chromosome engineering
gene mapping
insertional mutagenesis
leukemia
lymphoma
mitotic recombination
mouse models

Research: One area of research in our laboratory uses inbred mouse strains that develop high spontaneous incidences of retrovirally induced leukemia as model systems for identifying new leukemia disease genes. The expectation is that some of these genes will also represent human disease genes that might not be easy to identify directly in humans. Once these genes are identified, we use gene knock-out and transgenic mice, genetic criteria, and cell biological approaches to understand how these genes function in disease induction, to place these genes into different biochemical pathways, and to develop new mouse models for human leukemia. The BXH2 and AKXD recombinant inbred strains have been particularly valuable for this research because they develop high incidences of myeloid or B and T cell leukemia, respectively, and the proviral integration sites in these leukemias provide powerful genetic tags for disease gene identification. During the past several years, we have identified many new leukemia disease genes in these strains by proviral tagging. Importantly, some of these genes are also involved in human leukemia, validating the usefulness of this approach for human disease gene identification. Despite this success, however, it is apparent that there are many more disease genes to be identified. During the past year, we developed an inverse PCR (IPCR) method for proviral tagging that makes use of automated DNA sequencing and the genetic tools provided by the Mouse Genome Project, thereby increasing the throughput for disease gene identification. We also used this IPCR method to clone and analyze more than 400 proviral integration sites from BXH2 and AKXD leukemias and, in the process, identify more than 90 new candidate leukemia disease genes. Some of these genes appear to function in pathways already implicated in leukemia, whereas others are likely to define new disease pathways. Our studies highlight the power of the mouse as a tool for gene discovery and functional genomics in the modern genome era. Future studies will validate the role of these genes in leukemia and determine whether any are involved in human disease.

Studies are also in progress to develop a dense gene-based interspecific backcross linkage map of the mouse genome. Such studies have many important applications for mouse genome research, including (1) providing insights into mammalian genome evolution through comparative mapping, (2) facilitating the candidate positional cloning of mouse mutations, (3) helping to develop new mouse models of human disease, and (4) identifying new human disease genes through comparative mapping. Large-scale DNA sequencing and homologous recombination in yeast and bacteria are also being used to expedite the generation of targeting vectors for embryonic stem cell knockouts and for modifying bacterial artificial chromosomes for transgenesis.

Finally, we are attempting to develop an efficient Cre/*lox*P-mediated mitotic recombination system for mouse genome research. In *Drosophila*, mitotic recombination has been a powerful tool for addressing biological questions such as the developmental potential of celi clones, the autonomy of gene action, cell fact restriction, and patterns of cell division. An efficient mitotic recombination system also has enormous potential for mouse genetics. For example, it could greatly simplify ethylnitrosourea mutagenesis screens that are designed to uncover new recessive mutations as well as facilitate studies of genomic imprinting.

Collaborators include David A. Largaespada, University of Minneapolis; Takuro Nakamura, Japanese Foundation for Cancer Research; Stephen J. O'Brien, NIH; and John D. Shaughnessy, Jr., University of Arkansas for Medical Sciences.

Recent Publications:

Li J, et al. *Nat Genet* 1999;23:348–53.

Shaughnessy JD Jr, et al. *Oncogene* 1999;18:2069–84.

O'Brien SJ, et al. *Science* 1999;286:458–81.

Nakamura T, et al. *Mol Cell Biol* 2000;20:3178–86.

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Biography: *Dr. Jenkins received her Ph.D. in molecular and cellular biology from Indiana University. After carrying out postdoctoral research at the Dana-Farber Cancer Center and Harvard Medical School, she became an associate staff scientist at The Jackson Laboratory and an associate professor of microbiology and molecular genetics at the University of Cincinnati College of Medicine. In 1985, Dr. Jenkins joined the ABL–Basic Research Program as head of the Molecular Genetics of Development Section, which was part of the Mammalian Genetics Laboratory (MGL). She was appointed chair of the FCRDC–Animal Care and Use Committee in 1998 and editor-in-chief of the journal* Genomics *in 1997. In 1999, the MGL was incorporated into the NCI's Center for Cancer Research. Concurrent with this incorporation, the MGL was renamed the Mouse Cancer Genetics Program (MCGP). Dr. Jenkins is now head of the Molecular Genetics of Development Section within the MCGP.*

Mouse Cancer Genetics Program
Molecular Genetics of Mouse Development

Keywords:
absence epilepsy
bHLH–Zip proteins
calcium channel
cerebellar development
Griscelli's disease
microphthalmia
neurological disease
pigment genetics
vesicle transport
Waardenburg syndrome

Research: Members of the Molecular Genetics of Development Section are using classical mouse coat-color mutations [*dilute (d), ashen (ash), leaden (ln)*], as well as reverse genetics, to molecularly dissect the biochemical pathway(s) important for pigment granule transport. The *d*, *ash*, and *ln* mutations cause a lightening of coat color due to defects in pigment granule transport, while *d* also causes a fatal neurological disease due to endoplasmic reticulum (ER) transport defects in cerebellar Purkinje cells. Previously, we showed that these three mutations are suppressed by another mutation [*dilute suppressor (dsu)*] and provided evidence suggesting that all three mutations function in a common biochemical pathway. We also showed that *dsu* can suppress the eye color but not the diluted coat color associated with two other mutations [*ruby-eye (ru)* and *ruby-eye-2 (ru2)*]. In addition, we showed that *d* encodes unconventional myosin VA (MyoVA), a major cellular vesicle transport motor, while others showed that human *MYOVA* mutations produce Griscelli's disease, a rare autosomal recessive disorder characterized by pigment dilution, variable cellular immunodeficiency, neurological disorders, and acute phases of uncontrolled lymphocyte and macrophage activation. In more recent studies, we used RT–PCR-based sequencing to identify the mutations responsible for 17 viable *dilute* alleles and yeast two-hybrid assays to identify proteins that interact with MyoVA. These studies identified important functional domains of the protein and provided support for the notion that the different MyoVA isoforms produced by alternative splicing encode important cell-type-specific functions. These studies also showed that MyoVA can bind a major cellular microtubule vesicle transport motor, ubiquitous kinesin heavy chain (KhcU), and suggested for the first time that vesicle transport can be coordinated in the cell via the direct interaction of the different motor molecules. Future studies are aimed at using positional cloning to identify the gene products encoded by the other coat-color mutations and then to determine how they function in the cell.

Studies are also under way to determine the function of the Mi-Tfe family of basic helix-loop-helix leucine zipper (bHLH–Zip) transcription factors in mammalian development. The Mi-Tfe family consists of four related genes—*Mitf, Tfe3, Tfeb,* and *Tfec*. The *Mitf* gene encodes the *microphthalmia transcription factor* (*Mift*). Mutations in the human *MITF* gene are responsible for a common human pigmentation and hearing disorder, Waardenburg syndrome type 2 (WS2). More than 20 independent *Mitf* mutations have been isolated in the mouse and shown to affect a number of cell types, including melanocytes, osteoclasts, and mast cells. In contrast, *Tfeb, Tfe3,* and *Tfec* were isolated by biochemical means and little is known about their function in vivo. In collaborative studies, we showed that the Mitf-Tfe proteins can bind the E-box sequence as homodimers or as heterodimers with other family members. To investigate the function of the *Tfe* genes in mammalian development, we used embryonic stem (ES) cell knock-out technology to make germline null mutations in the three mouse *Tfe* genes. The effect of each mutation on development was then measured alone or in combination with mutations in other *Mitf-Tfe* family members. Surprisingly, these studies did not identify any essential functions for Mitf-Tfe heterodimers in development. This is in marked contrast to what has been observed for the Myc/Max/Mad family of bHLH–Zip proteins, where the heterodimers are thought to have essential functions. Future studies will attempt to use chemical mutagenesis and genetic modifier screens to identify other proteins in the Mitf signal transduction pathway.

We are also using classical mouse neurological mutations [*tottering* (*tg*), *nervous* (*nr*), *meander tail* (*mea*)] that affect the cerebellum, combined with reverse genetics, as a model system for understanding the cellular and molecular mechanisms that guide development of the mammalian brain. The *tg* mice were chosen for study because they are one of the few mouse models for absence epilepsy. Although half of all human epilepsies have a genetic component, and as much as one percent of the population suffers from epilepsy, no genes had been identified for absence epilepsy. By positional cloning, we showed that *tg* encodes the *Cacnl1a4* voltage-sensitive calcium channel. Contemporaneously, mutations in human *CACNL1A4* were found in patients with episodic ataxia 2 (EA2) and familial hemiplegic migraine (FHM). Future studies are aimed at understanding the pathophysiology of these diseases. We chose *nr* mice for study because they have an unusual pathology that initially includes rounding and clumping of the mitochondria and a disruption in the organization of the ER and Golgi in cerebellar Purkinje neurons. Subsequently, *nr* Purkinje cells degenerate in a striped pattern that is the mirror image of the Purkinje cell degeneration found in *tg* mice carrying the *leaner* allele. Positional cloning experiments are currently under way to identify the gene encoded by the *nr* locus. We chose *mea* mice because they have a morphogenetic defect that appears to define a discrete compartment of the cerebellum that has not been previously defined by histological or functional criteria. Future studies are aimed at positionally cloning the *mea* gene product.

All of these studies were done in collaboration with Scott Brady, University of Texas Southwestern Medical Center; Jamie Cope and John Kendrick-Jones, Cambridge University; Wayne Frankel, The Jackson Laboratory; Richard Hawkes, University of Calgary; Mark Mooseker, Yale University; and Liane Russell, Oak Ridge National Laboratory.

Recent Publications:

Steingrimsson E, et al. *Development* 1998;125:4607–16.

Huang J–D, et al. *Genetics* 1998;148:1951–61.

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Wilson SM, et al. *Proc Natl Acad Sci USA* 2000;97:7933–8.

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Biography: *Dr. Sharan obtained his B.Sc. in botany and M.Sc. in genetics from Delhi University, Delhi. In 1994, he obtained his Ph.D. in genetics under Dr. Terry Magnuson at Case Western Reserve University. He then joined Dr. Allan Bradley's laboratory at Baylor College of Medicine as a Howard Hughes Medical Institute Associate. In 1998, Dr. Sharan established the Genetics of Cancer Susceptibility Group in the ABL–Basic Research Program. His group is involved in the functional analysis of tumor suppressor genes in mice. In 1999, Dr. Sharan joined the Center for Cancer Research, NCI.*

Mouse Cancer Genetics Program
Functional Analysis of Breast Cancer Susceptibility Genes in Mice

Keywords:
BAC engineering
Brca1
Brca2
breast cancer
gene targeting
mouse models
transgenic mice

Research: Breast cancer is the most common disease and leading cause of lethality in women. The identification and cloning of the *BRCA1* and *BRCA2* genes have demonstrated that mutations in these two genes account for almost all of the families with multiple cases of breast and ovarian cancer. To understand the biological function of these genes, mutations that result in loss of function have been generated in mice. These studies have demonstrated the involvement of these two genes in cell proliferation and normal development and also in the Rad51-mediated DNA recombination repair machinery. Mutation in the *Brca1* or *Brca2* genes may result in defects in the DNA repair/monitor system, causing an accumulation of mutations in growth control genes and leading to tumorigenesis.

One of the goals of our research is to conduct a functional dissection of the Brca1 and Brca2 protein and to determine other components of the pathway(s) in which these function. To achieve this, we are generating subtle mutations in regions of the gene encoding these two proteins to study their phenotypic effect. To generate these multiple alleles, we are using bacterial artificial chromosomes (BACs). We have identified BAC clones that contain the functional *Brca1* and *Brca2* gene, as they can rescue the embryonic lethality associated with mutation in these genes. We are using BACs with mutation in these genes to generate transgenic mice on a *Brca1* or *Brca2* mutant background. We are manipulating the BACs by using the bacteriophage recombination system. We have developed a rapid method to manipulate BACs to generate deletions, insertions, and single base changes

using oligonucleotides as targeting vectors obviating the use of selection markers. We have developed a PCR-based approach to identify the correctly targeted clone.

In human cancer patients, in addition to mutations that clearly disrupt the gene, several missense mutations have been identified in the *BRCA1* and *BRCA2* genes. The functional significance of these mutations is unknown. These may result in an unstable protein, alter a subset of its function, or represent a rare polymorphic variant. Our aim is to generate missense mutations in murine *Brca1* and *Brca2* genes and study their functional significance using the BAC transgenic approach. We have examined the possibility of using human genes in mice to generate missense mutations. We have generated humanized mice using human *BRCA1* as well as the *BRCA2* gene. These transgenic mice are able to rescue the embryonic lethality associated with mutation in the endogenous *Brca1* or *Brca2* genes. We are generating missense mutations in the human gene to examine their functional significance. Such mutations will serve as valuable tool for functional dissection of these genes.

Interaction between Brca2 and Rad51 proteins was identified in a yeast two-hybrid screen, which proved to be valuable in understanding the role of Brca2 as a tumor suppressor. We are using the yeast two-hybrid system to identify proteins that interact with various conserve domains of Brca2 protein. These will help us in understanding their functional significance.

Our collaborators include Peter Devilee, Riccardo Fodde, Peter Hohestein, and Margaret Zdzienicka, Leiden University, The Netherlands; Jodi Flaws, University of Maryland, Baltimore; and Mary Ann Handel, University of Tennessee, Knoxville.

Recent Publications:

Sharan SK, et al. *Nature* 1997;386:804–10.

Swaminathan S, et al. *Genesis* 2001;29:14–21.

Chandler J, et al. *Genesis* 2001;29:72–7.

Leasure CS, et al. *Gene* 2001;271:59–67.

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Biography: *Dr. Tessarollo received his Ph.D. in biological sciences from the University of Padua, Italy, in 1987. He carried out postdoctoral training in the laboratory of Dr. Chieco-Bianchi at the Institute of Oncology, Padua, and Dr. Parada at the ABL–Basic Research Program, NCI–Frederick. In 1994, Dr. Tessarollo established the Special Program in Germline Mutation and, in 1996, he formed the Neural Development Group. In 1997, while continuing his research effort, Dr. Tessarollo established a mouse gene targeting program for the National Cancer Institute. In 1999, Dr. Tessarollo joined the Center for Cancer Research, NCI.*

Mouse Cancer Genetics Program
Role of Neurotrophins and Their Receptors in Mammalian Development

Keywords:
animal models
gene targeting
growth factors
mouse development
neurotrophins
receptor tyrosine kinase
Trk receptors

Research: Neurotrophins are a highly homologous family of secreted growth factors that have been extensively studied for their roles in the proliferation, survival, and differentiation of various cell populations in the mammalian nervous system. The activities of these molecules have led to an increasing interest in their use as therapeutic agents for certain neurodegenerative diseases. More recently, neurotrophins have also been shown to exert a variety of pleiotropic responses on malignant cells, and the expression of their receptors in specific cancers (such as neuroblastomas and medulloblastomas) has been correlated to a specific patient prognosis.

Neurotrophin function is mediated by two types of receptors: the unique p75 neurotrophin receptor, a member of the tumor necrosis factor receptor family that binds all neurotrophins with similar affinity, and the tyrosine kinase receptors of the *Trk* gene family. Trk receptors (in addition to the well-studied full-length tyrosine kinase receptors) include several isoforms, some of which lack the kinase domain. However, there is very little or no information about the developmental functions of specific isoforms of Trk receptors. In vitro studies have provided clues to the mechanism of function of these receptor isoforms, but have proved insufficient in unraveling their role in development. Genetic ablation experiments in mice have confirmed the essential role of neurotrophins and Trk receptors in the development of specific classes of neurons of the peripheral nervous system. Recently, we have shown that they also contribute to the functional phenotype of subpopulations of neurons of the central nervous system, namely of the serotonergic system.

One focus of our laboratory is the trkC receptors and their ligand neurotrophin-3 (NT–3). Both the *trkC* and *NT–3* genes have unique features among their gene families. The *trkC* gene generates several receptors, some of which are unique among the *Trk* genes (e.g., isoforms with an insertion in the catalytic region). NT–3 is the only neurotrophin that binds all Trk receptors, although with different affinities. Over the past few years, we have generated

in vivo data supporting specific roles for trkC receptors. We have also provided evidence that, in addition to trkC, NT–3 also activates other Trk receptors during development. We are now dissecting neurotrophin functions in vivo by generating and analyzing murine models in which specific isoforms are deleted by gene-targeting technology. Furthermore, by introducing these targeted deletions with an inducible system, we hope to overcome the reduced life span of the current mouse models that precludes the analysis of neurotrophin function in adulthood and, consequently, the dissection of the full range of NT–3 effects in vivo.

Since the discovery of neurotrophins, their critical role in neuronal development has been meticulously dissected by many laboratories, yet the widespread expression of neurotrophins and their receptors in several nonneuronal systems has been virtually ignored. The severity of deficiencies in mice lacking specific neurotrophin functions suggests that activation of these signaling pathways is required in a variety of organs during ontogenesis. For example, our analysis of mice with mutations in trkC or NT–3 has unveiled their crucial role in the normal development of the mammalian heart and suggests that NT–3 and trkC function in the survival and/or migration of the cardiac neural crest (NC) early in embryogenesis. The NC cells contribute to the mesenchymal components of numerous organs in development. Thus, NT–3 and trkC may play a role in the development of several organs via their effects on migration and/or survival of NC cells. A comprehensive analysis of trkC isoform mouse models should provide more definitive answers on the role of NT–3 outside the nervous system and whether trkC isoforms have a role in differentiating the complex function of NC cells. The integration of information on neurotrophin activities from different organ systems, especially data concerning their maturation and function, should improve our understanding of the possibilities and risks associated with manipulating neurotrophin signaling for therapeutic purposes.

Our collaborators include Bernd Fritzsch, Creighton University, Omaha; Michael Gershon, Columbia University; and Jan Kucera, Boston University School of Medicine.

Recent Publications:

Lyons WE, et al. *Proc Natl Acad Sci USA* 1999;96:15239–44.

Palko ME, et al. *J Neurosci* 1999;19:775–82.

Maier DL, et al. *Proc Natl Acad Sci USA* 1999;96:9397–402.

Mamounas LA, et al. *J Neurosci* 2000;20:771–82.

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Jihan Shogen	Administrative Technician
Christine Spates	Administrative Officer
Mary Stinson	Purchasing Agent
Mary Weiermiller	Personnel Management Specialist
Jeaninne Wingire	Purchasing Agent

IT Administrative Resource Center Staff

Patrick Miller	ARC Manager
Pritee Agarwal	Administrative Officer
Julie Cummings	Comprehensive Administrative Assistant
Marie Hirsch	Administrative Officer
Pam Robbins	Procurement Program Specialist
Toni Shepherd	Administrative Technician
Carrie Watkins	Administrative Officer

Appendix A: Areas of Expertise by Investigator

Bioinformatics and Computational Biology

Burt, Stanley 821, 823
Dimitrov, Dimiter 500
Friedman, Fred 631
Guy, Robert 501
Jernigan, Robert 502
Kohn, Kurt 698
Lee, Byungkook 659
Lemkin, Peter 506
Maizel, Jacob 493, 497
Misteli, Tom 759
Schneider, Thomas 510
Shapiro, Bruce 511
Shaw, Stephen 98
Weinstein, John 700

Cancer Genetics, Genomics, and Proteomics

Aladjem, Mirit 693
Aplan, Peter 129
Arthur, Diane 710
Austin, Stuart 543
Basrai, Munira 131
Callahan, Robert 306
Chanock, Stephen 214
Cheng, Sheue-yann 647
Copeland, Neal 847, 851
Court, Donald 545
Davidson, Florence 370
Dean, Michael 571
DeClue, Jeffrey 468
Emmert-Buck, Michael 712
Felber, Barbara 344
Fornace, Albert 312
Gottesman, Michael 403, 407
Gottesman, Susan 651
Green, Jeffrey 425
Hager, Gordon 751, 753
Hamer, Dean 371
Hennings, Henry 453
Hickstein, Dennis 113
Hou, Steven 594
Jenkins, Nancy 853
Johnson, Peter 773
Kaldis, Philipp 775
Kaye, Frederic 133
Khan, Javed 218
Kirsch, Ilan 123, 127
Kleiner, David 717
Kraemer, Kenneth 318
Kuehl, W. Michael 134
Kuehn, Michael 89
Leach, Frederick 293
Lerman, Michael 596
Lewandoski, Mark 398
Libutti, Steven 273
Linehan, W. Marston 289, 291
Liotta, Lance 701, 707
Lipkowski, Stanley 136
Miller, Mark 519
Mock, Beverly 558
Mortin, Mark 376
Mushinski, Frederic 560
Neckers, Leonard 60
Nedospasov, Sergei 682
Nussenzweig, Andre 91
O'Brien, Stephen 563, 567
Popescu, Nicholas 521
Potter, Michael 553, 555
Raffeld, Mark 727
Ried, Thomas 137
Roberts, David 729
Sharan, Shyam 855
Smith, Michael 572
Staudt, Louis 195
Steeg, Patricia 731
Stewart, Colin 395, 397
Strathern, Jeffrey 537, 541
Tessarollo, Lino 857
Thorgeirsson, Snorri 515, 517
Vogel, Jonathan 76
Waalkes, Michael 490
Wang, Xin Wei 582
Weinstein, John 700
Winkler, Cheryl 575
Yamaguchi, Terry 400
Zbar, Berton 591, 593

Cell, Molecular, and Developmental Oncology

Acharya, Jairaj 769
Adhya, Sankar 645
Aladjem, Mirit 693
Ambudkar, Suresh 409
Anderson, Lucy 482
Anderson, Wayne 465
Aplan, Peter 129
Appella, Ettore 411
Baker, Carl 301
Bates, Susan 29
Beck, George 335
Birrer, Michael 44
Blair, Donald 339
Blumberg, Peter 448
Bonner, William 695
Brady, John 304
Bustin, Michael 630
Callahan, Robert 306
Chattoraj, Dhruba 368
Cheng, Sheue-yann 647
Cho-Chung, Yoon 308
Clark, Geoffrey 47
Colburn, Nancy 341
Cuttitta, Frank 49
Daar, Ira 770
Davidson, Florence 370
DeClue, Jeffrey 468
Dennis, Phillip 30
Dhar, Ravi 311
Duckett, Colin 187
Farrar, William 675
Fearnhead, Howard 772
Ferris, Douglas 346
FitzGerald, David 649

Cell, Molecular, and Developmental Oncology (continued)

Fojo, Tito 34
Fornace, Albert 312
Gardner, Kevin 713
Garfinkel, David 547
Garges, Susan 650
Glick, Adam 452
Gonzalez, Frank 625, 629
Gottesman, Michael 403, 407
Gottesman, Susan 651
Green, Jeffrey 425
Hatfield, Dolph 315
Hearing, Vincent 413
Hegde, Ramanujan 469
Helman, Lee 2, 205, 211
Henkart, Pierre 86
Hou, Steven 594
Jakowlew, Sonia 51
Janz, Siegfried 556
Jin, Ding Jun 653
Johnson, Alfred 655
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Kashlev, Mikhail 549
Keller, Jonathan 677
Kelly, Kathleen 39, 43
Khan, Javed 218
Kim, Seong-Jin 427
Kimura, Shioko 633
Klar, Amar 551
Kohn, Elise 719
Kohn, Kurt 698
Krishna, M.C. 238
Kuehn, Michael 89
Lee, Kyung 634
Letterio, John 429
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Lichten, Michael 373
Linnekin, Diana 319
Linnoila, Ilona 55
Lipkowski, Stanley 136
Liu, Zheng-gang 57
Lowy, Douglas 2, 297, 461, 463
Mackem, Susan 723
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Merlino, Glenn 661
Miki, Toru 321
Milne, Jacqueline 416
Misteli, Tom 759
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Morrison, Deborah 776
Mortin, Mark 376
Mushinski, Frederic 560
Neckers, Leonard 60
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Parent, Carole 437
Paterson, Bruce 378
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Roberts, Anita 421, 423
Roberts, David 729
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Sharrow, Susan 96
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Vogel, Jonathan 76
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Wang, Xin Wei 582
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Wickner, Susan 663
Wolff, Linda 475
Wu, Carl 665, 667
Xia, Di 418
Yamaguchi, Terry 400
Yarmolinsky, Michael 383
Yeh, Grace 359
Yuspa, Stuart 3, 443, 447
Zhang, Ying 441

Chemistry and Structural Biology

Ambudkar, Suresh 409
Bai, Yawen 366
Blumberg, Peter 448
Boyd, Michael 845
Brechbiel, Martin 251
Burke, Terrence 616
Burt, Stanley 821, 823
Byrd, Andrew 785, 789
Dauter, Zbigniew 603
Friedman, Fred 631
Garges, Susan 650
Guy, Robert 501
Jernigan, Robert 502
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Kelley, James 618
Klee, Claude 361, 365
Le Grice, Stuart 829, 831
Leuba, Sanford 755
Marquez, Victor 611, 613
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Michejda, Christopher 791
Milne, Jacqueline 416
Moschel, Robert 486
Qasba, Pradman 508
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Roller, Peter 622
Smith-Gill, Sandra 357
Stetler-Stevenson, William 735
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Chemistry and Structural Biology (continued)

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Wink, David 243
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Clinical and Translational Oncology

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Restifo, Nicholas 276
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Topalian, Suzanne 282
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Turner, Maria Chanco 75
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Wayne, Alan 228
Wigginton, Jon 230
Wilson, Wyndham 119
Yang, James 284
Yarchoan, Robert 143, 147
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Epidemiology, Carcinogenesis, and Cancer Prevention

Anderson, Lucy 482
Baker, Carl 301
Barrett, J. Carl 1, 385, 387
Blumberg, Peter 448
Colburn, Nancy 341
Dawsey, Sanford 19
De Luca, Luigi 450
Dean, Michael 571
Forman, Michele 20
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Hearing, Vincent 413
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Larionov, Vladimir 391
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Moschel, Robert 486
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HIV and Tumor Virology

Arthur, Larry 807, 811
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Benveniste, Raoul 337
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Blauvelt, Andrew 69
Blumenthal, Robert 498
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Carrington, Mary 569
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HIV and Tumor Virology (continued)

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Henderson, Louis 814
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Immunology

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Anderson, Stephen 528
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Wiltrout, Robert 2, 331, 532
Wood, Lauren 152
Yang, James 284
Yoshimura, Teizo 685
Young, Howard 534

Appendix B:
NCI Faculties and Faculty Web Sites

Cellular, Molecular, and Developmental Biology
celmoldev-l@list.nih.gov

HIV and Virology
hivvirol-l@list.nih.gov

Immunology
immuno-l@list.nih.gov

Genetics, Genomics, and Proteomics
genprot-l@list.nih.gov

Bioinformatics, Biostatistics, and Computational Biology
bioinfcomp-l@list.nih.gov

Chemistry and Structural Biology
chemstruct-l@list.nih.gov

Epidemiology, Carcinogenesis, and Prevention
epicarprev-l@list.nih.gov

Molecular Targets
moltarg-l@list.nih.gov

Breast Cancer
breastca-l@list.nih.gov

Gynecologic Malignancies
gynonco-l@list.nih.gov

Genitourinary Malignancies
genimalig-l@list.nih.gov

Appendix C: Core Facilities and Resources

Centralized scientific resources have been established to give NCI investigators access to new technologies, special expertise, and additional technical support in fields such as imaging, animal sciences, analytical/protein chemistry, genetics/genomics, and structural biology. Cores—located at the Bethesda campus, NCI-Frederick and at the Applied Technology Center, are designed to support, complement, and advance the Institute's research efforts. Each Core is listed under the discipline or technology that it supports. Cores are listed on the CCR web site at: **http://ccr.cancer.gov/research/core_facilities.asp**. General inquiries about the NCI/SAIC Research Technology Program (RTP, **http://web.ncifcrf.gov/rtp**) at NCI-Frederick should be directed to Dr. David Goldstein, goldsted@mail.ncifcrf.gov.

Analytical Chemistry

Separation Technologies Group, NCI/SAIC RTP

Haleem Issaq, Ph.D., Head
issaqh@mail.ncifcrf.gov

Gas chromatography, HPLC, capillary electrophoresis, mass spectrometry, sample preparation, methods development and validation

Animal Resources

Germline Mutation Core Facility, CCR, NCI
http://rex.nci.nih.gov/gmcf/

Liya Shen, Ph.D., Manager
shenl@mail.nih.gov

Production of genetically altered mice, including R&D focusing on new techniques

Laboratory Animal Sciences Program, NCI/SAIC RTP
http://web.ncifcrf.gov/rtp/LASP.asp

Hendrick Bedigian, Ph.D., Director
bedigianh@ncifcrf.gov

Animal Models Services: Scientific support for animal-based research studies, including:

- Histotechnology
- Pathology
- Transgenic mouse models services
- Knockout mouse services
- Mouse models of human cancers consortium (MMHCC) repository: **http://web.ncifcrf.gov/researchresources/mmhcc/**
- Cryopreservation and assisted breeding program

Animal Resources (continued)

Animal Technical Services: Animal care, housing, and health-related services, including:

Animal holding/technical support
Animal health diagnostic lab
Laboratory animal medicine
Receiving and quarantine

Molecular Mouse Pathology Unit

Mark Simpson, D.V.M., Ph.D.
ms43b@nih.gov

Collaborative-based research support in comparative molecular pathology

Office of Mice Advice

Susan Silk, Ph.D.
silks@mail.nih.gov

General assistance with protocol development, mouse genetic counseling, and training

Veterinary and Tumor Pathology Section, CCR, NCI
http://www.ncifcrf.gov/vetpath/index.html

Jerrold Ward, D.V.M., Ph.D., Head
ward@mail.ncifcrf.gov

Pathology expertise in the design, conduct, and interpretation of animal-based studies

Bioinformatics

Advanced Biomedical Computing Center, NCI/SAIC
http://www-fbsc.ncifcrf.gov/

Stan Burt, Ph.D., Director
burt@ncifcrf.gov

High-performance supercomputing support for biological research problems

Biostatistics and Data Management Section, CCR, NCI

Seth Steinberg, Ph.D., Head
steinbes@mail.nih.gov

Statistical design, review, and analysis of clinical trials focused on treating cancer, AIDS, and AIDS malignancies

Genome Analysis Unit, CCR, NCI

Peter Fitzgerald, Ph.D., Head
pf4q@nih.gov

Bioinformatics support for genomic database analysis

Clinical Trials Support

Clinical Services Program, NCI/SAIC
http://www.ncifcrf.gov/fcrdc/home/csp/index.html

Michael Baseler, Ph.D., Director
mbaseler@nih.gov

Sequential studies of immune function in diseases such as cancer and AIDS during treatment with anticancer or antiviral agents

Flow Cytometry

EIB Flow Cytometry Facility
http://rex.nci.nih.gov/RESEARCH/basic/eib/sharrow.htm

Susan Sharrow, B.Sc., Manager
ss71k@nih.gov

Flow cytometry support, applications, and resource development for immunological research

ETIB Flow Cytometry Core Laboratory, CCR, NCI
http://dbs.nci.nih.gov/branches/medicine/flowcore/welcome.htm

Bill Telford, Ph.D., Manager
telfordw@box-t.nih.gov

Research flow and image cytometry services, R&D focusing on developing new techniques

Flow Cytometry Core, NCI/SAIC

Kathleen Noer, Manager
noer@ncifcrf.gov

Flow Cytometry Core, CCR, NCI

Barbara Taylor, Manager
taylorba@pop.nci.nih.gov

Genetics/Genomics and DNA Sequencing

High-Throughput Genome Analysis Center, LPG–ATC, NCI

Victor Llaca, Ph.D., Director
llacav@mail.nih.gov

High-throughput sequencing and genotyping; development of new genomic analysis methods and applications

DNA Sequencing MiniCore Facility, CCR, NCI
http://neoplasia.nci.nih.gov/dna/index.html

Mark Miller, Ph.D., Manager
mark@mail.nih.gov

Rapid, accurate sequencing of DNA samples; analysis software; consulting services

Genetics/Genomics and DNA Sequencing (continued)

Genome Analysis Unit, CCR, NCI

Peter Fitzgerald, Ph.D., Head
pf4q@nih.gov

Computational molecular biology and bioinformatics support for genomic database analysis

Laboratory of Molecular Technology, NCI/SAIC RTP

David Munroe, Ph.D., Head
mtlseq@ncifcrf.gov

Standard and high-throughput gene sequencing, molecular diagnostics, genomics/proteomics, microarrays, and bioinformatics

Comparative Molecular Cytogenetic Core, CCR, NCI

Buddy Chen, Manager
buddyc@mail.nih.gov

Spectral karyotyping (SKY), FISH, comparative genomic hybridization (CGH)

Imaging/Microscopy

Confocal Microscopy Core Facility, CCR, NCI
http://neoplasia.nci.nih.gov/confocal/index.html

Susan Garfield, M.S., Manager
susan_garfield@nih.gov

Laser scanning confocal microscopy for fixed specimens and live cells

Fluorescence Imaging Core Facility, CCR, NCI
http://rex.nci.nih.gov/RESEARCH/basic/lrbge/imaging.html

James McNally, Ph.D., Imaging Director
mcnallyj@exchange.nih.gov

3D confocal, deconvolution, FISH, and other techniques; image data analysis; consultation services; seminars and tutorials

Image Analysis Laboratory, NCI/SAIC RTP
http://web.ncifcrf.gov/rtp/labs/IAL/ial.asp

Stephen Lockett, Ph.D., Head
slockett@ncifcrf.gov

3D confocal and electron microscopy; computational resources, development of new imaging technologies

Laser Capture Microdissection Core Lab, NIH
http://dir.nichd.nih.gov/lcm/lcm.htm

Lance Liotta, M.D., Ph.D., Manager
lance@helix.nih.gov

DNA, RNA, or protein can be procured from selected pure cells to analyze normal cell function and development as well as disease progression

Microarrays

ATC Microarray Facility, NCI

Ernest Kawasaki, Ph.D., Head
nciarrays-r@mail.nih.gov

Laboratory of Molecular Technology, NCI/SAIC RTP

David Munroe, Ph.D., Head
mtlseq@ncifcrf.gov

Microarrays, molecular diagnostics, bioinformatics, genomics/proteomics

Tissue Array Research Project, NHGRI, NIH
http://www.nhgri.nih.gov/DIR/CGB/TMA/

David Kleiner, M.D., Director
dkleiner@helix.nih.gov

Multitumor tissue microarrays for high-throughput molecular profiling of tumor tissues

Protein Chemistry and Biophysics

Protein Chemistry Core, NCI, CCR

Terry Copeland, Ph.D., Manager
copelandt@ncifcrf.gov

Protein Chemistry Laboratory, NCI/SAIC RTP

Robert Fisher, Ph.D., Head
fisher@ncifcrf.gov

MALDI-TOF MS, amino acid microsequencing, HPLC, BIA core analysis of macromolecular interactions, molecular mechanism-based screens for small molecule inhibitors

Biophysics Core, NCI, CCR

Sergey Tarasov, Ph.D., Manager
tarasovs@ncifcrf.gov

Protein Expression/Purification

Protein Expression Laboratory, NCI/SAIC RTP
http://web.ncifcrf.gov/rtp/PEL.asp

Michael Sveda, Ph.D., Acting Head
mimsved@ncifcrf.gov

Vector engineering and recombinant protein expression, large-scale production of recombinant proteins, protein purification

Protein Purification Core, CCR, NCI

Joseph Tropea, Ph.D., Manager
jtropea@ncifcrf.gov

Publications Support

Publications Department, NCI/SAIC RTP
http://web.ncifcrf.gov/campus/publications/

Ken Michaels, Manager
kvm@ncifcrf.gov

Scientific illustration, Web page design/graphics, editing, photography, desktop publishing/word processing, reprographics

Repository Services

Central Repository at NCI-Frederick, NCI/SAIC RTP
http://web.ncifcrf.gov/rtp/repos-serv.stm

Carmen Anderson, Acting Manager
canderson@ncifcrf.gov

Cryogenic storage, inventory, and distribution of research samples

Structural Biology

Nuclear Magnetic Resonance (NMR) Group, NCI/SAIC RTP
http://web.ncifcrf.gov/rtp/labs/ACL/nmr/default.asp

Gwen Chmurny, Ph.D., Head
chmurny@ncifcrf.gov

1D, 2D, and 3D NMR detection methods; computer-assisted analysis; novel structure building/searching capabilities

Nuclear Magnetic Resonance (NMR) Facility, CCR, NCI

Joseph Barchi, Jr., Ph.D., Head
barchi@helix.nih.gov

Synthetic organic chemistry related to carbohydrate-based drug design; high-resolution NMR analysis of sugars, peptides, and glycopeptides

Appendix D:
2002 Intramural Research Award and Collaborative Project Award Recipients NCI Center for Cancer Research

Wei-Shau Hu, Ph.D.
HIV Drug Resistance Program
Dynamics of HIV–1 Double Infection In Vivo

Javed Khan, Ph.D.
Pediatric Oncology Branch
Development of Comprehensive Tools for the Analysis of Gene Expression Data

Elise Kohn, M.D.
Laboratory of Pathology
Intact Ovarian Cancer NER Pathway Predicts Sensitivity to ET743

Murali Krishna, Ph.D.
Radiation Biology Branch
Multi-Modality Small Animal Image Fusion

Jacek Lubkowski, Ph.D.
Macromolecular Crystallography Laboratory
Structure-Function Relationship for Human Beta-Defensins and MIP3 Alpha

Thomas Misteli, Ph.D.
Laboratory of Receptor Biology and Gene Expression
Heterochromatin Formation and Maintenance: An In Vivo Analysis

Vinay Pathak, Ph.D.
HIV Drug Resistance Program
Development of HIV–1 Integrase Inhibitors as Novel Antiretroviral Drugs

William Stetler-Stevenson, M.D., Ph.D.
Laboratory of Pathology
Development of a Directed, In Vivo Angiogenesis Assay

Berton Zbar, M.D.
Laboratory of Immunobiology
A Mouse Model of VHL–Mediated Renal Cell Carcinoma

Appendix E:
National Cancer Advisory Board
Board of Scientific Counselors–A

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Corixa Corporation

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Stanford University School of Medicine

Deborah Collyar
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The Johns Hopkins University School of Medicine

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Washington University School of Medicine

Elizabeth Fontham, Dr.P.H.
Louisiana State University Medical Center

Stanley R. Hamilton
University of Texas

Elizabeth A. Holly, Ph.D., M.P.H.
University of California at San Francisco

David J. Hunter, M.B.B.S., Sc.D.
Harvard School of Public Health

Michael B. Kastan, Ph.D., M.D.
St. Jude Children's Research Hospital

Laurence N. Kolonel, M.D., Ph.D.
University of Hawaii

Frank McCormick, Ph.D.
University of California at San Francisco

Daniel Medina, Ph.D.
Baylor College of Medicine

Beverly Mitchell, M.D.
University of North Carolina at Chapel Hill

James J. Mule, Ph.D.
University of Michigan

Richard J. O'Reilly, M.D.
Memorial Sloan-Kettering Cancer Center

Olufunmilayo Olopade, M.B.B.S., F.A.C.P.
University of Chicago Medical Center

Alice P. Pentland, M.D.
University of Rochester School of Medicine and Dentistry

Arthur T. Porter, M.D.
Detroit Medical Center

David A. Savitz, Ph.D.
University of North Carolina

Steven Self, Ph.D.
Fred Hutchinson Cancer Research Center

Margaret Ann Tempero, M.D.
University of California at San Francisco

Michael Thun, M.S., M.D.
American Cancer Society

Executive Secretary
Abby Sandler, Ph.D.

Appendix F:
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Cornell University

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University of Pennsylvania School of Medicine

E. Peter Geiduschek, Ph.D.
University of California at San Diego

Sankar Ghosh, Ph.D.
Yale University School of Medicine

Stephen P. Goff, Ph.D.
Columbia University

Beatrice H. Hahn, M.D.
University of Alabama at Birmingham

Nouria Hernandez, Ph.D.
Cold Spring Harbor Laboratory

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A phase II study to evaluate radiofrequency ablation of renal cancer. 295

Laboratory of Pathology

2CDA for gliomas. 739

Epidemiology, infectivity, and natural history of hepatitis C virus infection in a blood donor program. 739

A phase I protocol for the evaluation of the safety and immunogenicity of vaccination with synthetic HIV envelope peptides in patients with early human immunodeficiency virus infection. Open for accrual. 739

GW/ROB BI collaborative efforts. 739

HU/PX for GBM. 739

Gemcitabine for pancreas. 739

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Stereotactic radiosurgery. 739

Treatment planning. 739

A phase I study of the combination of CAI and paclitaxel in adult patients with refractory cancers or lymphoma: This clinical trial has shown safety of combining pulse CAI with 3 weekly paclitaxel and is now testing daily CAI with 3 weekly paclitaxel. 739

A multi-institutional phase II study of cyclophosphamide, paclitaxel, cisplatin with G–CSF for patients with newly diagnosed advanced stage ovarian cancer: Molecular evidence of the supra-additive effects of the combination of cisplatin, cyclophosphamide, and paclitaxel has led to this phase II study for the treatment of newly diagnosed epithelial ovarian cancer. Molecular diagnostic questions related to ovarian cancer progression are posed in the phase II study and are under way. 739

A phase II time-to-progression study of orally administered CAI to patients with persistent epithelial ovarian cancer: This protocol follows our evaluation of CAI and asks whether the agent might provide disease stabilization to patients with advanced ovarian cancer. Translational endpoints include assessment of a CAI resistance-associated gene and markers of angiogenesis. 739

A pilot study of proteomic evaluation of epithelial ovarian cancer patients in first clinical remission. Development of a protein fingerprint profile associated with relapse: This protocol enrolls women in first clinical response to treatment for ovarian cancer and follows them serially until relapse is diagnosed. Advanced proteomic technologies are being applied to serum samples to identify proteomic patterns that are predictive of recurrent disease. This is in concert with similar work ongoing in study sets of sera from newly diagnosed women. 739

Collaborative Clinical Trials:

A randomized phase III trial of IV carboplatin (AUC 6) and paclitaxel 175 mg/m2 q 21 days x 3 courses plus low-dose paclitaxel 40 mg/m2/wk versus IV carboplatin (AUC 6) and paclitaxel 175mg/m2 q 21 days x 3 courses plus observation in patients with early stage ovarian carcinoma. 740

A phase II trial of ZD1839 (Iressa™) (NSC 715055) in the treatment of persistent or recurrent epithelial ovarian or primary peritoneal carcinoma. 740

A phase I study of Taxol, cisplatin, cyclophosphamide, and granulocyte colony-stimulating factor (G–CSF) in previously nontreated ovarian cancer patients. 740

A phase I study of the combination of CAI and paclitaxel in adult patients with refractory cancers or lymphoma. 740

A multi-institutional phase II study of cyclophosphamide, paclitaxel, cisplatin with G–CSF for patients with newly diagnosed advanced stage ovarian cancer. 740

A phase II study of continuous-infusion aminocamptothecin for advanced epithelial ovarian cancer recurrent after paclitaxel and either cisplatin or carboplatin. 740

Tissue acquisition for molecular diagnostics. 740

A phase II clinical trial of orally administered CAI for patients with advanced, relapsed, or refractory epithelial ovarian cancer. 740

A pilot study of proteomic evaluation of epithelial ovarian cancer patients in first clinical remission: development of a protein fingerprint profile associated with relapse. 740

A pilot study of tumor-specific peptide vaccination and IL–2 with or without autologous cell transplantation in recurrent pediatric sarcomas. 740

A pilot study of autologous cell transplantation with vaccine-driven expansion of antitumor effectors after cytoreductive therapy in metastatic pediatric sarcomas. 740

Laboratory of Tumor Immunology and Biology

CEA-Based Vaccines

H. Kaufman, principal investigator, A. Einstein Cancer Center. 802

M. von Mehren, principal investigator, Fox Chase Cancer Center: Phase I studies of the use of avipox-CEA/B7–1 dual gene recombinants in patients with advanced CEA-expressing carcinomas. These trials are designed to evaluate the safety and efficacy of a dual transgene vaccine to induce CEA-specific cell responses in patients with advanced carcinoma. Immune responses of all patients are evaluated both pre- and postvaccination. 802

Phase I studies of peptide-based vaccines in patients with advanced CEA-expressing carcinomas:

M. Hamilton and P. Arlen, principal investigators, NCI Medicine Branch: CAP–1 peptide in Ribi adjuvant. 802

D. Cole, principal investigator, Medical College of South Carolina: rV–CEA prime followed by CAP–1 peptide boost. 802

K. Lyerly, principal investigator, Duke Comprehensive Cancer Center: CAP–1 peptide-pulsed autologous dendritic cells. These studies are designed to determine the safety and immunogenicity of an immunodominant CEA peptide, delivered via different vaccine modalities, in patients with advanced carcinoma. Immune responses of all patients are evaluated both pre- and postvaccination. 802

J. Marshall, principal investigator, Georgetown University Cancer Center: The role of diversified vaccination and cytokines in patients with advanced CEA-expressing carcinomas. This is a randomized phase I/II trial to determine the safety and validity of the use of diversified vaccination protocols in patients with advanced cancer. It is designed to compare immune responses of patients randomized to one of two different vaccination protocols in which patients receive either recombinant vaccinia CEA (V) followed by boosts with avipox CEA (A) (i.e., VAAA) or the reciprocal AAAV regimen. Following evaluation of those results, additional cohorts of patients will receive either GM–CSF along with vaccinations or GM–CSF and low-dose IL–2. Immune responses of all patients are evaluated both pre- and postvaccination. 802

J. Marshall, principal investigator, Georgetown University Cancer Center: Phase I study of CEA/TRICOM vaccines in patients with advanced CEA-expressing carcinomas. This trial incorporates several principles learned from previous clinical trials and preclinical studies and is the first clinical trial to evaluate the safety and efficacy of vectors containing CEA along with three different costimulatory molecules (B7–1, ICAM–1, and LFA–3—i.e., TRICOM). Moreover, the vector contains the entire CEA gene containing an agonist (6D) epitope. The first cohorts will receive different doses of avipox-CEA(6D)/TRICOM (designated A). The second cohorts will receive a primary vaccination with different doses of rV–CEA(6D)/TRICOM (designated V) followed by boosts with avipox-CEA(6D)/TRICOM. The third cohorts will receive the VAAA regimen accompanied with local GM–CSF administration. Immune responses of all patients are evaluated both pre- and postvaccination. 802

MUC–1 Vaccine Studies

J.P. Eder and D. Kufe, principal investigators, Dana-Farber Cancer Center: Phase I study of rV–MUC–1 vaccine in patients with metastatic breast carcinoma. This is a phase I dose escalation trial in which patients with metastatic breast cancer are being administered rV–MUC–1 vaccine, which will be evaluated for safety and the ability to mount cell responses to MUC–1. Immune responses of all patients are evaluated both pre- and postvaccination. 803

Prostate Vaccine Program

J.P. Eder and D. Kufe, principal investigators, Dana-Farber Cancer Center; M. Sanda, principal investigator, University of Michigan: Phase I studies of the vaccination of prostate cancer patients with rV–PSA vaccine. These studies were designed to evaluate the safety of PSA-based vaccines and the ability of patients with prostate cancer to mount PSA-specific immune responses. Immune responses of all patients are evaluated both pre- and postvaccination. 803

J.P. Eder and D. Kufe, principal investigators, Dana-Farber Cancer Center: A phase II trial of diversified prime and boost vaccination using rV–PSA and avipox-PSA vaccines. This is a phase II trial in patients who had a previously resected or irradiated prostate cancer and now have rising serum PSA. The two vaccine regimens consist of patients receiving either two vaccinations of vaccinia PSA(V) followed by three vaccinations with avipox-PSA(A) (VVAAA) or the reciprocal AAAVV protocol. Immune responses of all patients are evaluated both pre- and postvaccination. 803

H. Kaufman, principal investigator, A. Einstein Cancer Center: A multicenter (six centers) randomized ECOG trial employing diversified vaccination of rV–PSA and avipox-PSA. This is a phase II trial in patients who had a previously resected or irradiated prostate cancer and now have rising serum PSA. Patients are randomized into one of two cohorts to receive either rV–PSA(V) followed by three vaccinations with avipox-PSA(A) (VAAA) or the reciprocal AAAV regimen. Immune responses of all patients are evaluated both pre- and postvaccination. 803

P. Arlen, principal investigator; W. Dahut and J. Gulley, associate investigators, NCI Navy Oncology Clinic: Vaccine versus second line hormonal therapy: A randomized phase II study of either immunotherapy with a regimen of recombinant poxviruses that express PSA/B7–1 plus adjuvant GM–CSF and IL–2, or hormone treatment with nilutamide, in patients with hormone refractory prostate cancer and no radiographic evidence of disease. Prostate cancer patients are being randomized into two arms: arm 1 receives vaccine; arm 2 receives hormonal therapy (nilutamide). Patients can cross over if their serum PSA is rising at 6 months. The vaccine employed is a priming vaccination with rV–PSA admixed with rV–B7–1 followed by booster vaccinations with avipox-PSA. All vaccinations will be given with rGM–CSF and followed by low-dose IL–2. This (and the trial described below) are the first vaccine trials to employ the concept of admixing pox vectors, and the first clinical trials to use costimulation in a prostate cancer vaccine. Immune responses of all patients are evaluated both pre- and postvaccination. 803

W. Dahut, principal investigator; J. Gulley, protocol chairperson; P. Arlen, associate investigator, NCI Navy Oncology Clinic: Vaccine with local radiotherapy: A randomized phase II study of a PSA-based vaccine in patients with localized prostate cancer receiving standard radiotherapy. This is a randomized trial for patients with localized prostate cancer. In arm 1, patients will receive radiotherapy only; in arm 2, patients will receive radiotherapy plus vaccine. The vaccine regimen is identical to that described above (i.e., primary vaccination with rV–PSA admixed with rV–B7–1 followed by booster vaccinations with avipox-PSA; all vaccinations will be given with rGM–CSF and followed by low-dose IL–2). Immune responses of all patients are evaluated both pre- and postvaccination. 804

Intratumoral Vaccine Studies (Vector-Based Costimulation and Cytokine Studies)

H. Kaufman, principal investigator, A. Einstein Cancer Center: A phase I study to determine the maximum tolerated dose for intralesional injections of rV–B7–1 in the treatment of malignant melanoma. This is a phase I trial in which increasing doses of rV–B7–1 are being injected directly into melanoma lesions. Immune responses of all patients are evaluated both pre- and postvaccination. 804

R. Freedman, principal investigator, M.D. Anderson: A phase I study of intraperitoneal therapeutic tumor vaccine consisting of autologous tumor cells infected with avipox-B7–1, plus interferon. Autologous ovarian cancer cells are infected with avipox-B7–1 and x-irradiated. Prior to x-irradiation, tumor cells are also treated with interferon to upregulate MHC Class I and putative AAs. The vaccine is administered intraperitoneally to patients with advanced ovarian cancer. Immune responses of all patients are evaluated both pre- and postvaccination. 804

E. Kass/C. Van Waes, principal investigators, National Institutes of Deafness and Communicative Disorders (NIDCD), NIH: This is a phase I dose escalation study in which avipox TRICOM will be administered intratumorally to patients with advanced head and neck carcinoma. Immune responses of all patients are evaluated both pre- and postvaccination. 804

Back Cover: *Center: Atomic model for pyruvate dehydrogenase, a molecular machine essential for energy production in cells.*

Jacqueline Milne, Laboratory of Cell Biology; and Sriram Subramaniam, Laboratory of Biochemistry

Left: Molecular interaction map of the control of protein kinase Src, a bistable regulatory system with intra- and intermolecular interactions at the plasma membrane. Mol Biol Cell *1999;10:2703;* CHAOS *2001;11:84; and* **http://discover.nci.nih.gov/kohnk/interaction_maps.html.**

Kurt Kohn, Laboratory of Molecular Pharmacology

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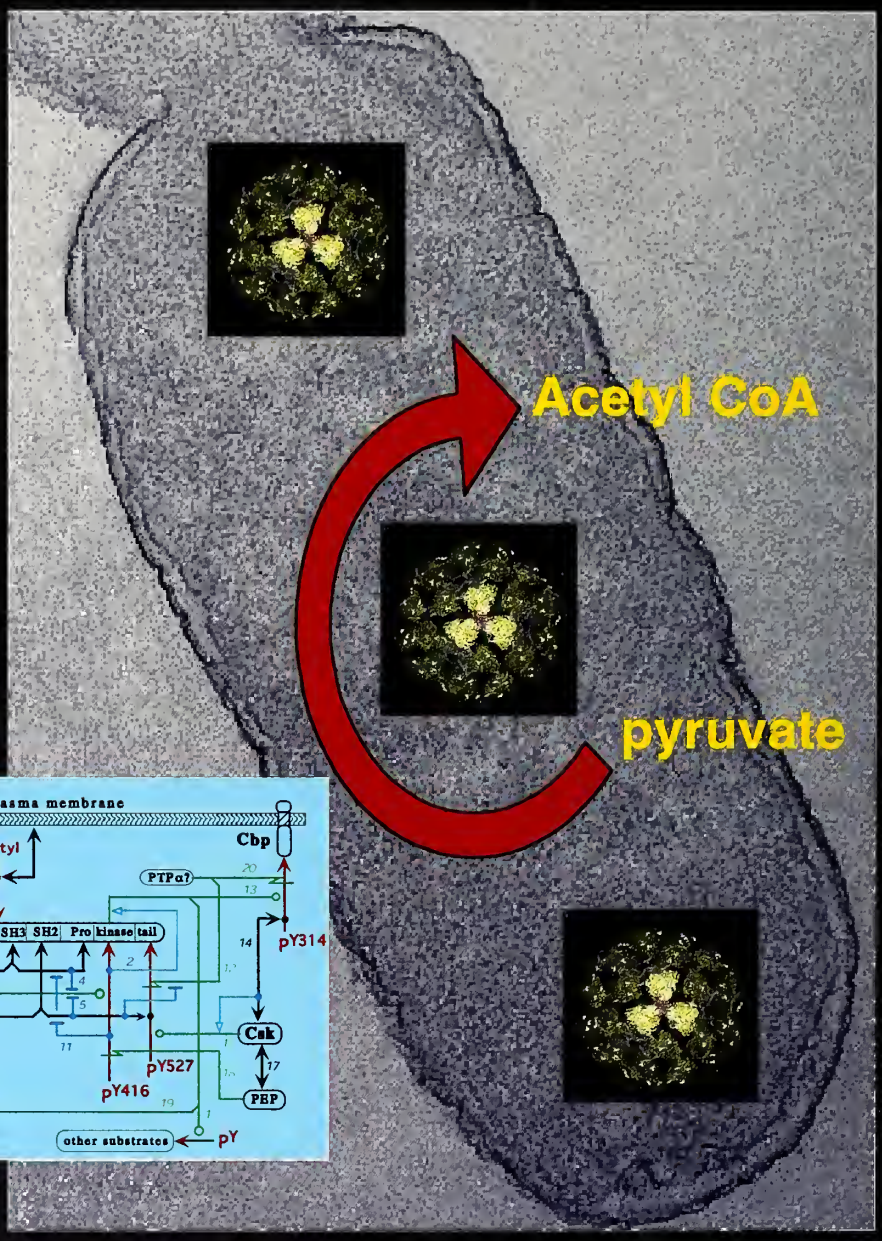

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