ANNUAL REPORT

DIVISION OF CANCER ETIOLOGY
NATIONAL CANCER INSTITUTE

October 1, 1986 through September 30, 1987

TABLE OF CONTENTS

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP03509-24 OD

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Carcinogenesis, Chemotherapy and Biological Markers in Nonhuman Primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. M. Sieber	Deputy Director	OD, DCE NCI
Others:	R. J. Parker	Expert	OD, DCE NCI

COOPERATING UNITS *(if any)*: Department of Pathology, Louisiana State University, New Orleans, LA (P. Correa); Hazleton Laboratories America, Inc., Vienna, VA (D. Dalgard)

LAB/BRANCH: Division of Cancer Etiology

SECTION: Office of the Director

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	1.5	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A wide variety of substances, including antitumor and antineoplastic agents; food additives, food components and environmental contaminants; "model" rodent carcinogens; and nitroso- compounds have been or are being evaluated in four species of nonhuman primates for their potential carcinogenicity and other long-term toxic effects. Of the 29 test compounds, 16 have not as yet demonstrated carcinogenic activity, although some have been on test for less than 4 years. Nine of the compounds are carcinogenic in nonhuman primates, producing tumors in 10-100% of the treated animals. 1-Methyl-1-nitrosourea (MNU) induced squamous cell carcinomas of the oropharynx and esophagus, with the esophageal tumors possessing clinical and morphologic similarities to human esophageal carcinoma. Long-term treatment with procarbazine produced malignant neoplasms, one-half of which were acute nonlymphocytic leukemia. The effects of seven of the compounds (diethylnitrosamine [DENA], dipropyl-nitrosamine [DPNA], 1-nitrosopiperidine, aflatoxin B-1, MAM-acetate, urethane and sterigmatocystin) were manifested primarily as hepatocarcinogenicity. Single cases of malignant tumors have been diagnosed in animals treated with adriamycin (acute myeloblastic leukemia), butter yellow (bronchioalveolar carcinoma), cyclophosphamide (transitional cell carcinoma of the urinary bladder), and 3-methyl-DAB (hepatocellular carcinoma).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04548-15 OD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Registry of Experimental Cancers/WHO Collab. Ctr. for Tumours of Lab Animals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Harold L. Stewart	Scientist Emeritus	DCE	NCI
Others:	Bernard Sass	Veterinary Medical Officer	DCE	NCI
	Margaret K. Deringer	Guest Researcher	DCE	NCI
	Carel F. Hollander	Guest Researcher	DCE	NCI
	Annabel G. Liebelt	Guest Researcher	DCE	NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Office of the Director

SECTION

INSTITUTE AND LOCATION

NIH, NCI, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	2.5	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The objectives of the Registry of Experimental Cancers are the storage and retrieval of pathologic material and data on cancers and other lesions of laboratory animals (primarily rodents) and the use of such information for research and educational purposes. The Registry has acquired a total of 4,137 (792 since the 1986 report) single or group accessions from investigators outside the NCI and approximately 64,330 records have been coded. Thirty investigators have come to the Registry for study and consultation on single or multiple visits.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP06134-12 OD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Lymphatic System in the Absorption and Distribution of Antitumor Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. M. Sieber	Deputy Director	OD, DCE	NCI
Others:	R. J. Parker	Expert	OD, DCE	NCI
	J. N. Weinstein	Senior Investigator	LMB	NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Division of Cancer Etiology

SECTION
Office of the Director

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of the lymphatic system in the absorption and biodistribution of antitumor agents and monoclonal antibodies is under investigation. Antitumor agents are delivered at high concentration to lymphatics and regional lymph nodes when entrapped in liposomes. Similarly, monoclonal antibodies given subcutaneously are delivered with high efficiency to regional lymph nodes where they bind specifically to lymphoid cells. Extensive metabolic and pharmacokinetic studies of antibodies directed against both normal and malignant cell types have been carried out in rodents. The pharmacological principles that have emerged from studies in rodents have been applied to the design of clinical protocols for the detection of malignant melanoma and T-cell lymphoma. In addition to studies on lymphatic malignancies, the carrier systems developed for selective delivery of antitumor agents and monoclonal antibodies to the lymphatics are being applied to therapy of human immunodeficiency virus (HIV)-induced acquired immunodeficiency syndrome. Initial studies indicate that liposome-entrapped dideoxycytidine triphosphate (ddCTP), a compound which blocks viral replication by inhibition of viral reverse transcriptase, is more effective in killing T-cells infected with HIV than is free ddCTP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04930-16 LCMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biology of Natural and Induced Neoplasia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. Arnstein	Veterinary Director	LCMB	NCI
Others:	S. A. Aaronson	Chief	LCMB	NCI
	J. S. Rhim	Research Microbiologist	LCMB	NCI
	K. C. Robbins	Chief, Mol. Genetics Section	LCMB	NCI
	J. Pierce	Research Microbiologist	LCMB	NCI
	A. Eva	Visiting Scientist	LCMB	NCI
	W. Taylor	Research Biologist	LCMB	NCI

COOPERATING UNITS *(if any)*
J. Riggs and R. Emmons, CA Dept. Health Services, Berkeley, CA; A. Hackett, Peralta Cancer Inst.; M. Gardner, J. Levy, H. Rubin and M. Stampfer, U. CA, San Francisco; and K. Walen, Children's Hospital Medical Ctr., San Francisco.

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Collaborative studies on the oncogenes sis, erbB, and TGF α are continuing. Two newly integrated oncogenes, mac and dbl, are also under intensive experimental analysis. Viral constructs containing the above genes inoculated into newborn mice result in distinct patterns of tumorigenicity. The sis-containing viruses are uniformly sarcomagenic; erbB-containing viruses tend to be more pleiomorphic in their carcinogenesis and induce hepatocellular carcinomas as well as sarcomas; mac seems to have a predilection for endothelial target cells in vivo. Fibroblast cell cultures morphologically transformed in vitro by these oncogenes are uniformly malignant by graft into nu/nu mice and, as expected, produce sarcomas. Epithelial cell cultures similarly transformed in vitro give rise to carcinomas in the nu/nu hosts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04940-20 LCMB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Viruses and Transforming Genes in Experimental Oncogenesis and Human Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. A. Aaronson	Chief	LCMB	NCI
Others:	S. R. Tronick	Chief, Gene Structure Section	LCMB	NCI
	K. C. Robbins	Chief, Molecular Genetics Section	LCMB	NCI
	J. H Pierce	Research Microbiologist	LCMB	NCI
	A. Eva	Visiting Scientist	LCMB	NCI
	J. C. Lacal	Visiting Associate	LCMB	NCI
	M. H. Kraus	Visiting Associate	LCMB	NCI

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Laboratory of Cellular and Molecular Biology

SECTION: Molecular Biology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	1.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goals of this project are to elucidate the mechanisms of action of tumor viruses and to determine the cellular alterations responsible for naturally occurring malignancies. Topics of present interest include: (1) transforming genes of retroviruses and cancer cells; (2) the biology of endogenous retroviruses; (3) the molecular biology of retrovirus replication and transformation; and (4) the application of knowledge gained from these studies to the search for the causes and mechanisms involved in human neoplastic transformation.

During the past year, we have isolated and partially characterized a number of new human oncogenes, and have gained important new insights regarding the structure and function of the sis and ras oncogenes. We have also made important progress in the exploitation of two animal lentiviruses as models for the human immunodeficiency viruses (HIV).

Newly described oncogenes include the erbB-2 gene, isolated from a human breast carcinoma; dbl, isolated in a human diffuse B cell lymphoma; and arg, a member of the tyrosine kinase family closely related to but distinct from c-abl. Another gene encoding transforming growth factor alpha (TGF α), a human growth factor, was characterized as having growth promoting potential but not to be a direct-acting oncogene.

Lentivirus studies revealed their evolutionary relatedness to HIV and resulted in a collaborative drug therapy study which demonstrated the broad spectrum antiretroviral activity of the dideoxynucleosides.

Protein kinase C was demonstrated to be activated by phorbol esters.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04941-15 LCMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemical Characterization of Retroviruses and onc Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. R. Tronick	Chief, Gene Structure Section	LCMB	NCI
Others:	S. A. Aaronson	Chief		
	K. C. Robbins	Chief, Molecular Genetics Section	LCMB	NCI
	J. E. Dahlberg	Research Microbiologist	LCMB	NCI
	A. Eva	Visiting Scientist	LCMB	NCI
	T. Kawakami	Visiting Associate	LCMB	NCI
	S. Katamine	Guest Researcher	LCMB	NCI
	D. Ron	Visiting Fellow	LCMB	NCI

COOPERATING UNITS *(if any)*

Sackler School of Medicine, Tel Aviv, Israel (A. Yaniv)

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Gene Structure Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	1.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The human dbl oncogene, isolated by transfection, has been characterized with respect to its transcribed sequences and the genomic rearrangements present at its termini. Its structure has been compared to dbl sequences present in the lymphoma cells from which it was isolated and also to the dbl proto-oncogene.

The mRNA expressed by the human c-fgr proto-oncogene has been isolated and shown to encode the entire fgr protein. Efforts are underway to localize its 5' coding and regulatory sequences in human genomic DNA.

Studies on animal lentiviruses have led to the production of the equine infectious anemia virus (EIAV) gag gene precursor in bacteria and the development of sensitive and specific assays for EIAV. Trans-activation of the EIAV LTR was demonstrated and sequences encoding its tat gene have been localized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04951-11 LCMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Characterization of Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. E. Dahlberg	Research Microbiologist	LCMB	NCI
Others:	S. A. Aaronson	Chief	LCMB	NCI
	S. R. Tronick	Chief, Gene Structure Section	LCMB	NCI
	M. Wang	Visiting Fellow	LCMB	NCI
	T. Kawakami	Visiting Fellow	LCMB	NCI
	D. Archambault	Guest Researcher	LCMB	NCI
	J. Hallum	Guest Researcher	LCMB	NCI
	S. Broder	Chief	COP	NCI

COOPERATING UNITS *(if any)*
Tel Aviv University (A. Yaniv); Hebrew University (K. Perk); Dept. Pathology, Colorado State University, Fort Collins (J. DeMartini); Dept. Microbiology, Pathology and Parasitology, North Carolina State University (L. Coggins).

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Molecular Genetics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lentiviruses of sheep, goats, and primates are a genetically distinct group of retroviruses that replicate in immune cells and usually cause disease with a long latent period and slowly progressive course, often leading to death. In order to understand how these viruses interact with their hosts and cause pathological change, as well as developing improved diagnostic and therapeutic methods, a molecular analysis of several of these viruses is being carried out. Sequence analysis of molecular clones of caprine arthritis encephalitis virus (CAEV) and equine infectious anemia virus (EIAV) revealed that the complex genomic organization observed with HIV and visna is common to all lentiviruses and suggests that their extra genes, such as tat, art, and sor, must be an important part of how these viruses interact with their hosts. We have developed sensitive ELISAs, using viral protein produced in bacteria, to detect antibodies to CAEV and EIAV, which are superior to existing assays. We have also determined that the replication of lentiviruses, and other retroviruses as well, can be effectively inhibited by 2',3'-dideoxynucleosides, which act to terminate the synthesis of retroviral DNA during reverse transcription of the viral genomic RNA. Currently, additional drugs are being evaluated to determine if they are superior to the dideoxynucleosides, or can be used in combination. Animal studies, using both mice and goats, have been initiated to determine optimal ways in which such drugs can be used to control viral spread and virus-induced disease. It is hoped that such studies will represent an important model for the use of such drugs on AIDS patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04976-10 LCMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Carcinogenesis of Mammalian Cells in Culture

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	K. K. Sanford	Chief, In Vitro Carcinogenesis Section	LCMB	NCI
Others:	S. Takai	Visiting Fellow	LCMB	NCI
	J. S. Rhim	Research Microbiologist	LCMB	NCI
	M. Potter	Chief	LG	NCI
	K. H. Kraemer	Research Scientist	LMC	NCI
	R. E. Tarone	Mathematical Statistician	BB	NCI
	M. A. Tucker	Oncologist	EEB	NCI

COOPERATING UNITS *(if any)*
Howard U. College Med. (R. Parshad); Childrens Hosp. of Los Angeles (W. E. Benedict); Tel Aviv U. (Y. Shiloh); U. NC (M. Swift); Walter Reed Dept. Med. (R. Knight).

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
In Vitro Carcinogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cultures of skin fibroblasts and peripheral lymphocytes from normal and cancer-prone individuals, as well as neoplastic cells transformed in culture or in vivo, are utilized in evaluating the relationship between radiation-induced chromosomal DNA damage, deficient DNA repair, cancer susceptibility and malignant neoplastic transformation. An increased incidence of chromatid damage after x-irradiation during the G-2 phase of the cell cycle is associated with both a predisposition to cancer and malignant transformation and can provide the basis of a test for cancer susceptibility. A genetic basis for this radiosensitivity with localization of genes to specific chromosomes is indicated from studies with somatic cell hybrids, inbred strains of mice, and congenic mouse strains. The chromosomal radiosensitivity appears to result from deficient DNA repair during G-2. Another aspect of this project is to develop a reproducible transformation system with human epidermal keratinocytes as an in vitro model for following the progression of biologic and biochemical changes leading to neoplastic transformation. An associated problem is to identify quantifiable cytomorphologic changes diagnostic of neoplastic transformation to facilitate transfection and transformation studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05060-09 LCMB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Studies on Oncogenic Transformation in Culture

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. S. Rhim	Research Microbiologist	LCMB	NCI
Others:	S. A. Aaronson	Chief	LCMB	NCI
	J. B. Park	Visiting Fellow	LCMB	NCI
	P. Arnstein	Veterinary Director	LCMB	NCI
	K. Sanford	Chief, In Vitro Carcinogenesis Section	LCMB	NCI

COOPERATING UNITS *(if any)*: Georgetown University (A. Dritschilo), Washington, D.C.

LAB/BRANCH: Laboratory of Cellular and Molecular Biology

SECTION: Office of the Chief

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Objectives of this project are (1) to establish and define a cell culture transformation system for identification of carcinogenic agents and humans at high risk for cancer; (2) to develop human cell transformation systems, with particular emphasis on epithelial cells, in order to study host factors regulating cell transformation and the mechanisms of carcinogenesis by chemicals, viruses, hormones and x-irradiation; and (3) to isolate and characterize oncogenes from human tumors.

In line with these objectives, we have (1) established a nontumorigenic human epidermal keratinocyte line immortalized by transfection with pSV3-neo; (2) demonstrated malignant transformation of human epidermal keratinocytes by the combined action of SV40 T antigens and K-MSV; (3) demonstrated enhanced G-2 chromatid radiosensitivity in continuous cell lines established by infection with adeno 12-SV40 or transfection with pSV3-neo; (4) established human epidermal keratinocyte lines expressing SV40 T antigens, malignantly transformed with chemicals for detection of new human cellular oncogenes; (5) demonstrated that human epidermal keratinocytes retain radiation resistance following in vitro immortalization and malignant transformation; and (6) demonstrated activation of a cellular transforming oncogene, H-ras, in the human 312H cell line transformed with the chemical carcinogen, 3-methylcholanthrene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05062-09 LCMB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Transforming Genes of Naturally-Occurring and Chemically-Induced Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Eva	Visiting Scientist	LCMB	NCI
Others:	S. A. Aaronson	Chief	LCMB	NCI
	S. R. Tronick	Chief, Gene Structure Section	LCMB	NCI
	S. K. Srivastava	Visiting Fellow	LCMB	NCI
	D. Ron	Visiting Fellow	LCMB	NCI
	L. Varesio	Visiting Scientist	LMI	NCI
	J. Ward	Chief, TPPS	LCC	NCI

COOPERATING UNITS (if any): NIEHS, Research Triangle Park, NC (M. Anderson); Dana-Farber Cancer Institute, Boston, MA (G. M. Cooper); Baylor College of Medicine, Houston, TX (P. Overbeek)

LAB/BRANCH: Laboratory of Cellular and Molecular Biology

SECTION: Molecular Biology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The human transforming gene, dbl, was isolated from the DNA of a primary human diffuse B-cell lymphoma by the DNA transfection assay on NIH/3T3 cells, and cloned in cosmid vector as a human DNA sequence of 45 kilobases. An independent isolate of a dbl-related transforming gene was obtained following transfection of NIH/3T3 cells with DNA of a human nodular poorly differentiated lymphoma (NPDL). Physical mapping indicated that this transforming gene, designated NPDL-dbl, shared considerable homology with the prototype dbl oncogene. A cDNA library was constructed with polyadenylated RNA purified from a dbl third-cycle transfectant. The full size cDNA was isolated and completely sequenced. No homology was found by computer search of published nucleic acid and protein sequences. We have also cloned and sequenced the cDNA of the dbl proto-oncogene. Both cDNA clones were introduced into eukaryotic expression vectors and are being analyzed and compared for their transforming activity.

Fifty percent of the DNAs of methylcholanthrene (MCA)-induced fibrosarcomas in mice were found to contain an activated K-ras gene. Analysis of cell lines established from the tumors for their growth capacity in vivo indicated that an activated K-ras gene was associated with a more malignant phenotype of the cells. Thymic lymphomas were induced in RFJ mice by percutaneous application of methylcholanthrene (MCA). DNAs from 83% of the tumors analyzed contained a transforming K-ras gene. The high frequency of K-ras activation in response to MCA seems to favor the concept that the activation of K-ras is related to the specificity of the mutagenic effect of MCA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05063-09 LCMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on Epstein-Barr Virus and HIV

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. V. Ablashi	Research Microbiologist	LCMB	NCI
Others:	S. Z. Salahuddin	Expert	LTCB	NCI
	R. C. Gallo	Chief	LTCB	NCI
	S. Joseph	Chemist	LTCB	NCI
	F. Wong-Staal	Chief, MGHC Section	LTCB	NCI
	C. Saxinger	Research Microbiologist	LTCB	NCI

COOPERATING UNITS *(if any)*

M. Kaplan, North Shore University Hospital, Long Island, NY; P. D. Markham, Litton Bionetics, Kensington, MD; P. Biberfeld, Karolinska Institute, Sweden; B. Kramarsky, Electro Nucleonics, Inc., Silver Spring, MD

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20982

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Acquired immunodeficiency syndrome (AIDS) B-cell lymphomas occur in individuals in the 30-year age group with a history of homosexuality, bisexuality, and intravenous drug use. Thirty percent of these lymphomas are, histologically, Burkitt's type and contain the Epstein-Barr virus (EBV) genome. The other B-cell lymphomas are diffuse, large cell type which lack EBV association. Based on these findings, the interaction of EBV, possible other human viruses, and HIV in B-cell lymphomas was investigated.

B-cell lymphomas associated with EBV developed in an HIV virus-positive and antibody-positive AIDS patient six months after significant increase in EBV-early antigen antibody (EA) titers were observed. This suggested that EBV may lead to polyclonal proliferation of B-cells, one of which may undergo transformation.

During the course of these investigations, a novel human B lymphotropic virus (HBLV) was isolated from peripheral blood lymphocytes from two AIDS patients with B-cell lymphomas. Later on, HBLV was also isolated from four patients with angioimmunoblastic lymphoadenopathy, immunoblastic lymphoma, and lymphocytic leukemia. HBLV is a new herpesvirus which is genetically and immunologically distinct from known herpesviruses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05164-07 LCMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interaction of Hematopoietic Cells and Mammalian Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. H. Pierce	Research Microbiologist	LCMB	NCI
Others:	S. A. Aaronson	Chief	LCMB	NCI
	P. Di Fiore	Visiting Associate	LCMB	NCI
	J. Falco	Medical Staff Fellow	LCMB	NCI
	M. Kraus	Visiting Associate	LCMB	NCI

COOPERATING UNITS *(if any)*

University of Virginia, Charlottesville (J. T. Parsons); University of Massachusetts Medical Center, Worcester (J. Greenberger)

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Molecular Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Betheda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A recombinant vector containing the normal human erbB-2 cDNA was generated to determine whether this growth factor receptor-like gene could transform in the NIH/3T3 transfection assay. erbB-2 was shown to be a potent oncogene when overexpressed in NIH/3T3 cells. These findings demonstrate a new mechanism for acquisition of oncogenic properties by genes encoding growth factor receptor-like proteins and provide a functional basis for the role of their overexpression in the development of human malignancies.

The interactions of murine mast cell lines with B-cell stimulatory factor-1 (BSF-1/IL-4) were explored. BSF-1 mRNA was expressed by a majority of transformed mast cell lines and by five IL-3-dependent mast cell lines. BSF-1 activity was detected in the supernatants of transformed mast cells. The role of BSF-1 as a mast cell growth factor and its constitutive production by transformed mast cells raises the possibility that BSF-1 may act as an autocrine growth factor for some transformed mast cells. Furthermore, production of BSF-1 mRNA by non-transformed cells indicates mast cells may be an important physiologic source of this factor.

The arrangement of immunoglobulin genes was examined in a series of lymphoid cell lines transformed with Harvey murine sarcoma virus in vitro. One fetal liver transformant was shown to possess a germline configuration for the immunoglobulin gene family. This line was shown to frequently rearrange either immunoglobulin or T-cell receptor genes during subcloning. Therefore, this transformant appears to represent the earliest stage in lymphoid development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05167-07 LCMB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Mechanisms of Transformation Induced by the sis Gene

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. C. Robbins	Chief, Molecular Genetics Section	LCMB	NCI
Others:	S. A. Aaronson	Chief	LCMB	NCI
	S. R. Tronick	Chief, Gene Structure Section	LCMB	NCI
	T. Miki	Guest Researcher	LCMB	NCI
	N. Giese	IRTA Fellow	LCMB	NCI
	T. Fleming	Guest Researcher	LCMB	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH: Laboratory of Cellular and Molecular Biology

SECTION: Molecular Genetics Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	0.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our previous studies have demonstrated the importance of human sis/PDGF-2 gene deregulation in its activation as an oncogene in cells responsive to PDGF stimulation. Current studies have focused on the structure, regulation, and function of this gene. We have shown that human tumor cells arising from PDGF-responsive cell types express sis/PDGF-2 mRNA and mitogenically active sis/PDGF-2 homodimers. Utilizing cDNA cloning, S1 nuclease mapping, and primer extension techniques, the normal human sis/PDGF-2 transcriptional unit has been defined. These studies also suggested the presence of transcriptional and translational regulatory signals within the sis/PDGF-2 locus, and have provided an approach for elucidating mechanisms by which this gene is controlled.

Knowledge that the v-sis oncogene encodes a PDGF-related product whose transforming activity requires functional interaction with the PDGF receptor has suggested the importance of identifying the active site of the v-sis translational product. Site-directed mutagenesis of v-sis has localized an 89 codon stretch as its minimum transforming region and has shown a requirement for each of 8 cysteine codons within the region for proper folding of the v-sis gene project. These studies have also predicted three testable models for the active conformation of this protein and represent an important step in identifying the receptor binding domain of this oncogenic growth factor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05362-04 LCMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Serum-free Culture of Transformed and Untransformed Mouse Keratinocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. P. Falco	Medical Staff Fellow	LCMB	NCI
Others:	S. A. Aaronson	Chief	LCMB	NCI
	W. G. Taylor	Research Biologist	LCMB	NCI
	P. P. Di Fiore	Visiting Associate	LCMB	NCI

COOPERATING UNITS *(if any)*
Childrens Hospital of Los Angeles (Dr. Bernard Weissman).

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

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Office of the Chief

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NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A chemically-defined tissue culture system developed for the BALB/MK epithelial cell line was used to study (1) how the introduction of activated viral oncogenes alters the cell line's growth factor requirements, and (2) what growth factors elicit a mitogenic response in this cell line and how these growth factors interact with each other.

In this defined media system, uninfected BALB/MK keratinocytes required only two growth factors for growth--insulin and epidermal growth factor (EGF). Oncovirally infected BALB/MK demonstrated four patterns of growth factor requirements: (1) requirements unaltered from parental line (v-raf); (2) partial escape from EGF requirement (v-mos, v-fms, v-erbB); (3) complete escape from EGF requirement (v-K-ras, v-H-ras); and (4) escape from all growth factor requirements (v-fgr).

Three of these viral infectants, v-K-ras, v-fgr, and v-fms, demonstrated release into the medium of EGF-like activity, presumably TGF α. No viral infectants produced insulin-like activity.

In defined medium mitogenic assays, the following growth factors were found to be BALB/MK mitogens, in descending order of potency: acidic fibroblast growth factor (FGF), basic FGF, EGF, and insulin. Synergism was noted between insulin and EGF or insulin and basic FGF.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05366-04 LCMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Activation of Proto-oncogenes Encoding Growth Factor Receptor Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. H. Kraus	Visiting Associate	LCMB	NCI
Others:	S. A. Aaronson	Chief	LCMB	NCI
	P. P. Di Fiore	Visiting Associate	LCMB	NCI
	J. H. Pierce	Research Microbiologist	LCMB	NCI
	O. Segatto	Visiting Fellow	LCMB	NCI
	H. Lacroix	Guest Researcher	LCMB	NCI
	N. C. Popescu	Microbiologist	LB	NCI

COOPERATING UNITS *(if any)*
Meloy Laboratories, Rockville, Maryland (C. R. King)

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Laboratory of Cellular and Molecular Biology

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NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Employing relaxed stringency hybridization conditions with v-erbB as probe, we previously had identified a novel member of the erbB/EGF receptor gene family in the human genome amplified in a mammary carcinoma. In a series of human mammary tumor cell lines, transcript analysis demonstrated elevated expression levels of erbB-2 ranging from 8- to 128-fold above those of normal controls in 8 out of 16 cases. An aberrantly sized erbB-2 transcript was not detected in these cell lines. Immunoblot analysis indicated elevated levels of the 185-kd product of erbB-2 expressed by these cells. In four lines, erbB-2 gene amplification in the absence of an apparent gene rearrangement was demonstrated. Amplified gene copies in a representative cell line, SK-BR-3, were localized in an aberrant chromosomal location by in situ hybridization. In four additional cell lines, 4- to 8-fold erbB-2 mRNA overexpression was observed in the absence of gene amplification. In a representative cell line, 7R-75-1, normal chromosomal location of erbB-2 was determined. Moreover, gene amplification of erbB-2 was observed in 10% of human mammary tumor tissues analyzed. These findings linked overexpression of an apparently normal erbB-2 gene product with human mammary neoplasia. In order to assess the transforming potential of this growth factor receptor-like gene, we introduced the normal coding sequence of erbB-2 in NIH/3T3 cells by DNA transfection expressing the gene product at different expression levels. Under SV40 promoter, the gene lacked transforming activity despite expression of erbB-2 protein levels. A five to tenfold increase in its expression under LTR influence was associated with activation of erbB-2 as a potent oncogene. The higher levels of erbB-2 protein were observed in mammary tumor cell lines with erbB-2 gene amplification.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05456-03 LCMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Transformation Induced by Viral and Cellular fgr Oncogenes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. S. C. Cheah	Medical Staff Fellow	LCMB	NCI
Others:	K. C. Robbins	Chief, Molecular Genetics Section	LCMB	NCI
	S. R. Tronick	Chief, Gene Structure Section	LCMB	NCI
	S. Katamine	Visiting Fellow	LCMB	NCI

COOPERATING UNITS *(if any)*

Division of Hematology/Oncology, Univiversity of Washington, St. Louis, MO (T. J. Ley).

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Molecular Biology Section

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NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The GR-FeSV onc gene, v-fgr, appears to contain genes coding for actin as well as a tyrosine-specific protein kinase. In an effort to understand the role of the actin domain in the transforming ability of the virus, a series of mutants with deletions in their gag and/or actin sequences were constructed and tested for their ability to transform NIH/3T3 cells. Preliminary data suggest that the actin domain has little effect on transforming activity in vitro but that the carboxy terminus of the gag sequences might be important for membrane binding and transformation. Expression of the human fgr proto-oncogene is limited to Burkitt's lymphomas naturally infected with Epstein-Barr virus (EBV) but not to EBV-negative Burkitt's lymphoma. Normal umbilical cord or peripheral blood lymphocyte lines established in vitro by EBV infection also contain detectable c-fgr mRNA. A 50-fold increase in steady state mRNA concentration is observed when uninfected Burkitt's lymphoma cell lines are deliberately infected with EBV. These findings demonstrate, for the first time, the induction of a proto-oncogene in response to infection by a DNA tumor virus. Effects to identify normal sources of fgr proto-oncogene expression have revealed that high levels of c-fgr mRNA are detected in monocytes as well as resting polymorphonuclear leukocytes (PMNs). Although high levels of c-fgr mRNA are present in resting PMNs, the fgr proto-oncogene is transcriptionally inactive, implying that it is synthesized at an earlier stage of granulocytic maturation and is found in the mature PMN as a stable mRNA species. These findings suggest an important role for the fgr proto-oncogene in some facet of granulocytic maturation or function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05457-03 LCMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Usage of Human Tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. P. Di Fiore	Visiting Associate	LCMB	NCI
Others:	S. A. Aaronson	Chief	LCMB	NCI
	J. H. Pierce	Research Microbiologist	LCMB	NCI
	M. Kraus	Visiting Associate	LCMB	NCI
	O. Segatto	Visiting Fellow	LCMB	NCI

COOPERATING UNITS *(if any)*

None

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SECTION
Molecular Biology Section

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NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanism of oncogenic activation of the newly discovered erbB-2 growth factor receptor-like gene is being studied. A wide variety of human tumors contain an amplified and/or overexpressed erbB-2 gene. To study the role of overexpression of this gene in the initiation of oncogene transformation in a controlled in vitro model system, we engineered eukaryotic expression vectors to direct the synthesis of erbB-2 mRNA either under the control of a strong promoter (LTR) or of a weak promoter (SV40 early promoter). When erb-2 cDNA was expressed in NIH/3T3 cells under the control of the SV40 promoter, the gene lacked transforming activity, despite expression of detectable levels of the erbB-2 protein. A further five- to tenfold increase in its expression, under LTR influence, was associated with activation of erbB-2 as a potent oncogene. The high levels of the erbB-2 product associated with malignant transformation of NIH/3T3 were observed in human mammary tumor cells that overexpressed this gene.

A murine pseudotype of the v-erbB gene has been employed to alter the growth properties and the differentiation program of an in vitro cell line of mouse keratinocytes (MKB). As already demonstrated for many other oncogenes, v-erbB is capable of relieving MKB cells from their dependence on EGF for growth. Nevertheless, the v-erbB oncogene is unable to block the expression of the differentiated phenotype when MKB cells are challenged with high calcium concentrations (a property displayed by all other oncogenes).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05459-03 LCMB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Structural and Functional Characterization of ras p21 Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	J. C. Lacal	Visiting Associate	LCMB	NCI
Others:	S. A. Aaronson	Chief	LCMB	NCI
	S. R. Tronick	Chief, Gene Structure Section	LCMB	NCI
	J. Moscat	Guest Researcher	LCMB	NCI
	P. Blumberg	Chief, Molecular Mechamisms of Tumor Promotion Section	LCCTP	NCI

COOPERATING UNITS (if any): Centro de Biologia Molecular, Universidad Autonoma, Madrid, Spain (L. Serrano); Department of Medicine, SUNY, Stony Brook, NY (N. Hagag); Departmento de Medicina y Cirugia Experimental, Hospital Provincial, Madrid (P. Garcia-Barreno).

LAB/BRANCH: Laboratory of Cellular and Molecular Biology

SECTION: Molecular Biology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Expression vectors were generated to produce large amounts of mammalian ras p21 proteins in E. coli. Purified proteins were analyzed for in vitro and in vivo activities. We have found that ras p21 proteins have at least two different mechanisms of activation of their transforming properties. One of these mechanisms implies an increase of the off-rate of p21 GTP-binding activity. We have found that mutations that substitute Thr 59 for Ala 59 (normal p21) increased the off-rate for GDP and GTP three to ninefold when compared to normal or mutated p21 at positions 12 or 61. The observation that monoclonal antibody Y13-259 specifically blocks the ability of normal and mutated ras proteins to interchange prebound guanine nucleotides suggests that this might be the mechanism by which Y13-259 interferes with the biological activity of both normal and mutated ras p21 proteins. In parallel studies, we have found that in phorbol ester down-regulated Swiss 3T3 cells, microinjection of purified protein kinase C restores the phorbol ester-induced mitogenic activity. Using this system, we have been able to demonstrate the requirement of functional protein kinase C for the mitogenic activity of ras proteins. Microinjection of p21 into down-regulated (protein kinase C-depleted) cells does not induce DNA synthesis. Coinjection of ras p21 with protein kinase C restored its activity. We have also demonstrated that transforming ras p21 induces a rapid rise in intracellular pH after microinjection which is mediated by the Na+/H+ antiporter system. These data, together with the requirement of protein kinase C, implies that ras p21 function is mediated by protein kinase C. Finally, we have observed that protein kinase C can phosphorylate ras p21 at serine residues. Attempts to correlate in vitro activities and results of microinjection studies are being made.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05460-03 LCMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Tissue-specific Expression of c-sis/PDGF-2 Gene

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. D. Rao	Visiting Associate	LCMB	NCI
Others:	S. A. Aaronson	Chief	LCMB	NCI
	M. W. Pech	Visiting Scientist	LCMB	NCI
	K. C. Robbins	Chief, Molecular Genetics Section	LCMB	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Molecular Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

(1) The structure and sequence of the human c-sis/PDGF-2 transcriptional unit has been determined. The role of various sequences in the gene locus, including the 5' and 3' flanking sequences, in the regulation of tissue-specific expression of this prototype growth factor with transforming potential was investigated in endothelial cells and fibroblasts that do and do not express the c-sis/PDGF-2 transcript, respectively. By utilizing the bacterial chloramphenicol acetyl transferase gene as reporter, we functionally localized the c-sis/PDGF-2 promoter 23 bp upstream of the mRNA cap site. Within a 4-kbp region upstream of the mRNA cap site, there were no sequences that conferred tissue-specific differences in reporter gene expression. Inhibiting sequences were detected upstream of the promoter but lacked cell specificity. The lack of tissue specificity of the c-sis/PDGF-2 gene promoter is further established by nuclear run-on analysis which demonstrated constitutive transcriptional activity of the endogenous c-sis/PDGF-2 promoter in fibroblasts. All of these findings imply that c-sis/PDGF-2 RNA expression is normally regulated at a post-transcriptional rather than transcriptional level. (2) The 5' untranslated sequence (5' UTS) of the c-sis mRNA was shown to exert a potent inhibitory effect on translation. Deletion of 5' UTS resulted in as much as a 40-fold increase in translation, independent of the reporter gene or cell type analyzed. A DNA construct containing the c-sis/PDGF-2 transcriptional unit lacked detectable biological activity upon transfection of NIH/3T3 fibroblasts, but deletion of the 5' UTS unmasked c-sis/PDGF-2 transforming activity. Thus, the normal mechanisms which inhibit transforming activity of the c-sis/PDGF-2 proto-oncogene in fibroblasts are exerted at post-transcriptional levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05461-03 LCMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of Normal Counterpart of dbl Oncogene

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Eva	Visiting Scientist	LCMB	NCI
Others:	D. Ron	Visiting Fellow	LCMB	NCI
	S. A. Aaronson	Chief	LCMB	NCI

COOPERATING UNITS *(if any)*

None

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The entire coding sequence of dbl proto-oncogene was determined. The sequence analysis revealed that dbl proto-oncogene codes for a 925-amino acid protein. It is a hydrophilic protein with a characteristic α-helical coiled-coil structure similar to that of intermediate filaments. The dbl proto-oncogene sequence showed no homology to any known oncogene and thus may represent a member of a new class of oncogenes.

Comparison of the proto-oncogene sequence with that of the activated dbl revealed that the transforming gene was rearranged with respect to its amino-terminal domain.

The oncogenic potential of the dbl proto-oncogene was examined by cloning the full length cDNA in several eukaryotic expression vectors and transfecting these vectors to NIH/3T3 cells. These studies showed that the dbl proto-oncogene is capable of transforming NIH/3T3 cells when it is driven by a strong promoter. However, this activity was lower than that found with the activated dbl driven by the same promoter.

The dbl proto-oncogene product was detected for the first time utilizing the COS cell system and an expression vector driven by SV40 early promoter. The size of the dbl proto-oncogene protein was determined to be ~110 kd.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05463-03 LCMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular and Oncogene Products which Participate in Growth Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. G. Taylor	Research Biologist	LCMB	NCI
Others:	S. A. Aaronson	Chief	LCMB	NCI
	J. P. Falco	Medical Staff Fellow	LCMB	NCI

COOPERATING UNITS *(if any)*
None

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this program is to understand the mechanism(s) of cellular changes fundamental to neoplastic transformation. Retroviral onc gene(s) or their mitogenic onc gene product(s) may subvert normal growth regulatory mechanisms and cause neoplastic transformation in culture. Nonneoplastic mammalian cells in culture have specific hormone and growth factor requirements for initiation of DNA synthesis and mitosis, and rigorous analysis is possible only in the absence of serum mitogens. A serum-free model system which supports proliferation and maintenance of nonneoplastic NIH/3T3 cells for up to three weeks was developed. Changes in insulin, epidermal growth factor and basic fibroblast growth factor requirements were assessed with a known prototype onc gene which codes for a potent mitogen (v-sis) or a defective cell membrane receptor (v-erbB), or is involved in intracellular transduction of mitotic stimuli. In this biologic assay system, nonneoplastic NIH/3T3 cells remain sensitive to the constraints of normal growth regulation when tested as single cells (clonal growth) or as proliferating population at higher density. Introduction of an onc gene causes unique changes in growth factor requirements. Cells transfected with v-sis and v-erbB generally are less dependent upon a set of competence and progression factors than cells transfected with v-ras, which, like progenitor NIH/3T3 cells, need at least two growth factors for survival and growth. Knowledge of the impact these genes have on normal cells will lead to strategies for counteracting their tumorigenic potential.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05466-02 LCMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Human Transforming Growth Factor α in Neoplasia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Finzi	Medical Staff Fellow	LCMB	NCI
Others:	S. H. Yuspa	Chief	LCCTP	NCI
	A. E. Kilkenny	Expert	LCCTP	NCI
	T. P. Fleming	Guest Researcher	LCMB	NCI
	O. Segatto	Visiting Fellow	LCMB	NCI
	S. A. Aaronson	Chief	LCMB	NCI
	J. H. Pierce	Research Microbiologist	LCMB	NCI

COOPERATING UNITS *(if any)*

Department of Molecular Biology, Genentech, Inc., South San Francisco, CA (T.S. Bringman and R.K. Derynck).

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previously, we have shown that TGF α-expression vectors failed to induce morphological transformation upon transfection of NIH/3T3 cells. Transfected cells were shown to secrete large amounts of TGF α into the medium, to grow to high saturation density, to have down-regulated EGF receptors, and to be growth-inhibited by TGF α monoclonal antibody. However, TGF α-expressing sublines were not tumorigenic in nude mice. These and other results suggest that the normal coding sequence for TGF α is not a direct-acting oncogene. To broaden our investigation of the potential transforming properties of TGF α, we constructed and characterized a recombinant murine retrovirus which expresses the human TGF α gene. Infection of NIH/3T3 cells with the TGF α virus did not induce foci formation; however, infected cells were shown to have integrated a transcriptionally active provirus and to secrete large amounts of biologically active TGF α. Results were obtained upon infection of six other types of fibroblasts. We showed that the TGF α retrovirus could not transform the clonal BALB/MK-2 epidermal keratinocyte cell line. However, BALB/MK-2 cells infected with the TGF α retrovirus were shown to synthesize and secrete TGF α. We are investigating the role of TGF α in epithelioid cancer using a nude mouse skin graft model. Recent work indicates that although infection of primary epithelial cells with the TGF α retrovirus does not lead to the formation of tumors, papilloma cells infected with the virus form papillomas which are five to ten times larger than those formed by control papilloma cells, suggesting a role for TGF α in the clonal expression of the premalignant lesion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05467-02 LCMB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Cloning of Human c-fgr Proto-oncogene cDNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. C. Robbins	Chief, Molecular Genetics Section	LCMB	NCI
Others:	S. Katamine	Visiting Fellow	LCMB	NCI
	S. R. Tronick	Chief, Gene Structure Section	LCMB	NCI
	M. S. C. Cheah	Medical Staff Fellow	LCMB	NCI
	C. D. Rao	Visiting Associate	LCMB	NCI
	T. Miki	Guest Researcher	LCMB	NCI
	T. Kawakami	Visiting Associate	LCMB	NCI

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Laboratory of Cellular and Molecular Biology

SECTION: Molecular Genetics Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to elucidate the structure and function of the human fgr proto-oncogene, studies are directed toward cloning and nucleotide sequence analysis of human c-fgr cDNA as well as identification of its translational product. Several overlapping c-fgr cDNA clones were isolated from a normal mononuclear cell cDNA library. Nucleotide sequence analysis revealed an open reading frame of 529 codons in length. Both antibodies directed against peptides representing amino and carboxy terminal regions of the predicted c-fgr protein specifically immunoprecipitated a 55-kd protein from lysates of COS cells transfected with an expression vector containing the entire c-fgr cDNA open reading frame.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05468-02 LCMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Implications of Human Tyrosine Kinase Gene, c-fyn, on Tumorigenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. Kawakami	Visiting Associate	LCMB	NCI
Others:	K. C. Robbins	Chief, Molecular Genetics Section	LCMB	NCI
	Y. Kawakami	Guest Researcher	LCMB	NCI
	T. Matsui	Visiting Fellow	LCMB	NCI
	S. A. Aaronson	Chief	LCMB	NCI
	N. C. Popescu	Microbiologist	LB	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recently, a novel human src-like gene, designated fyn, has been isolated and the nucleotide sequence of its coding region has been determined. Based upon nucleotide sequence information, the predicted fyn translational product is 537 amino acids in length and shares a number of structural features with p60 c-src, including identity at 337 of the 455 amino acid residuals at its carboxy terminus. In an effort to identify the fyn translational product for further study, fyn transcripts synthesized from cDNA templates were translated in vitro. The major translational product observed was a protein of 59 kd, a size in good agreement with the extent of the fyn cDNA open reading frame. Moreover, using antibodies prepared against peptides representing fyn amino and carboxy terminal coding sequences, it was possible to immunoprecipitate the 59-kd protein, designated p59 fyn, in in vitro translational products and lysates of NIH/3T3 cells transfected with constructs containing fyn cDNA in retroviral expression vectors. p59 fyn was found to possess protein-tyrosine kinase activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05469-02 LCMB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Identification of New Tyrosine Kinase Oncogenes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G. Kruh	Medical Staff Fellow	LCMB	NCI
Others:	S. A. Aaronson	Chief	LCMB	NCI
	M. H. Kraus	Visiting Associate	LCMB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH: Laboratory of Cellular and Molecular Biology

SECTION: Office of the Chief

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In order to understand the role of growth factor receptors in neoplasia, the identification of new oncogenes was attempted. A new gene with extensive homology to v-abl, termed arg (Abelson-related gene), was identified in normal human DNA. This new gene was found to be expressed in several human tissues, as well as a variety of tumor cell lines. Thus, based upon nucleotide sequence diversity and identification of a distinct RNA transcript, arg represents a new functional human gene of the tyrosine kinase family. The coding sequence of arg is currently being investigated. cDNA clones are being isolated to allow elucidation of the complete coding sequence. The chromosomal localization of this gene was identified on the long arm of chromosome 1, and tumors with abnormalities in this region are under investigation to determine if rearrangements of arg are involved.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05472-02 LCMB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders.): Structural Characterization of Putative Growth Factor Receptor Gene c-erbB-2

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. A. Aaronson	Chief	LCMB	NCI
Others:	O. Segatto	Visiting Fellow	LCMB	NCI
	P. P. Di Fiore	Visiting Fellow	LCMB	NCI
	J. H. Pierce	Research Microbiologist	LCMB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH: Laboratory of Cellular and Molecular Biology

SECTION: Molecular Biology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In order to study the molecular mechanisms involved in signal transduction and regulation of catalytic activity of the putative growth factor receptor gene, c-erbB-2, a series of mutants in different structural domains of the mature gene product have been generated by means of site-directed mutagenesis techniques. Mutant molecular clones were then inserted into eukaryotic expression vectors and expressed in NIH/3T3 cells in order to assess the biologic activity in a focus assay. A nonconservative amino acid substitution in the transmembrane domain leading to a change from valine to either aspartic or glutamic acid activates the transforming potential of the gene. This finding suggests that the transmembrane domain is important in signal transduction and that specific molecular lesions might irreversibly mimic informational changes which usually take place reversibly upon ligand binding to the receptor. Further work is aimed at correlating biological differences between these mutants and the wild-type molecule in a variety of biochemical assays. Experiments are also in progress to evaluate the biologic activity of another series of mutants generated in the COOH terminal region of the protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05473-02 LCMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Mechanisms of Pathogenesis of Animal Lentiviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. R. Tronick	Chief, Gene Structure Section	LCMB	NCI
Others:	M. C. Wang	Visiting Fellow	LCMB	NCI
	S. A. Aaronson	Chief	LCMB	NCI
	J. E. Dahlberg	Research Microbiologist	LCMB	NCI
	T. Kawakami	Visiting Associate	LCMB	NCI
	J. C. Lacal	Visiting Associate	LCMB	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanisms of pathogenesis of equine infectious anemia virus (EIAV) is being investigated. The role of related viruses in other diseases is also being assessed. To pursue these problems, EIAV proteins are being produced by using prokaryotic expression systems. The EIAV gag gene precursor has been expressed in E. coli and milligram quantities have been obtained which have made possible development of sensitive radioimmunoassays for EIAV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05511-01 LCMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Purification and Characterization of Epithelial Cell Mitogens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. A. Aaronson	Chief	LCMB	NCI
Others:	J. S. Rubin	Biotechnology Fellow	LCMB	NCI
	P. W. Finch	Visiting Fellow	LCMB	NCI
	W. G. Taylor	Research Biologist	LCMB	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Using heparin-Sepharose affinity chromatography (HSAC), we have isolated a highly enriched preparation of an epithelial cell mitogen from the conditioned media of M426 fibroblasts (derived from embryonic human lung tissue). Other cell lines are being screened for this mitogenic activity. The factor, which appears to be distinct from any previously characterized mitogen, can stimulate DNA synthesis in responsive cells (BALB/MK) at an estimated concentration of 0.1 ng/ml. Determination of an amino terminal protein sequence should be forthcoming and the generation of monoclonal and polyclonal antibodies will facilitate isolation of the factor's cDNA.

Another epithelial cell mitogen has been partially purified from a commercial source of bovine pancreatic ribonuclease type 1. It is retained on HSAC but elutes at a different position than the mitogen from M426. Heparin, itself, is an inhibitor of DNA synthesis in BALB/MK cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05512-01 LCMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Cloning of Gene(s) Encoding an Epithelial Cell Growth Factor

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. A. Aaronson	Chief	LCMB	NCI
Others:	P. W. Finch	Visiting Fellow	LCMB	NCI
	J. S. Rubin	Biotechnology Fellow	LCMB	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An epithelial cell polypeptide growth factor has been partially purified from conditioned media from a human embryonic lung fibroblast cell line, M426. Poly A+ RNA from M426 fibroblasts has been isolated and used to construct a cDNA expression library in the Okayama-Berg plasmid vector, pcDV1, which promotes expression of the cloned cDNA in mammalian cells. This library will be used to screen for expression of the cDNA coding for the mitogen using COS cells as hosts. The latter are capable of greatly amplifying transfected DNAs and therefore the amount of gene product synthesized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05513-01 LCMB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Mechanisms of Transformation Induced by fgr and Related Oncogenes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. C. Robbins	Chief, Molecular Genetics Section	LCMB	NCI
Others:	S. A. Aaronson	Chief	LCMB	NCI
	S. R. Tronick	Chief, Gene Structure Section	LCMB	NCI
	S. Katamine	Visiting Fellow	LCMB	NCI
	M. Cheah	Medical Staff Fellow	LCMB	NCI
	T. Kawakami	Visiting Fellow	LCMB	NCI

COOPERATING UNITS *(if any)*: Division of Hematology/Oncology, Washington Univ., St. Louis, Missouri (T. Ley)

LAB/BRANCH: Laboratory of Cellular and Molecular Biology

SECTION: Molecular Genetics Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Efforts to determine normal functions for protein-tyrosine kinases encoded by human fgr and related proto-oncogenes have focused on the isolation of cDNA molecules representing their transcriptional units and complete coding sequences. We have isolated and sequenced human c-fgr and fyn cDNAs and have deduced the primary amino acid sequence of their encoded product. These findings have made it possible to identify the products of both genes, designated p55 c-fgr and p59 fyn. These gene products are protein-tyrosine kinases with conserved catalytic domains and unique amino terminal regions. We have shown that expression of the human c-fgr gene is limited to normal monocytes, granulocytes, macrophages and Epstein-Barr virus-infected B lymphocytes; and cultured granulocyte precursor cells express c-fgr mRNA only when induced to differentiate. Kinetic studies of p55 c-fgr expression in differentiating granulocytic cells imply that this protein functions in mature cells that no longer are capable of proliferating.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05514-01 LCMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Analysis of an Oncogene Related to a Growth Factor and Its Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. Kawakami	Visiting Associate	LCMB	NCI
Others:	T. Matsui	Visiting Fellow	LCMB	NCI
	E. Finzi	Medical Staff Fellow	LCMB	NCI
	M. H. Kraus	Visiting Associate	LCMB	NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Molecular Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have analyzed the abnormalities of growth factor and its receptor genes in human tumor samples by Southern hybridization. No remarkable gene amplification or rearrangement of platelet-derived growth factor (PDGF)-A chain, c-fms, c-ros or fyn was detected in 124 (22), 99 (21), 99 (21) and 72 (20) tumor cells (tissue species), respectively.

Recently, we have isolated five genomic DNA clones homologous to the proto-oncogene family, which includes fms, kit and PDGF receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04899-15 LMO

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Transforming Genes of Avian RNA Tumor Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. S. Papas	Chief	LMO	NCI
Others:	R. J. Fisher	Expert	LMO	NCI
	J. A. Lautenberger	Senior Staff Fellow	LMO	NCI
	D. K. Watson	Senior Staff Fellow	LMO	NCI
	N. Sacchi	Visiting Associate	LMO	NCI
	N. K. Bhat	Visiting Fellow	LMO	NCI
	S. Fujiwara	Visiting Fellow	LMO	NCI

COOPERATING UNITS *(if any)*
Department of Biology, Johns Hopkins University School of Medicine, Baltimore, MD (E. Moudrianakis); Department of Biology, University of California, Berkeley, CA (P. Duesberg)

LAB/BRANCH
Laboratory of Molecular Oncology

SECTION
Carcinogenesis Regulation Section

INSTITUTE AND LOCATION
NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A major effort of the LMO is to elucidate the processes by which specific retroviral oncogenes, as well as their cellular homologs, are able to impact on critical cellular events. Using the oncogenes, ets and myc, as probes, we have detected, isolated and cloned the cellular homologs of these genes from evolutionarily diverse organisms such as humans, mice, cats, fish, sea urchin and Drosophila. Specific regions of these cellular genes are retained at very high levels of homology and each proto-oncogene was compared to their viral homologs. In all cases, the proto-oncogenes were significantly larger than their corresponding viral oncogenes. This consistent truncation of the viral oncogene and its products may implicate this damage as a general mechanism in events controlled by these highly conserved genes. We have also developed and exploited several expression vector systems, both prokaryotic and eukaryotic, to produce oncogene-specific products in quantity. These expressed products were used to purify, characterize and develop immunologic reagents to locate and characterize the cellular proto-oncogene products. Such reagents have also been used to probe for the expression of oncogene-specific products in normal and malignant tissues and related them to specific human pathologies. In certain leukemias, we have noted an alteration in the chromosomal location of the ets genes and compared their expression in normal and leukemic cells. We have also been able to isolate and characterize a new gene related to the human ets proto-oncogene and chromosomally locate this gene to the same region of chromosome 21. Therefore, it would seem that this gene is a new member of a family of ets genes and these may play a significant role, by their location, in diseases other than cancer. In particular, genes in this region appear to be implicated in Down's syndrome, in addition to leukemia disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04963-11 LMO

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Toward a Molecular Description of Malignant Transformation by p21 ras Oncogenes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. Y. Shih	Research Chemist	LMO	NCI
Others:	L. S. Ulsh	Microbiologist	LMO	NCI
	D. J. Clanton	Senior Staff Fellow	LMO	NCI
	P. Saikumar	Visiting Fellow	LMO	NCI
	D. G. Blair	Supv. Research Chemist	LMO	NCI
	Y. Lu	Visiting Fellow	LMO	NCI

COOPERATING UNITS *(if any)*
ERRB, NICHD, NIH (K. P. Huang); U. of Tokyo, Tokyo, Japan (S. Hattori); NAPS, PRI, Frederick, MD (G. DuBois)

LAB/BRANCH
Laboratory of Molecular Oncology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major focus of this project is to investigate the molecular biology and biochemistry of the ras oncogenes and the ras p21 proteins. The long-range objective is to elucidate molecular mechanisms of cell transformation induced by these genes and their products. We have found novel phosphorylation of p21 of H- and K-ras genes, distinct from autophosphorylation previously known in Harvey and Kirsten viral ras oncogene products. The phosphorylation of ras proteins in cells was stimulated by phorbol ester. Protein kinase C phosphorylated p21 in vitro. The present results suggest that these novel phosphorylations were mediated by kinase C. Structure-function of ras proteins were investigated by methods of site-directed mutagenesis, enzymology, and immunochemistry. Results indicate that the structure of the GTP-binding domain of p21 is very similar to that of a super family of G-proteins important in cellular signal transduction. The GTP-binding domain functions as a switch region for the regulatory roles of p21. Classes of mutants have been found that either render p21 in a permanent on mode, or inactivate p21. Studies on a neutralizing monoclonal antibody, which has been shown to block p21 cellular activities, indicates that the dissociation off-rate of pre-bound GDP for the exchange with GTP is important for p21 function. The nucleotide exchange rate is significantly higher in the viral ras p21 than that of the proto-oncogene p21, suggesting its role in high oncogenicity of viral oncogenes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04970-11 LMO

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemistry of Cellular Transformation by Avian Tumor Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. P. Bader	Research Microbiologist	LMO	NCI
Others:	D. A. Ray	Chemist	LMO	NCI
	F. A. Hausman	Chemist	LMO	NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Oncology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	1.0	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The product of the myc oncogene is responsible for the alteration of growth potential and induction of malignancy in cells in which the oncogene is active. The function of the myc protein has been studied by localizing the protein within the cell and examining the intracellular properties of the protein. Cells infected with the avian MC29 virus produce a hybrid protein, p110, which contains elements of both avian retrovirus and myc protein, and can be detected and quantitated by using antisera to either of these elements. Radiolabeled p110 migrates rapidly to the nucleus where it can be found in both chromatin-containing and nucleoplasmic fractions. The nucleoplasmic fraction contains about two-thirds of the initially labeled p110, which is degraded with a half-life of 30-40 minutes. The p110 in the chromatin-containing fraction has an extended half-life, about two hours. Steady-state analyses revealed a greater amount of p110 in the chromatin fraction than in the nucleoplasm, and the p110 associated with chromatin was found to be more highly phosphorylated than that in the nucleoplasm. Other studies indicate that p110 is associated with DNA, consistent with the in vitro binding properties of this protein. We suggest that the stability of the myc protein is dependent upon its association with DNA, and results with inhibitors of transcription suggest that the association of myc protein with chromatin is dependent upon transcription.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05120-08 LMO

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Expression of Retroviral and Oncogene Proteins in Bacterial and Mammalian Vectors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. A. Lautenberger	Research Chemist	LMO	NCI
Others:	F. Wong-Staal	Biologist	LTCB	NCI
	T. S. Papas	Chief	LMO	NCI
	Z-Q. Chen	Visiting Associate	LMO	NCI

COOPERATING UNITS *(if any)*

BRI-Basic Research Program, Frederick, MD (A. Seth); School of Life and Health Sciences, University of Delaware, Newark, DE (L. Levinger);

LAB/BRANCH

Laboratory of Molecular Oncology

SECTION

Carcinogenesis Regulation Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A protein has been synthesized in E. coli that contains HTLV-I gag gene sequences. The HTLV-I gag gene was placed into the mos gene coding sequences in the expression vector, pA28, a derivative of pJL6 developed in our laboratory. A 30 kDa protein can be detected by anti-mos peptide antibody. By use of an inclusion body purification protocol, this protein can be made 50% pure without the use of column chromatography. This protein is potentially useful as a diagnostic reagent since it is recognized by antibodies in patient serum.

A protein was identified in nuclear extracts of the HTLV-I cell line, C10/MJ, that specifically binds a region on the LTR near the polyadenylation site. The specificity of binding was verified by demonstrating that it could be abolished by the addition of unlabeled LTR DNA as a competitor, but not by pBR322 DNA. Little or none of this activity was found in other cell lines tested, including MJ leukemic T-cells, indicating that this phenomena is specific to the C10/MJ line.

DNA sequences from the sea urchin, Lytechinas variegatus, related to the v-ets oncogene from avian erythroblastosis virus, E26, were molecularly cloned. They were shown to have a high degree of sequence homology with the region of v-ets that is also homologous with the Hu-ets-2 domain found on human chromosome 21. Northern blot analysis of sea urchin tissues and developing embryos indicated that the gene is actively transcribed in the early stages of embryonic development and somewhat less so in unfertilized eggs. No transcript was detected in adult somatic tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05238-06 LMO

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Transforming Genes of Acute Leukemia Viruses and Their Cellular Homologues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. K. Watson	Senior Staff Fellow	LMO	NCI
Others:	T. S. Papas	Chief	LMO	NCI
	S. J. O'Brien	Chief	LVC	NCI
	L. J. Pribyl	Biologist	LMO	NCI
	R. J. Van Beneden	Guest Researcher	LMO	NCI

COOPERATING UNITS *(if any)* Developmental Genetics Lab., Johns Hopkins Hospital, Baltimore, MD (R. Reeves); Dept. Molecular Biology, U. California, Berkeley, CA (P. H. Duesberg); Program Resources, Inc., Frederick, MD (S. Reddy, S. Showalter, M. J. Smith); LBI-Basic Research Program, Frederick, MD (A. Seth)

LAB/BRANCH

Laboratory of Molecular Oncology

SECTION

Carcinogenesis Regulation Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	0.9	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To provide an initial step toward understanding the functional relationship between the onc genes of transforming retroviruses and their cellular prototypes, structural comparisons at the nucleic acid and protein levels have been carried out. We have determined the complete nucleotide sequence of the chicken ets gene and compared it to the ets gene of E26. E26 is a genetic hybrid with sequences derived from viral structural genes and parts of essential cellular proto-onc genes. The chicken ets gene is present as a single locus with v-ets homologous sequences found in nine regions over 60 kb of genomic DNA. The major difference between v-ets and c-ets sequences is found at the 3' end, resulting in different carboxy-termini of p135 (gag-myb-ets transforming protein of E26) and the cellular proto-ets product. The cellular gene contains additional 5' sequences that can be found in chicken cDNA. The first two viral homologous regions are not found in the major ets transcript, suggesting that they are not true exons. Thus, the E26 virus demonstrates: (1) substitution of viral genes for parts of normal cellular genes; (2) truncation of the gene; and (3) acquisition of non-cellular coding proto-ets sequences. These structural differences may be responsible for the oncogenic potential of this retrovirus. We have previously determined that the mammalian homologs of v-ets consist of two distinct domains located on different chromosomes. The mammalian ets genes from man and mouse encode for identical amino acids and are over 90% conserved relative to the chicken ets gene. Ets-related genes have been isolated from Drosophila and sequence analysis indicates that the ets-2 gene of Drosophila has been highly conserved and differentially expressed during development. Because ets sequences can be found either on different chromosomes (mammals) or as contiguous sequences (chicken), we can conclude that the v-ets contains at least two domains. As a further means to characterize the ets genes, viral ets and human ets gene regions have been expressed in bacteria.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05295-06 LMO

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Activation of onc Genes in Viruses and Human Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. G. Blair	Supv. Research Chemist	LMO	NCI
Others:	T. S. Papas	Chief	LMO	NCI
	K. J. Dunn	Bio. Lab. Tech. (Micro.)	LMO	NCI
	Q. Yuan	Visiting Fellow	LMO	NCI
	Y. Lu	Visiting Fellow	LMO	NCI
	D. J. Clanton	Senior Staff Fellow	LMO	NCI

COOPERATING UNITS *(if any)*
Mol. Mech. of Car. Lab., Basic Research Program, BRI, Frederick, MD (G. F. Vande Woude, A. Seth, M. K. Oskarsson); Nucl. Acid & Protein Syn. Lab., PRI, Frederick, MD (M. Zweig, S. D. Showalter, D. O. Halverson, L. A. Eader)

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SECTION
Microbiology Section

INSTITUTE AND LOCATION
NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.75	1.75	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have constructed a murine homolog (ME26) of the avian acute leukemia virus, E26, which replicates efficiently in murine cells and expresses transforming functions both in vitro and in vivo. The ME26 p135 gag-myb-ets fusion protein is associated with the nucleus and is at least partially myristilated. NIH 3T3 mouse fibroblasts infected with ME26 form foci of overgrowing cells at low serum concentrations in tissue culture and form small colonies in agar suspension. Newborn NFS mice infected with ME26 rescued with amphotropic MuLV develop leukemia beginning about 100 days after infection, while animals infected with helper virus alone show no incidence of disease.

We have identified a novel human DNA sequence with transforming potential which appears to have been generated as the result of the fusion of two human sequences during NIH 3T3 transfection. Portions of the sequences involved have been mapped to human chromosomes 8 and 9. These transforming sequences are not related to known oncogenic sequences located on these two chromosomes, nor to any of 10 other oncogenes tested. NIH 3T3 cells transformed by these sequences acquire the ability to grow in serum-free media, and conditioned media from these cells allow normal NIH 3T3 cells to grow in the absence of serum.

Treatment of mouse fibroblasts with tunicamycin, an inhibitor of N-linked glycosylation, renders it susceptible to infection by the cat endogenous virus, RD114. The induction of the susceptible state is rapid and transient, and requires only subtoxic doses of tunicamycin. The effect is specific for RD114, and treated mouse cells remain resistant to GaLV, FeLV, or murine xenotropic viruses. An RD114 recombinant with an altered gp70 is unable to infect treated mouse cells, suggesting that the acquired susceptibility involves some specific interaction between the RD114 envelope and a cellular receptor protein.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05440-03 LMO

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Site-Directed Mutagenesis of ras Oncogenes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. J. Clanton	Senior Staff Fellow	LMO	NCI
Others:	T. Y. Shih	Research Chemist	LMO	NCI
	L. S. Ulsh	Microbiologist	LMO	NCI

COOPERATING UNITS *(if any)* Department of Pure and Applied Research, University of Tokyo, Tokyo, Japan (S. Hattori)

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NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Oligonucleotide-directed, site-specific mutagenesis is used to dissect the biochemical basis of oncogenic activation and of enzymatic activity of the ras oncogene. Studies are directed toward an understanding of the interrelationship between the known properties of the ras gene product. Mutagenesis of the ras oncogene in specific regions of the protein has been designed to explore the active center which is believed to be responsible for these properties.

Point mutations of p21 proteins were constructed by oligonucleotide-directed mutagenesis of the v-ras-H oncogene, which substituted amino acid residues within the nucleotide-binding consensus sequence, GXXXXGK. When the glycine residue at position 10, 13, or 15 was substituted with valine, the viral ras-H product, p21, lost its GTP-binding and autokinase activities. Other substitutions at position 22, 33, 51 or 59 did not impair its binding activity. G418-resistant NIH 3T3 cell lines were derived by transfection with constructs obtained by inserting the mutant proviral DNA into the pSV2neo plasmid. Clones with valine mutation at position 13 or 15 were incapable of transforming cells, while all other mutants with GTP-binding activity were competent. Ras, with a valine mutation at glycine-10, which had lost its ability to bind GTP and its autokinase activity in vitro and in vivo, was fully capable of transforming NIH 3T3 cells. These cells grew in soft agar and formed tumors in nude mice. The p21 of cell lines derived from tumor explants still lacked the autokinase activity. These findings suggest that the glycine-rich consensus sequence is important in controlling p21 activities and that certain mutations may confer p21 its active conformation without participation of ligand binding.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05441-03 LMO

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of the Gene Products of the c-myc Locus and the c-ets Locus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. J. Fisher	Expert	LMO	NCI
Others:	S. Fujiwara	Visiting Fellow	LMO	NCI
	N. Bhat	Visiting Fellow	LMO	NCI
	T. S. Papas	Chief	LMO	NCI

COOPERATING UNITS *(if any)*

Nucleic Acid and Protein Synthesis Laboratory, Program Resources, Inc., Frederick, MD (M. Zweig, G. DuBois and S. Showalter)

LAB/BRANCH

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Transgenic Analysis Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, MD 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The protein products of the ets-1 and ets-2 genes have been identified and characterized with polyclonal anti-peptide antibodies and with a monoclonal antibody prepared against a bacterially-expressed human ets-2 protein. The ets-1 protein is 52 kDa and found primarily in cells of lymphoid origin; the p56 ets-2 protein was found to be widely distributed and located in the nucleus. In addition, a nuclear p60 and p53 were identified as ets-2-related proteins which share a limited homology by two-dimensional peptide mapping. The nuclear p56 was purified to homogeneity and its N-terminal 20 amino acid sequence determined. An oligonucleotide probe was made from this sequence to reprobe cDNA and genomic libraries for additional clones of ets-2-related proteins. The next part of the work is to purify the native form of the ets-2 protein in order to give insight into its function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05442-03 LMO

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Human ets Genes in Human and Cancer Genetics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	N. Sacchi	Visiting Scientist	LMO	NCI
Other:	T. S. Papas	Chief	LMO	NCI

COOPERATING UNITS (if any)
Dept. Neurogenetics, Harvard Univ., Boston, MA (J. F. Gusella); Eleanor Roosevelt Cancer Inst., Denver CO (H. D. Drabkin); School of Medicine, Univ. Milan, Milan, Italy (G. Bigi); Down Syndrome Center, Genova, Italy (L. Perroni)

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SECTION
Carcinogenesis Regulation Section

INSTITUTE AND LOCATION
NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The localization of human ets genes at the 11q23 and 21q22 regions suggested a possible involvement of these genes in both constitutional and acquired (neoplasia) diseases, presenting a known cytogenetic abnormality. The 11q23 region is involved in a number of chromosome abnormalities peculiar to acute leukemias of the myelomonocytic lineage. In two of these abnormalities, the translocations (4;11)(q21;q23) and (9;11)(p21;q23), transpositions of the ets-1 gene from its normal position on chromosome 11 to chromosomes 4 and 9, is evident. On the other side, the 21q22 region is relevant both in human and cancer genetics. In an acquired cytogenetic abnormality specific to AML-M2 leukemias, we found ets-2 transposed from chromosome 21 to chromosome 8. Despite the repositioning of the ets genes, neither one was found structurally involved by the chromosome rearrangements. The role of these genes in the pathogenesis of these leukemias is, therefore, not directly demonstrated, even if "position effect," well known to affect gene regulation at a distance, may somehow alter their expression. The real "cancer genes" involved by the above-mentioned abnormalities, therefore, have to be identified.

As far as the ets-2 gene is concerned, part of the work was aimed at demonstrating whether or not it does belong to the obligate genetic region necessary for the expression of the constitutional aneuploidy known as Down's syndrome. This work led to a preliminary observation relative to one patient (only very rare Down's syndrome patients are informative) of three copies of ets alleles in the region. It is, therefore, possible that the ets-2 gene belongs to the set of genes needed for the expression of the multitrait Down's syndrome clinical picture.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05443-03 LMO

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Oncogene Expression During Cell Differentiation and Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. J. Fisher	Expert	LMO	NCI
Others:	N. K. Bhat	Visiting Fellow	LMO	NCI
	S. Fujiwara	Visiting Fellow	LMO	NCI
	R. Ascione	Research Chemist	LMO	NCI
	T. S. Papas	Chief	LMO	NCI

COOPERATING UNITS *(if any)*

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NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Analysis of c-ets gene expression during cell proliferation and differentiation indicate that (i) the ets-1 and ets-2 genes are activated by serum addition to quiescent fibroblast cells before DNA synthesis; (ii) the increase in the level of ets mRNAs is due to an increase in the transcription of these genes and stabilization of their mRNA; (iii) during hepatic regeneration, only the ets-2 mRNA, but not the ets-1 mRNA, level increases before DNA synthesis; (iv) the ets-1 and ets-2 genes are differentially regulated; (v) in vivo ets-2 gene expression is regulated mainly at the post-transcriptional level; (vi) addition of TPA to HL60 cells appears to stabilize both ets-1 and ets-2 mRNAs; and (vii) subcellular fractionation indicates that 56 kDa protein is localized in the nucleus, whereas 55 kDa ets-1 protein localized in the cytoplasm and the nucleus.

These results suggest that the ets-2 gene products accumulate well before DNA synthesis and its expression is intrinsically linked with cell proliferation and follows a pattern similar to other members of the nuclear oncogene family. Depending on particular cell or tissue type, different control mechanisms may be operative in regulating ets gene loci.

The role of ets gene products in T-cell proliferation in different types of T-cells, hematopoietic tumors and in hepatoma are under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05483-02 LMO

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

RNA Processing, Transcription Termination and Gene Control

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. L. Court	Research Biologist	LMO	NCI
Others:	H. E. Takiff	Guest Researcher	LMO	NCI
	R. J. Fisher	Expert	LMO	NCI
	T. A. Patterson	Biotechnology Fellow	LMO	NCI
	S-M. Chen	Guest Researcher	LMO	NCI
	T. L. Wigle	Biologist	LMO	NCI

COOPERATING UNITS *(if any)* Program Resources, Inc., Frederick, MD (M. Zweig, N. Costantino, K. Johnson); Dept. of Gen. & Mol. Biol., Cent. de Invest. y de Estudios Avanzados Del IPN, Mexico City, Mexico (G. Guarneros); Inst. of Medical Science, University of Tokyo, Tokyo, Japan (Y. Nakamura)

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Laboratory of Molecular Oncology

SECTION

Molecular Control and Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

RNaseIII is a double-strand specific endoribonuclease that has different functions in E. coli. It processes rRNA precursors for efficient maturation into ribosomes. It processes some mRNAs either to activate gene expression or to reduce gene expression. It regulates mRNA degradation.

The int gene of phage λ is transcribed from two promoters yielding different mRNA transcripts. Int expression from one is reduced by RNaseIII; from the other, expression is enhanced. In both cases, control of expression by RNaseIII occurs from a single site beyond the gene. This form of control is named retroregulation. The site present on the RNA is able to form a special stem and loop structure that is recognized by RNaseIII. This site is also a transcription termination signal for RNA polymerase.

In order to understand how RNaseIII levels in the cells are modulated, its gene in E. coli, rnc, has been cloned on λ vectors and on pBR322 plasmid. Sequence analysis indicates a second gene in an operon with rnc. This gene produces a protein with significant homologies to the yeast ras genes and is called era (E. coli ras). Both rnc and era have been placed on expression vectors and their proteins have been purified and antibodies have been made. Era is an essential gene in E. coli. The purified protein binds GTP. E. coli mutants have been isolated that are conditionally lethal because of mutations in rnc and era. Suppression mutants that restore growth are being analyzed to determine proteins that may interact or compensate for the products of these genes.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05484-02 LMO

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Proto-oncogene ets in Sea Urchin and Xenopus larvis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

PI:	Z.-Q. Chen	Visiting Associate	LMO	NCI
Others:	J. A. Lautenberger	Research Chemist	LMO	NCI
	S. Fujiwara	Visiting Fellow	LMO	NCI
	R. J. Fisher	Expert	LMO	NCI
	R. Ascione	Research Chemist	LMO	NCI
	T. S. Papas	Chief	LMO	NCI

COOPERATING UNITS *(if any)*

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NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
1.8	1.8	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Southern blot analysis of DNA derived from sea urchin, Lytechinus variegatus, that had been cleaved by EcoRI or HindIII revealed a major strong hybridization band using a v-ets probe. The band obtained was constructed from a charon 28 library from DNA fragments of this size. A phage (12E3) containing sequences hybridizing to the E26 v-ets probe was isolated from this library and the ets-homologous region was sequenced by the dideoxynucleotide chain-termination method. A highly homologous sequence to E26 v-ets was found; this region is the same one that also corresponds to the human (Hu-ets-2) homologous sequences defined in our lab. The sea urchin homology with v-ets begins at a consensus splice acceptor sequence and ends at the point where it is known that v-ets and Hu-ets homology diverge. Ninety-one out of 97 (or 94%) predicted amino acids share identity between the sea urchin c-ets and E26 v-ets over their region of homology. A somewhat weaker homology with the Hu-ets-2 sequences continues beyond this point for 13 more codons, ending at a common termination codon. Methods for culturing the embryos of sea urchin and Xenopus larvis have been established. A single 6.8 kb ets-related RNA was observed by Northern blot analysis from the unfertilized egg stage until the blastula stage of development in the sea urchin embryos. The maximal level of expression occurred in the early stages of embryonic sea urchin development (16 cells to morula stage). Western blot and immunoprecipitation analysis of sea urchin embryo protein extracts revealed a 72 kDa band that is identifiable by anti-human ets-2 peptide antibody. Microinjection of antibody (anti-ets-2) into sea urchin embryos has been started in order to find some clues to the ets gene functions in these cells. Several positive clones have already been found from screening of the Xenopus larvis DNA library with the v-ets probe. Both cDNA library constructions have been started.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05485-02 LMO

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Application of Monoclonal Antibodies to the Study of Oncogene Products

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. J. Fisher	Expert	LMO	NCI
Others:	S. Fujiwara	Visiting Fellow	LMO	NCI
	N. K. Bhat	Visiting Fellow	LMO	NCI
	T. S. Papas	Chief	LMO	NCI

COOPERATING UNITS *(if any)*
BRI-Basic Research Program, Frederick, MD (A. Seth)

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Carcinogenesis Regulation Section

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NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

c-myc and c-ets (1 and 2) genes are cellular homologues of the oncogenes carried by the avian myelocytomatosis virus MC29 and the avian acute leukemia virus E26, respectively. These genes are suspected to have some roles in the pathogenesis of certain types of human malignancy. Production of monoclonal antibodies against products of these genes was planned for application to the biological and biochemical characterization of these products. A monoclonal antibody against the human myc gene products has already been produced and described in last year's report. This year, two monoclonal antibodies have been generated against the human ets-2 gene product. These antibodies recognize a 56 Kd nuclear protein from various human cell lines, which was identified as a product of the human ets-2 gene. One monoclonal antibody recognizes two other proteins of 60 Kd and 53 Kd. These two proteins appear to be antigenically related to the 56 Kd ets-2 protein. The monoclonal antibodies also react with the ets-2 proteins from mouse, chicken and sea urchin. Thus, it is likely that the antibodies detect epitopes that are highly conserved in evolution. This high degree of conservation implies the functional importance of the domain detected by the antibodies. These antibodies are being used as immunological probes in the screening of cDNA expression library for ets-2-specific clones.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05515-01 LMO

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
cDNA Cloning, Sequencing, Expression and Chromosomal Localization of Human erg Gene

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. S. Papas	Chief	LMO	NCI
Other:	V. N. Rao	Visiting Fellow	LMO	NCI

COOPERATING UNITS *(if any)*
Nucleic Acid and Protein Synthesis Laboratory, Program Resources, Inc., Frederick, MD (E. S. P. Reddy)

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NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The replication-defective avian erythroblastosis virus, E26, induces a mixed erythroid/myeloid leukemia in chickens. E26 includes elements from two proto-oncogenes, chicken proto-myb and chicken proto-ets, and Δgag from the viral gag gene. Human genomic clones homologous to the ets region were cloned and shown to be related to the v-ets region by partial sequence analysis. The human ets-1 locus on chromosome 11 encodes a single mRNA of 6.8 kb; the human ets-2 locus encodes three mRNAs of 4.7 kb, 3.2 kb and 2.7 kb. The Hu-ets-1 and Hu-ets-2 genes have recently been shown to be transposed in certain leukemias. Because of the significance of ets in neoplasia, we embarked on a search for other human genes closely related to ets. A cDNA library was prepared from a human COLO 320 cell line which expresses very high levels of ets-specific transcripts. Two cDNA clones reactive with the Hu-ets-2 probe were isolated. Characterization of these clones by restriction mapping and sequence analysis revealed that they represented the complete coding sequence of a novel human gene named erg (ets-related gene). The erg gene shows a homology of ~40% and ~70% to two domains of the 5' and 3' regions of the v-ets oncogene. One of the cDNAs (erg-2) differs from erg-1 by a splicing event that causes a coding frameshift near the NH-2 terminus, resulting in an additional 99 a-a insertion at the amino terminal end. There is preliminary evidence to state that erg-2 may use a different translation initiation and poly-adenylation signal. The full-length cDNA clones, erg-1 and erg-2, are being expressed in vitro and in vivo in E. coli and in mammalian cells. The erg gene has been localized on human chromosome 21. In situ hybridization studies for chromosomal localization of the erg gene are in progress. Thus, the precise location of the erg gene and analysis of the erg locus in different human cancers, Down's syndrome and Alzheimer's disease should make it possible to determine if amplification, translocation or rearrangement of this gene can be linked to any of these diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05516-01 LMO

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of Normal and Oncogenic ras Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. Y. Shih	Research Chemist	LMO	NCI
Others:	P. Saikumar	Visiting Fellow	LMO	NCI
	D. J. Clanton	Senior Staff Fellow	LMO	NCI
	L. S. Ulsh	Microbiologist	LMO	NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Oncology

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NCI, NIH, Frederick, MD 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To understand the molecular principles involved in the cellular transformation by ras oncogenes has been the major focus of this project. The understanding of the structure-function relationship of ras protein is important in this regard. As a necessary prelude, we have changed v-H-ras DNA at two positions with point mutations by oligonucleotide-directed mutagenesis. The v-ras protein differs from the cellular proto-oncogene product at the amino acid positions 12 and 59. We have obtained mutants 12R/59T (equivalent to v-ras), 12R/59A, 12R/59S, 12G/59T, 12G/59S and 12G/59A (equivalent to c-ras). We compared some of the biochemical properties of these proteins, especially guanine nucleotide binding, nucleotide exchange and GTPase activities. Our preliminary results indicate that single point mutations, either at positions 12 or 59, produce oncogenic activation reflected in their GTPase activity (lowered). Position 59 is important in the nucleotide exchange of ras proteins. Threonine or serine are required for higher rate of exchange. Alanine at position 59 renders a poor exchange of nucleotides. Thus, altered amino acids at positions 12 and 59 sustain the v-ras protein in an activated state. Our studies are continuing in the direction of understanding the signal-transducing role of p21.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05517-01 LMO

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Changes in Transcription Induced by myc Protein

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. P. Bader	Research Microbiologist	LMO	NCI
Others:	M. Ohtsuka	Visiting Fellow	LMO	NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Oncology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cells transformed by the avian MC29 virus and related viruses assume a characteristic microscopic appearance which differs from the changes seen in cells transformed by other viruses or other agents. We are interested in identifying transcriptional changes which occur specifically as a result of infection with these myc-containing viruses, which may differ from general changes which occur in all cells transformed to malignancy. Rat embryo cells were infected with SV40 virus, and clones of transformed cells were superinfected with murine retrovirus constructs containing the myc oncogene. Polyadenylated messenger RNA was isolated, and cDNA libraries were constructed and cloned in bacteriophage. The induction or repression of mRNAs by myc using these libraries and radiolabeled cDNAs is under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05101-09 LMV

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Molecular Mechanisms for Malignant Transformation of Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Gilbert Jay	Chief, Cell Physiology Section	LMV	NCI
Others:	Steven Hinrichs	Medical Staff Fellow	LMV	NCI
	Michael Nerenberg	Medical Staff Fellow	LMV	NCI
	Kazuhiko Koike	Visiting Fellow	LMV	NCI

COOPERATING UNITS *(if any)*

Department of Biology, The Johns Hopkins University (Charles Bieberich)

LAB/BRANCH

Laboratory of Molecular Virology

SECTION

Cell Physiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.4	3.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to investigate the molecular mechanisms underlying the malignant transformation of cells. In our studies, we have made use of a RNA tumor virus (human T-lymphotropic virus type 1) and a DNA tumor virus (human adenovirus type 12). We have succeeded in deriving transgenic mouse models for the study of neurofibromatosis, gastric carcinoma, and mammary carcinoma. These experimental models will be particularly useful not only for improved diagnosis and treatment of the corresponding human malignancy, but also for a detailed analysis of the molecular basis for their etiology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05216-07 LMV

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ras Oncogene Regulation in Yeast

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ravi Dhar	Visiting Scientifist	LMV	NCI
Others:	T.L.V. Sreenath	Visiting Fellow	LMV	NCI
	Richard Koller	Biologist	LMV	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Virology

SECTION

Virus Tumor Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Expression of the ras1 and ras2 genes of Saccharomyces cerevisiae has been examined at the transcriptional and translational levels. In cells grown with glucose as carbon source, ras1 mRNA and ras1 protein synthesis were detected only in the early exponential phase of growth. By contrast, ras2 protein synthesis was low in the early exponential phase, increased 10-fold and remained nearly constant into the stationary phase. The ras2 mRNA level was high and nearly constant until late in the exponential phase and decreased considerably as cells entered the stationary phase. Taken together, these data suggest that translational control is important in regulating ras2 gene expression in cells grown on glucose. Nutrient starvation, leading to G1-arrest and sporulation in diploids, had little effect on the rate of ras2 protein synthesis, but lead to decreased amounts of ras2 mRNA. This decrease was accomplished in part by selective repression of ras2 transcripts with particular 5' ends. Our data also suggest that nutrient starvation is another condition in which translational control is prominent in regulation of ras2 expression. The fact that a large decrease in the amount of ras2 mRNA occurs in the stationary phase and starvation conditions, but is associated with little effect on ras2 protein synthesis, suggests that ras2 transcriptional control in these conditions is designed primarily to offset changes in translational efficiency.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05217-07 LMV

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Regulation of SV40 Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	John Brady	Expert	LMV	NCI
Others:	Kamel Khalili	Visiting Fellow	LMV	NCI
	Jeffrey Green	Biotechnology Fellow	LMV	NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Molecular Virology

SECTION
Virus Tumor Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Simian virus 40 (SV40) has two early transcriptional units that are transcribed from a region near the origin of replication. The early-early (EE) transcription unit, whose RNA encodes a large T-antigen and small t-antigen, predominates at early times post infection. The late-early (LE) transcription unit RNA initiation sites are located upstream of the EE TATA box and function at late times post infection. We have characterized the in vitro translational efficiency of SV40 early-early (EE) and two late-early (LE) RNAs. We demonstrated that the presence of one or two potenial AUG initiator codons in the leader sequences of the LE RNAs inhibits efficient translation from the downstream T-antigen initiator, AUG. In addition, translation of the LE RNA resulted in the synthesis of new viral proteins, 2.7 Kd in size. The role of this protein is currently under investigation. Carboxy terminal mutants of T-antigen cause a minimal decrease in the efficiency of viral DNA replication but under appropriate conditions significantly decrease the yield of infectious virus particles by three orders of magnitude. Our studies have demonstrated that a reduction in viral late RNA is, in part, responsible for the lower titers produced by these mutants in CV-1P cells. Furthermore, we have demonstrated that the viral late protein, agnoprotein, is not produced in CV-1P cells infected with C-terminal mutants. This suggest that T-antigen plays a role in the translation of agnoprotein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05220-07 LMV

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Studies on the Structure and Function of Cell Surface Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Gilbert Jay	Chief, Cell Physiology Section	LMV	NCI
Others:	Jonathan Vogel	Medical Staff Fellow	LMV	NCI
	Roberta Reynolds	Research Microbiologist	LMV	NCI

COOPERATING UNITS *(if any)*
Department of Biology, The Johns Hopkins University (George Scangos)

LAB/BRANCH
Laboratory of Molecular Virology

SECTION
Cell Physiology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have cloned and analyzed cDNA sequences derived from a family of genes which encode the classical transplantation antigens. Our findings have led to a better understanding of the structure and function of these cell surface antigens, particularly with regard to the regulation of their expression in both normal and cancer cells.

We have studied the expression and function of the human interleukin-2 receptor. Our findings suggest the existence of a secreted interleukin-2 receptor which can bind interleukin-2 efficiently, and may function to regulate the interaction between interleukin-2 and its cell surface receptor. By using DNA-mediated gene transfer, we have demonstrated that the interleukin-2 receptor can function effectively in nonlymphoid cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05254-06 LMV

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kuan-Teh Jeang	Medical Staff Fellow	LMV	NCI
Others:	John Brady	Expert	LMV	NCI

COOPERATING UNITS *(if any)*

Dana Farber Cancer Institute, Boston, MA (David Livingston)

LAB/BRANCH

Laboratory of Molecular Virology

SECTION

Virus Tumor Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are interested in understanding the regulation of enhancer-dependent gene expression in vivo. Specifically, there are two major areas of interest: (1) regulation of gene expression in undifferentiated cells and (2) interaction(s) of protein factors that may effect enhancer-dependent expression. Our studies have focused on the role of DNA binding proteins in the regulation of gene transcription. We have found that a prokaryotic DNA-binding protein can be functional in eukaryotic cells. Specifically, the placement of DNA-binding sites such that the E. coli lac repressor molecule surrounds the SV40 enhancer sequences bidirectionally can negatively modulate the expression of a linked gene. This result suggests certain models that may explain the actions of enhancer sequences.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05354-05 LMV

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Activated Form of the Human Proto-oncogene, c-Ha-ras

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John Brady	Expert	LMV	NCI
Others:	Rudy Pozzatti	Guest Researcher	LMV	NCI
	Mary McCormick	Senior Staff Fellow	LMV	NCI
	Lance Liotta	Chief	LP	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Molecular Virology

SECTION
Virus Tumor Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have transfected various viral and cellular oncogenes into primary cultures of rat embryo cells and have obtained lines of morphologically transformed cells. Transformation with the ras oncogene alone was observed; however, a 10-fold increase in the transformation frequency was obtained when ras was cotransfected with the adenovirus E1A gene. We have examined cell lines transformed by the ras oncogene alone, and by ras plus E1A and have observed a striking difference in their metastatic potential as assayed in nude mice. Specifically, the ras alone transformants are highly metastatic, while the two gene transformants show a very low metastatic potential. Transfection of the serotype 2 E1A gene, but not the serotype 12 E1A gene, into the ras alone transformants results in a substantial reduction (at least 10-fold) in the metastatic potential of these cell lines. Experiments are in progress to investigate the mechanism by which the adenovirus type 2 E1A gene reduces the metastatic potential of the ras alone transformants. In addition, we have constructed two cDNA libraries from both a high metastatic and a low metastatic cell line. These libraries will be screened with cDNA probes in order to isolate genes that are uniquely or preferentially expressed in either cell line.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05355-05 LMV

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Immune Surveillance Against Tumor Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Gilbert Jay	Chief, Cell Physiology Section	LMV	NCI
Others:	Roberta Reynolds	Research Microbiologist	LMV	NCI
	Takayuki Yoshioka	Visiting Fellow	LMV	NCI

COOPERATING UNITS *(if any)*
Department of Pharmacology, State University of New York at Stony Brook (Sidney Strickland)

LAB/BRANCH
Laboratory of Molecular Virology

SECTION
Cell Physiology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.7	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Since the class I molecules are self antigens present on the surface of all cells in the body, the immune system must be rendered tolerant to them. Yet, these class I antigens must be recognized by cytotoxic T-cells in association with virus-infected and tumor cells. In our analysis of class I genes, we have identified a related gene which may function to regulate this self-nonself recognition. This class I gene is expressed only in the liver and encodes a secreted class I antigen. Our demonstration of the secretion of a class I antigen by the liver has explained a previous observation that liver grafts across histocompatibility barriers were never rejected and has led us to suggest that this molecule serves to modulate class I restriction. We reasoned that a molecule with class I specificity that is constantly secreted into the circulation could act as a "blocking" factor, leading to suppression of class I recognition. The level of expression of such a blocking factor may act directly to modulate self-nonself recognition that will destroy aberrant cell types but not normal cells. This hypothesis has significant implications and suggests a means to modulate the host's response to neoplastic and autoimmune diseases. Attempts are being made to determine what regulates the expression of this particular class I gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05390-04 LMV

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
How do tumor Cells Escape Immune Surveillance?

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Gilbert Jay	Chief, Cell Physiology Section	LMV	NCI
Others:	Jonathan Vogel	Medical Staff Fellow	LMV	NCI
	Lian-Sheng Chen	Visiting Fellow	LMV	NCI

COOPERATING UNITS *(if any)*

Department of Medicine, Harvard Medical School, Massachusetts General Hospital (Kurt J. Isselbacher)

LAB/BRANCH
Laboratory of Molecular Virology

SECTION
Cell Physiology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The classical transplantation antigens (the major histocompatibility complex class I antigens) play a key role in host defense against cells expressing foreign antigens. Several naturally occurring tumors and virally transformed cells show an overall suppression of these surface antigens. Since the class I molecules are required in the presentation of neoantigens on tumor cells to the cytotoxic T-lymphocytes, their absence from the cell surface may lead to the escape of these tumors from immunosurveillance. To test this possibility, a functional class I gene was transfected into human adenovirus 12-transformed mouse cells which do not express detectable levels of class I antigens; the transformants were tested for expression of the transfected gene and for changes in tumorigenicity. The expression of a single class I gene, introduced by DNA-mediated gene transfer into highly tumorigenic adenovirus 12-transformed cells, was sufficient to abrogate the tumorigenicity of these cells. Treatment of adenovirus 12-transformed cells with interferon led to derepression of the endogenous class I genes. Rejection of human adenovirus (Ad12) tumors was observed with intramuscular injections of interferon. Interestingly, Ad12 tumor cells treated with interferon can immunize mice against untreated Ad12 tumors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05391-04 LMV

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transcription Analysis of the SV40 Early and Late Promoter

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John Brady	Expert	LMV	NCI
Others:	Lionel Feigenbaum	Microbiologist	LMV	NCI
	Kamel Khalili	Visiting Fellow	LMV	NCI

COOPERATING UNITS *(if any)*

National Institute of Neurological and Communicative Disorders and Stroke, NIH (Eugene Major)

LAB/BRANCH

Laboratory of Molecular Virology

SECTION

Virus Tumor Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human papovavirus, JCV, is associated with the human demyelinating disorder progressive multifocal leukoencephalopathy. In tissue culture, the virus is largely restricted to growth in primary human fetal glial cells. In this study, we demonstrate two levels of regulation of the viral host range. Expression of the early JCV mRNA, which encodes the essential viral protein, large tumor antigen (T-antigen), depends on recognition of the early enhancer/promoter elements by tissue-specific factors found in both human and rodent glial cells. In the presence of JCV T-antigen, viral DNA replication requires a species-specific factor, presumably a component of DNA polymerase, which is found in a wide range of primate cells. We further demonstrated that simian virus 40 T-antigen has sufficient homology to efficiently substitute for the analogous JCV protein in initiating viral DNA replication. We have used primer extension and S_1 analysis to localize the 5'-termini of JC virus (JCV) early RNAs in infected primary human glial cells at various times postinfection and in stable JCV-transformed hamster fetal glial cells. At early times postinfection (days 1-5), two early transcripts are initiated at nucleotides 5122 and 5082. A major shift in 5'-ends at later times results in the synthesis of a new series of early mRNAs beginning upstream at nucleotide 35 and downstream at nucleotides 5047, 5037, and 5012. In the transformed hamster cells, however, only one RNA species was detected, starting at nucleotide 5122. The mechanism underlying the shift in the initiation site of JCV early RNAs during a lytic infection remains unclear but appears analogous to that which occurs in the SV40 lytic cycle. Since the shift occurs during DNA replication, when T-antigen is at maximal levels, it is possible that T-antigen binding to JCV DNA and/or alterations in chromatin structure contribute to this event.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05392-04 LMV

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of SV40 Late Transcription by Large T-Antigen

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John Brady	Expert	LMV	NCI
Others:	Mary Loeken	Guest Researcher	LMV	NCI
	Mary Ann Thompson	Staff Fellow	LMV	NCI

COOPERATING UNITS *(if any)*

Stony Brook University, New York, NY (Peter Tegtmeyer)

LAB/BRANCH

Laboratory of Molecular Virology

SECTION

Virus Tumor Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The simian virus 40 (SV40) late promoter can be trans-activated by SV40 T-antigen in the absence of DNA replication. Transfection experiments suggest that T-antigen trans-activation may involve either direct promoter binding or induction of one or more cellular transcription factors. In collaboration with Dr. Peter Tegtmeyer, we have demonstrated a role for T-antigen binding site I, as well as II, in the T-antigen binding dependent pathway.

To gain further understanding of the mechanisms by which trans-acting factors interact to recognize transcriptional regulatory sequences, we have examined the ability of SV40 T-antigen and adenovirus E1A protein to stimulate the adenovirus E2 promoter. Chemically synthesized mutants of the E2 promoter function as an inducible enhancer. By insertion of 5, 10, 15 or 20 base pairs of non-specific DNA between inverted repeats in the E2 enhancer, we have found that a specific spatial arrangement of sequences on the E2 promoter are required for trans-activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05393-04 LMV

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of JC Virus Early Region in Transgenic Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John Brady	Expert	LMV	NCI
Others:	Judy Small	Guest Researcher	LMV	NCI
	Lionel Feigenbaum	Microbiologist	LMV	NCI
	Jeffrey Green	Biotechnology Fellow	LMV	NCI
	Kamel Khalili	Visiting Fellow	LMV	NCI

COOPERATING UNITS *(if any)*

Department of Biology, The Johns Hopkins University, Baltimore, MD (G. Scangos)

LAB/BRANCH

Laboratory of Molecular Virology

SECTION

Virus Tumor Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

JC virus (JCV) is a ubiquitous human papovavirus and is strongly associated with the demyelinating disease, progressive multifocal leukoencephalopathy (PML). PML occurs in patients who are immunosuppressed by illness, immunosuppressive therapy or genetic disorders. JCV exhibits a highly specific host range and tissue specificity. In immunosuppressed humans, viral particles are detected in brain cells of glial origin, specifically oliodendrocytes and astrocytes. It is the intent of this study to determine if introduction of JCV into transgenic mice would provide an animal model to study these human diseases. Transgenic mice have been produced containing JC virus early region genes under the control of the JCV promoter/enhancer element. Five mice were obtained containing the JCV sequences. Three female founder mice succumbed to tumors, resulting from metastasis of an adrenal medullary neuroblastoma. Two of five mice produced offspring which developed a neurological disorder related to a myelin deficiency. Neuropathological analysis indicated a myelin deficiency in the central nervous system apparently correlated with the expression of JCV T-antigen in brain tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05394-04 LMV

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Enhancer Elements in B-Lymphocytes and T-Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John Brady	Expert	LMV	NCI
Others:	Imre Boros	Visiting Fellow	LMV	NCI
	Chou-zen Giam	Guest Researcher	LMV	NCI
	Kuan-Teh Jeang	Medical Staff Fellow	LMV	NCI
	Michael Nerenberg	Medical Staff Fellow	LMV	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Molecular Virology

SECTION
Virus Tumor Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.4	3.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A recent focus of this project has been the role of the 3' long open reading frame of the human T-cell leukemia virus type-I (HTLV-I) which encodes a 40-Kd protein (p40x). This protein positively regulates transcription directed by the HTLV-I long terminal repeat (LTR) in a phenomenon known as trans-activation. We have succeeded in expressing the complete p40x coding sequence in E. coli and in a baculovirus vector. Both p40x proteins are capable of stimulating transcription from the HTLV-I LTR. Significant purification of the p40x proteins has been achieved. We have been unable to attribute any sequence-specific DNA binding properties to p40x, suggesting that the protein activates the HTLV-I promoter in an indirect fashion using cellular transcription factors. Our objective is to understand the biochemical mechanism of trans-activation by the p40x protein and the involvement of cellular transcription factors in this process. Distinct transcriptional regulatory sequences located in the upstream sequences of the HTLV-I LTR have been identified and chemically synthesized. These sequences, which have the properties of enhancer sequences, have been cloned and are trans-activated by the HTLV-1 p40x protein. Using a novel DNA-protein cross-linking protocol developed in this laboratory, we have identified cellular factors that interact with the 21 bp p40x-responsive sequence. We have demonstrated, by mutational analysis, that the binding of the cellular proteins in vitro correlates with the in vivo biological activity in response to p40x trans-activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05534-01 LTCB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Mononuclear Phagocytes and Accessory Cells in HIV-1 Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Popovic	Visiting Scientist	LTCB NCI
Others:	R.C. Gallo	Chief	LTCB NCI
	S. Gartner	Senior Staff Fellow	LTCB NCI
	A. Minassian	Guest Researcher	LTCB NCI
	H. Buchow	Guest Researcher	LTCB NCI

COOPERATING UNITS *(if any)*
Institute for Tropical Disease, Hamburg, Germany (P. Racz); Karolinska Institute, Stockholm, Sweden (E.-M. Fenyo); Temple University, Philadelphia, PA (H. Uschner); Cornell University, NY, NY (S. Pahwa)

LAB/BRANCH
Laboratory of Tumor Cell Biology

SECTION
Hematopoietic Cellular Control Mechanisms

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	2.0	3.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

Our studies have focused on the role of monocyte/macrophages and accessory cells (reticuloendothelial system) in the pathogenesis of AIDS. An in vitro cell system of peripheral blood (PB)-derived monocyte/macrophages has been developed. It has been established that PB-derived monocyte/macrophages are as susceptible targets for HIV-1 as PB-derived T-cells. Moreover, this cell system can be successfully applied for virus isolation in situations where T-cell systems fail. Using the monocyte/macrophages as targets, HIV-1 has been isolated from cells of the mononuclear phagocyte lineage from various tissues. These isolates are being characterized with respect to biological behavior, nucleic acid properties of the viral genome, and viral protein expression in monocyte/macrophages vs. T-cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05535-01 LTCB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Retrovirus Infection, Treatment, Prevention and Etiology of TSP

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.S. Sarin	Research Chemist	LTCB NCI
Others:	R.C. Gallo	Chief	LTCB NCI
	Y. Taguchi	Visiting Fellow	LTCB NCI
	M. Civeira	Guest Researcher	LTCB NCI
	C.C. Gajdusek	Chief	CNSS NINCDS
	C.J. Gibbs	Deputy Chief	CNSS NINCDS
	P.R. Johnson	Visiting Scientist	CNSS NINCDS

COOPERATING UNITS *(if any)*
George Washington University Medical Center, Washington, D.C. (A. Goldstein, P. Naylor and R. Schulof); Worcester Fdn. for Experimental Biology, Shrewsbury, MA (P. Zamecnik); Northwestern University, Chicago, IL (R. Letsinger)

LAB/BRANCH
Laboratory of Tumor Cell Biology

SECTION
Hematopoietic Cellular Control Mechanisms

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several drugs have been examined for their capacity to block HIV-1 replication in cell culture. Preliminary studies indicate that foscarnet, D-penicillamine, amphotericin analogs and avarol may be useful in the treatment of acquired immunodeficiency syndrome (AIDS). Antisense oligonucleotides have also been found to be effective in blocking human immunodeficiency virus (HIV-1) replication. A syncytia assay has been developed and is being utilized to measure the effect of these drugs in HIV-1 replication. Antibodies made against a 30 amino acid HIV-1 p17 synthetic peptide (HGP30) were found to inhibit syncytia formation as well as HIV-1 replication in H9 and Molt3 cells. HIV-1 inoculation studies in chimpanzees indicate the development of antibodies against the HIV-1 envelope and core antigens and persistent viremia, but none of the animals have, so far, developed the disease. HTLV-I has been isolated from a patient with tropical spastic paraperesis (TSP) and is being characterized further.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05536-01 LTCB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunologic Studies: HIV Neutralizing Antibodies and Vaccine Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Robert-Guroff	Research Biologist	LTCB NCI
Others:	R.C. Gallo	Chief	LTCB NCI
	M. Reitz	Research Chemist	LTCB NCI
	B. Moss	Chief	LVD, NIAID
	J. Goedert	Medical Officer	EEB, NCI

COOPERATING UNITS *(if any)* Kumamoto Univ., Kumamoto, Japan (S. Matsushita; NY Hosp, Cornell Med. Ctr, NY, NY (P. Giardina; Univ. of Med. & Dentistry, Newark, NJ (. Oleske); Repligen Corp., Cambridge, MA (S. Putney and J. Rusche); Univ. of Paris, Paris, France (D. Zagury); Univ. of Essen, Essen, Germany (O. Thraenhart)

LAB/BRANCH
Laboratory of Tumor Cell Biology

SECTION
Hematopoietic Cellular Control Mechanisms

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In studying immune surveillance mechanisms operative following human immunodeficiency virus (HIV-1) infection, we have concentrated on HIV-1 neutralizing antibodies which we first detected in 1985. In a limited survey, HIV-1-infected individuals with high geometric mean neutralizing antibody titers had lesser disease manifestations. Subsequently in pediatric AIDS cases, a relationship of neutralizing antibodies and a stable clinical state, as opposed to a poor one, was observed. In a retrospective investigation of HIV-1-seropositive thalassemia patients, neutralizing antibodies also were associated with a better clinical outcome. Ongoing long-term prospective studies of HIV-1-infected individuals are aimed at elucidating any protective role of HIV-1 neutralizing antibodies. In related studies, the effect of HIV-1 envelope heterogeneity on the elicitation and function of neutralizing antibodies is being pursued. An HIV-1 variant virus was obtained by transmitting and propagating a cloned virus isolate in the presence of a neutralizing serum, indicating that type-specific neutralizing antibodies occur naturally. Whether such antibodies can cause immune selection of mutant viruses arising in vivo and influence disease progression remains to be determined. Genetic analysis of the variant has shown that a minor change was responsible for its loss of neutralizability. Further studies will pinpoint important epitopes. Other approaches for identification of neutralizing epitopes include use of a neutralizing monoclonal antibody to the viral gp120. In collaborative studies, the ability of various potential vaccine preparations to elicit high-titer, broadly reactive, neutralizing antibodies is being examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05537-01 LTCB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*: Immunopathogenesis of Human RNA and DNA Viruses

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principel Investigetor.) (Name, title, laboretory, end institute affiliation)*

PI:	W.C. Saxinger	Research Microbiologist	LTCB NCI
Others:	R.C. Gallo	Chief	LTCB NCI
	S. Weiss	Medical Staff Fellow	EEB NCI
	P. Levine	Medical Officer	EEB NCI
	E. Murphy	Medical Staff Fellow	EEB NCI
	W. Blattner	Chief, Family Studies Section	EEB NCI

COOPERATING UNITS *(if any)*: Howard University Hospital, Washington, D.C. (W. Frederick); North Shore Hospital, Long Island, NY (S. Pahwa)

LAB/BRANCH: Laboratory of Tumor Cell Biology

SECTION: Hematopoietic Cellular Control Mechanisms

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

HIV serological testing: Work on the development of an improved serological test has been successfully concluded. The competition ELISA test is superior in both sensitivity and specificity to licensed tests, including the Western blot.

Viral pathogenesis: Work on the chimpanzee HIV model has suggested new directions for approaches to intervention. Findings are that infection appears to progress by discrete stages which may be variably immunoregulated and that cofactors or cellular immunity, or target cell selection may be fundamental. Also, in vitro tests of B- and T-cell immunosuppression by viral proteins and fragments produced by molecular biological techniques have been successful in the preliminary phase. Detailed characterization of mechanisms of immunosuppression are in progress.

U.S. HTLV-I prevalence: A retrospective random sampling of the U.S. population (HANES-II) and a retrospective geographic drug-abuser population have been tested for HTLV-I antibody. Analysis in progress will indicate frequency of infection and its rate of change in these populations.

HBLV prevalence: ELISA tests have been successfully developed. Prevalence studies, geographically and epidemiologically oriented, are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05538-01 LTCB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Structure and Function of HIV Genomes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	M. Reitz	Research Chemist	LTCB NCI
Others:	R.C. Gallo	Chief	LTCB NCI
	F. Wong-Staal	Research Microbiologist	LTCB NCI
	M. Robert-Guroff	Research Biologist	LTCB NCI
	M. Popovic	Visiting Scientist	LTCB NCI
	G. Franchini	Guest Researcher	LTCB NCI
	H.-G. Guo	Visiting Scientist	LTCB NCI

COOPERATING UNITS *(if any)*
NONE

LAB/BRANCH
Laboratory of Tumor Cell Biology

SECTION
Molecular Genetics of Hematopoietics Cells

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Nucleotide sequence of the genome of simian T-lymphotropic virus type III from African green monkeys ($STLV\text{-}III_{agm}$) and of an HIV-2 isolate (HIV-2 SBL6669) have been carried out in order to compare them with HIV-1 and help understand the natural history and pathobiology of these viruses, as well as their role in the AIDS epidemic. Work has been initiated to try to construct biologically active clones of STLV-III and HIV-2. A second project has been to study the ability of HIV-1 clonal populations to generate mutants resistant to the host immune response. One such mutant has been obtained and thoroughly characterized and the specific mutation responsible for neutralization resistance is being identified. A third project is to identify the genetic determinants of macrophage-tropic HIV-1 which confer the ability to grow in macrophages. Several DNA clones of such viruses have been obtained, and are currently being tested for their biological activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05539-01 LTCB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Mapping of the Regulatory Elements of Human Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. Arya	Research Biologist	LTCB NCI
Others:	R.C. Gallo	Chief	LTCB NCI
	F. Wong-Staal	Research Microbiologist	LTCB NCI

COOPERATING UNITS *(if any)*: NONE

LAB/BRANCH: Laboratory of Tumor Cell Biology

SECTION: Molecular Genetics of Hematopoietic Cells

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

This project involves the molecular cloning, characterization, and functional mapping of the regulatory elements and regulatory genes (e.g., tat) of human immunodeficiency virus -1 and -2 (HIV-1 and HIV-2). We have recently shown that several isolates of HIV-2, as well as simian immunodeficiency virus (SIV), possess a function tat gene, irrespective of their pathogenic potential in the natural host. Possibly relevant to virus latency, HIV-1 and HIV-2 gene expression can be stimulated by immune activation and heterologous trans-activators such as human T-lymphotropic virus-1 (HTLV-I) and oncogenic DNA viruses. DNA sequencing and functional mapping of the long terminal repeats (LTR), 3'orf and tat genes have revealed that the regulatory elements and genes of HIV-2 are related to HIV-1 and that various isolates of HIV-2, as well as SIV, are more related among themselves than to HIV-1.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP07148-04 LTCB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on T-Cell Malignancies, Lymphomas and AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.C. Gallo	Chief	LTCB NCI
Others:	S.Z. Salahuddin	Cancer Expert	LTCB NCI
	S. Nakamura	Visiting Scientist	LTCB NCI
	K. Krohn	Visiting Scientist	LTCB NCI
	A. Ranki	Guest Researcher	LTCB NCI
	P.S. Sarin	Research Chemist	LTCB NCI
	W.C. Saxinger	Research Microbiologist	LTCB NCI
	M. Robert-Guroff	Research Biologist	LTCB NCI

COOPERATING UNITS *(if any)*

Imperial Cancer Research Fund, London, England (Robin Weiss); Duke University, Durham, NC (Bart Haynes); M.D. Anderson Hospital and Tumor Inst., Houston, TX (Ken McCredie); Harvard University, Boston, MA (Myron Essex)

LAB/BRANCH

Laboratory of Tumor Cell Biology

SECTION

Hematopoietic Cellular Control Mechanisms

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	2.0	4.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cell biology studies have been focused on: (1) the role of human T-lymphotropic retroviruses (HTLV) in human T-cell malignancies and acquired immunodeficiency syndrome (AIDS), and (2) a B-lymphotropic DNA virus (HBLV). HTLV-I has been shown to be a transforming virus, whereas human immunodeficiency virus (HIV-1) is cytopathic. HTLV-I, HTLV-II and HIV-1 have specificity for OKT4 positive T helper cells. The involvement of these viruses in neuropathy is being examined. HTLV-1 has recently been isolated from patients with tropical spastic paraparesis (TSP). HIV-1 has been shown to be associated with cells of monocyte-macrophage lineage. HIV-1 isolates obtained from different patients show some genetic variations in the envelope region. Drugs that block HIV-1 replication are being tested in in vitro systems. Studies in chimpanzees show the development of antibodies against HIV-1 antigens and viremia in these animals on inoculation with HIV-1. Vaccine studies indicate the development of neutralizing antibodies against the virus envelope, and more recently antibody against a synthetic HIV-1 p17 peptide (HGP30) was found to block HIV-1 replication in cell culture. The worldwide distribution of HTLV infection and the mechanism of its transmission in patients with AIDS and AIDS-related complex (ARC) have been extensively studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP07149-04 LTCB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular Biological Studies on HTLV and Oncogenes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	F. Wong-Staal	Research Microbiologist	LTCB NCI
Others:	R.C. Gallo	Chief	LTCB NCI
	S. Josephs	Research Chemist	LTCB NCI
	B. Starcich	Visiting Associate	LTCB NCI
	A. Aldovini	Visiting Fellow	LTCB NCI
	E. Collalti	Visiting Fellow	LTCB NCI

COOPERATING UNITS (if any)
Cold Spring Harbor laboratory, Cold Spring Harbor, NY (R. Franza); Duke Univ., Durham, NC (W. Greene); Showa Biomedical Univ., Miami, FL (M. Nonayama); NICHD, Bethesda, MD (W. Leonard)

LAB/BRANCH
Laboratory of Tumor Cell Biology

SECTION
Molecular Genetics of Hematopoietic Cells

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10.0	5.0	5.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are pursuing several broad areas relating to pathogenic human viruses, principally the T-lymphotropic retroviruses and a new DNA herpesvirus, human B lymphotropic virus (HBLV). There are two distinct subgroups of human T-lymphotropic retroviruses: the leukemia viruses, human T lymphotropic virus (HTLV-I and HTLV-2) and the human immunodeficiency viruses (HIV-1 and HIV-2). In the past, complementing LTCB's pivotal discovery of HTLV-I and -II, we have contributed to the molecular analysis of these genomes. The major findings can be summarized as follows: (1) all adult T-cell leukemia (ATL) cells contain monoclonally integrated HTLV-I; (2) the site of provirus integration is different from patient to patient, suggesting a transacting mechanism for transformation; and (3) presence of a conserved gene, tat, responsible for transcriptional activation. More recently, in collaboration with Warren Leonard (NICHD) and Warner Green's (Duke University) laboratories, we demonstrated that HTLV-I tat turns on expression of IL-2R and IL-2 in T lymphocytes. The target sequences for tat-1 are distinct from those for antigen/mitogen activation. The major efforts of our group at present are directed at studies on the HIVs. The following areas are addressed: (a) analysis of structure and function of the HIV-1 genome, with emphasis on the novel accessory genes of this virus; (b) analysis of the env gene, in detail, to define epitopes for neutralization, T4 binding, and viral cytopathic effect (CPE). Of relevance is our group's first demonstration of conserved and non-conserved domains in env; (c) molecular approaches to vaccine development. This work is currently carried out in collaboration with several industrial groups; and (d) comparative analysis of the new virus subgroup, HIV-2, and the related simian virus, STLV-III.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP00543-09 LTVB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Characterization of the Papillomaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. M. Howley	Chief	LTVB	NCI
Others:	B. Spalholz	Senior Staff Fellow	LTVB	NCI
	V. Lindgren	Guest Researcher	LTVB	NCI
	P. Lambert	Guest Researcher	LTVB	NCI
	P. Hermonat	Guest Researcher	LTVB	NCI
	M. Sippola-Thiele	Visiting Fellow	LTVB	NCI
	A. McBride	Visiting Fellow	LTVB	NCI
	J. Byrne	Biologist	LTVB	NCI

COOPERATING UNITS *(if any)*

Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (Doug Hanahan).

LAB/BRANCH

Laboratory of Tumor Virus Biology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.9	6.4	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The papillomaviruses are a group of small DNA viruses associated with benign and malignant proliferative lesions in a variety of higher vertebrates. Currently, there are recognized to be 46 distinct human papillomaviruses (HPVs) and six bovine papillomaviruses (BPVs). The lytic expression of these viruses is linked to the state of differentiation of squamous epithelial cells and to date no tissue culture system exists for their propagation in the laboratory. The bovine papillomavirus type 1 (BPV-1) is one of a subgroup of papillomaviruses which is capable of inducing fibroblastic tumors when inoculated into hamsters and is capable of inducing morphologic transformation of certain rodent cells in tissue culture. To date, transformation of rodent cells remains the only in vitro assay for the systematic study of the papillomaviruses. Because of this property, BPV-1 has become the prototype for unravelling the molecular biology of the papillomaviruses. A unique feature of this papillomavirus transformation system is that the viral DNA does not integrate into the host chromosome. The DNA remains extrachromosomal as a stable multiple copy plasmid. The factors involved in stable transformation, as well as for stable plasmid maintenance, are being extensively studied. A second characteristic associated with the papillomavirus infection is the propensity of certain viruses to be associated with lesions which may progress to carcinomas. What factors, either viral or host, which are involved in such a progression from a benign lesions to a carcinoma are as yet unknown. Our studies are designed to unravel the molecular biology of the normal virus infection of cells as well as for understanding the viral and cellular factors involved in carcinogenic progression. We have determined that BPV-1 encodes at least two genes which can independently transform mouse cells. Also, we have mapped transcriptional regulatory elements in the LCR of BPV-1 which are trans-activated by the full viral E2 gene product. A transcriptional repressor is encoded by the 3' portion of the E2 ORF. This domain of E2 contains the DNA binding site.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP00547-07-LTVB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Use of Papillomavirus DNAs as Eukaryotic Cloning Vectors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. M. Howley	Chief	LTVB	NCI
Others:	J. C. Byrne	Biologist	LTVB	NCI

COOPERATING UNITS *(if any)*

Revlon Health Care Research and Development, Springfield, VA (N. Sarver)

LAB/BRANCH

Laboratory of Tumor Virus Biology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The bovine papillomavirus (BPV) is capable of transforming certain rodent fibroblast lines in which the viral DNA remains as a stable extrachromosomal plasmid. These properties have been exploited in developing BPV into a stable extrachromosomal mammalian cell vector. The complete genome cloned into pML2, which is a deletion derivative of pBR322, is capable of serving as a shuttle vector which can replicate as a plasmid in mouse C127 cells or in bacteria. We have studied the expression of the rat preproinsulin gene in BPV vectors in C127 cells and have shown that the expression of the gene is enhancer-dependent. An "enhancer" element is located in the BPV-1 genome at the 3' end of the transforming region, downstream from the polyadenylation recognition sequence. Using this vector system, a variety of exogenous genes have been expressed. A portion of the human T-cell lymphotropic virus type 1 (HTLV-1) has been expressed off of the mouse metallothionein promoter in a BPV vector. The extrachromosomal state of the DNA should provide a physical characteristic to permit the purification of chromatin complexes of mammalian genes. Using the lac operator, we have developed a technique for rapid purification and identification of sequence-specific binding proteins. This technique, combined with the extrachromosomal papillomavirus plasmid vector system, should facilitate the identification of important viral and cellular gene regulatory proteins. During this year, this project has been phased out and this represents the terminal report on this project.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP00565-05 LTVB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transforming Activities and Proteins of the Papillomaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Schlegel	Chief, CRT Section	LTVB	NCI
Others:	A. Burkhardt	Guest Researcher	LTVB	NCI
	V. Bubb	Visiting Fellow	LTVB	NCI
	Y. Zhang	Visiting Fellow	LTVB	NCI
	M. Glass	Biologist	LTVB	NCI

COOPERATING UNITS *(if any)*

Department of Human Genetics, Yale University, School of Medicine, New Haven, CT. (Dr. Daniel DiMaio)

LAB/BRANCH

Laboratory of Tumor Virus Biology

SECTION

Cellular Regulation and Transformation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Papillomaviruses induce benign tumors in a variety of vertebrate species including man and, in some cases, these viral-induced lesions can progress to carcinomas. The intent of our laboratory's investigations is to define the mechanisms by which the papillomaviruses "transform" both immortalized or primary cells in vitro and to determine how they contribute to tumorigenesis in vivo. Specifically, we are studying the transforming activities of bovine and human papillomavirus DNA as determined by focus formation and immortalization assays of cultured murine and human epithelial cells. These studies also involve genetic definition of the viral genes responsible for in vitro transformation. We are also committed to identifying the protein products of the papillomavirus transforming genes and to characterizing their mode of action. To date, we have been able to demonstrate that the bovine papillomavirus (BPV) E5 ORF directs the synthesis of a small, hydrophobic transforming protein which is responsible for the major in vitro transforming activity of BPV. Cell fractionation studies have shown that most of the E5 protein is present in cell membranes but that some remains associated with the nucleus (presumeably with nuclear membranes). We have also shown that the E5 protein forms dimers via cysteines which are located near the COOH terminus of the molecule. By mutational analysis of the E5 ORF, we also have defined the initiation codon for E5 protein translation and demonstrated that NH2 terminal deletions, insertions, or substitutions do not interfere with the ability of the E5 protein to associate with cell membranes or to form dimers. Analysis of BPV-transformed hamster cells indicates that there is a direct correspondence between viral protein expression and the tumorigenic phenotype and that there is a threshold level of viral protein expression which is required for cellular transformation. Finally, we have shown that BPV-transformed hamster cells can resist allograft rejection, unlike hamster cells transformed by adenovirus type 2 or 12.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP00898-04 LTVB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Role of Human Papillomaviruses in Human Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. M. Howley	Chief	LTVB NCI
	C. C. Baker	Senior Investigator	LTVB NCI
Others:	W. Phelps	Guest Researcher	LTVB NCI
	K. Munger	Visiting Fellow	LTVB NCI
	C. Yee	Biologist	LTVB NCI
	J. Byrne	Biologist	LTVB NCI

COOPERATING UNITS *(if any)*
Frederick Cancer Research Facility, NCI (Mike Braun and Matt Gonda)

LAB/BRANCH
Laboratory of Tumor Virus Biology

SECTION
Viral Oncology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.9	2.5	1.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The papillomaviruses are associated with naturally occurring carcinomas in a variety of species, including man. There are now 46 human papillomaviruses (HPVs) which have been identified in man. Twelve of these have been associated with human genital tract lesions. Of these, HPV-6 and HPV-11 have been found associated with a high percentage of benign genital warts. HPV-16, HPV-18, HPV-31, and HPV-33 have been found in a high percentage of cervical carcinomas. We have previously identified several human cervical carcinoma lines which contain either of the integrated HPV DNA sequences. Two of the cell lines contained integrated HPV-16 DNA, and in each of these cell lines the genomes were transcriptionally active. Genomic clones have been made from each of these HPV-16 positive lines and have been characterized. In the SiHa cell line in which only a single copy of the HPV-16 genome is integrated, the cellular flanking sequences have been sequenced. Integration has occurred in the E2 ORF of the HPV-16 genome. We have characterized a conditional enhancer in the control region of the HPV-16 genome and have shown that the E2 gene of HPV-16 encodes a transcriptional transactivating function that induces this enhancer element. Further genetic analysis has mapped an additional transacting function to the HPV-16 E7 gene. This factor can transactivate the adenovirus E1a gene product. It can also complement ras in the transformation of primary rat embryo cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05420-03-LTVB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transformation by Polyomaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. B. Bolen	Senior Staff Fellow	LTVB	NCI
Others:	S. Amini	Visiting Fellow	LTVB	NCI
	V. DeSeau	Biologist	LTVB	NCI
	J. O'Shaughnessy	Medical Staff Fellow	MB	NCI

COOPERATING UNITS *(if any)*

Department of Molecular and Cellular Biology, Pennsylvania State University, University Park, Pennsylvania (D. Shalloway)

LAB/BRANCH

Laboratory of Tumor Virus Biology

SECTION

Cellular Regulation and Transformation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The polyomaviruses comprise a class of small DNA tumor viruses within the papovavirus group of DNA viruses. Members of the polyomavirus class include polyomavirus (Py) of mice, simian virus 40 of monkeys, hamster papovavirus, and JC and BK viruses of humans. Of these viruses, Py has been most thoroughly characterized with respect to the genetic elements and proteins involved in oncogenic transformation of mammalian cells. Oncogenic transformation of rodent cells by Py requires the continued expression of the Py-encoded middle tumor antigen (MTAg). The MTAg is a membrane-associated phosphoprotein with an associated tyrosine-specific protein kinase activity that has been demonstrated to be, at least in part, a property of the c-src gene product, $pp60^{c\text{-}src}$. The importance of MTAg-associated tyrosine-specific protein kinase activity is suggested by the finding that all known transformation-competent strains of Py encode MTAg molecules which possess this associated activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05481-02-LTVB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Regulation of pp60^{c-src} Protein Kinase Activity in Human Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. B. Bolen	Senior Staff Fellow	LTVB	NCI
Others:	S. Amini	Visiting Fellow	LTVB	NCI
	A. Veillette	Guest Researcher	LTVB	NCI
	G. DeSeau	Biologist	LTVB	NCI
	N. Rosen	Senior Investigator	MB	NCI
	J. O'Shaughnessy	Medical Staff Fellow	MB	NCI

COOPERATING UNITS *(if any)*

Department of Pathology, The George Washington University Medical Center, Washington, D. C. (A. M. Schwartz)

LAB/BRANCH

Laboratory of Tumor Virus Biology

SECTION

Cellular Regulation and Transformation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The protein products of proto-oncogenes possess functions (e.g., enzymatic activity, nucleic acid binding activity) that are believed to play a role in the regulation of normal cellular growth and differentiation. Thus, analysis of the functional state of proto-oncogene-encoded proteins in human malignancies represents one experimental approach that may provide insights into the biochemical alterations within cells that contribute to oncogenic transformation. While the biochemical functions of most proto-oncogene products are not known, several have been shown to be tyrosine-specific protein kinases. Of these proto-oncogene-encoded tyrosine kinases, the most extensively characterized is the product of the c-src gene, pp60^{c-src}. This protein is the normal cellular homolog of the Rous sarcoma virus oncogene, v-src. The transforming potential of pp60^{v-src} and mutated species of pp60^{c-src} appears to be related to elevations in the specific activity of the v-src- and c-src-encoded protein kinases. We have determined, in a variety of human tumor cell lines, human tumor and normal human tissues, the activity and abundance of pp60^{c-src}. Our results show that while pp60^{c-src} protein kinase activity is low in most types of human tumors, significant elevation of pp60^{c-src} kinase activity can be found in all human tumors of neural origin, several sarcomas, all human breast and all colon carcinomas tested.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05482-02 LTVB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Control of Papillomavirus Late Transcription

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. C. Baker	Senior Investigator	LTVB	NCI
Others:	P. M. Howley	Chief	LTVB	NCI
	L. M. Cowsert	Biotechnology Fellow	LTVB	NCI
	U. Linz	Visiting Fellow	LTVB	NCI
	J. S. Noe	Biologist	LTVB	NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Tumor Virus Biology

SECTION
Viral Oncology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.75	2.75	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The papillomaviruses cause benign and malignant lesions of squamous epithelia in higher vertebrates. The complete lytic cycle of these viruses (including late gene expression) occurs only in the differentiated cells of the squamous epithelium. Malignant lesions and infected cells in culture do not produce virus. An understanding of the transcriptional regulation of the papillomaviruses and its relationship to the control of epithelial cell differentiation is necessary for the elucidation of the role of the papillomaviruses in carcinogenesis. We have used bovine papillomavirus type 1 (BPV-1) as a model system for the study of late transcription and its control. BPV-1 transcription in productively infected tissue has been mapped by a combination of cDNA cloning, nuclease S1 protection, and primer extension and compared to similar analyses for BPV-1 fibroma tissue and BPV-1-transformed C127 cells. A strong viral transcriptional promoter (called the late promoter) has been identified which is active only in productively infected epithelium. All other viral promoters are active in both the fibropapilloma and in BPV-1-transformed cells. We are currently attempting to identify the *cis*- and *trans*-acting elements which are involved in the control of the late promoter and to determine the role which these *trans*-acting factors may play in epithelial cell differentiation. Control of late transcription is also mediated through *cis*-acting elements in the late region. These elements most likely function through transcription termination, polyadenylation, and/or mRNA destabilization. One element has been identified which decreases gene expression when placed upstream of the polyadenylation site in a eukaryotic expression vector. Additional *cis*-acting elements are being mapped and their mechanisms of action determined. Viral and/or cellular factors which interact in *trans* with these elements will also be identified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05518-01 LTVB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transformation and Gene Regulation of the Hamster Papovavirus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. B. Bolen	Senior Staff Fellow	LTVB	NCI
	P. M. Howley	Chief	LTVB	NCI
Others:	J. Pyper	Guest Researcher	LTVB	NCI
	S. Mackem	Medical Staff Fellow	LP	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Tumor Virus Biology

SECTION

Cellular Regulation and Transformation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The hamster papovavirus (HaPV) was originally isolated from skin epitheliomas originating from hair follicle epithelial cells in Syrian hamsters. The HaPV virions are found in the keratinized layer of the epithelium from infected animals, but are not found in the basal layers. Thus, the maturation of this virus is limited to terminally differentiated keratinocytes thereby resembling the tissue-specific tropism of the papillomaviruses. However, the morphology of HaPV virions, the DNA sequence of the HaPV genome, and the genetic organization of the HaPV genome clearly show that this virus is a member of the polyomavirus family. In contrast with other members of the polyomaviruses and papillomaviruses, HaPV injection into newborn hamsters produces rapid and acute lymphomas and leukemias which are thought to be of T-cell origin. Thymectomy of the animals severely reduces the incidence of this disease but results in formation of sarcomas at the site of injection. Thus, the HaPV is capable of inducing tumors of lymphoid, mesenchymal, and epithelial origin in its natural host. The viral genes responsible for this broad tumor potential and the control of the expression of these genes is currently unknown.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05330-05 LVC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Urinary Transforming Growth Factors (TGFs) in Human Neoplasia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Kurt J. Stromberg Medical Director LVC NCI

Others: None

COOPERATING UNITS *(if any)*

Division of Endocrinology, Vanderbilt University, Nashville, TN (D. N. Orth); Department of Biochemistry, George Washington University, Washington, DC (W. R. Hudgins)

LAB/BRANCH

Laboratory of Viral Carcinogenesis

SECTION

Viral Leukemia and Lymphoma Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	1.0	1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Urinary TGF-alpha, EGF, and TGF-beta were efficiently concentrated on microparticulate silica and separated by acetonitrile elution. Further resolution was obtained by sequential chromatography based on molecular size (Bio-Gel), charge (CM-cellulose), and hydrophobicity (RP-HPLC). The high molecular weight (HMW) TGF of 30,000 to 35,000 Mr, previously reported in the urine of various cancer patients is, in patients with malignant astrocytomas, indistinguishable from the HMW form of hEGF in terms of apparent molecular size, EGF receptor binding activity, EGF immunoreactivity and clonogenic activity. However, in comparison to bulk (25 liters) urine from normal individuals, equivalently large urine samples from these brain tumor patients contained about fourfold more HMW hTGF/hEGF. hTGF-alpha was not identified in either source of bulk urine. Secondly, in an in vitro study of HMW TGFs, conditioned medium of A673 cells (a human rhabdomyosarcoma cell line) was found to contain principal peaks of EGF radioreceptor and clonogenic activity in sodium dodecyl sulfate-polyacrylamide gel electrophoresis slices corresponding to Mr 15,000 and 22,000 in an RP-HPLC sample eluting at 25-26% acetonitrile, and two additional higher Mr activities in a 22-23% acetonitrile eluting region. Neither of these active regions from HPLC competed in radioimmunoassay under reduced and denatured conditions for hEGF or rTGF-alpha. Evaluation of TGF-alpha mRNA content in A673 cells is currently in progress. Thirdly, the pooled urine of patients with disseminated breast cancer contains immunoreactive TGF-alpha which is not present in comparable control urine from normal individuals. Fourthly, urinary proteins from individual 24-hour urine samples were concentrated, fractionated, and scored for immunoreactive TGF-alpha by RIA. The scattergram results, in order of decreasing nanograms of urinary TGF-alpha per gram creatinine, were samples from (1) patients with disseminated breast carcinoma and lactating females, (2) pregnant women, (3) patients with small primary breast cancers with no or minimal evidence of regional metastasis, and (4) healthy normal women.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05367-03 LVC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

The Genetic Structure of Natural Populations of Past and Present

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Stephen J. O'Brien	Chief	LVC	NCI
Others:	Janice S. Martenson	Microbiologist	LVC	NCI
	Mary A. Eichelberger	Microbiologist	LVC	NCI
	Lisa Forman	Guest Researcher	LVC	NCI
	Hector Seuanez	Visiting Scientist	LVC	NCI

COOPERATING UNITS *(if any)*

Laboratory of Clinical Studies, ALC, NIH, Bethesda, MD (D.Goldman); National Zoological Park, Washington, DC (M.Bush, D.E.Wildt); National Museums of Kenya, Nairobi, Kenya (R.Leakey); PRI, Frederick, MD(W.Modi,D.Gilbert,D.Janczewski)

LAB/BRANCH

Laboratory of Viral Carcinogenesis

SECTION

Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.1	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the spece provided.)*

Genetic analysis of human and animal populations has been used to study the genetic health and disease susceptibility of several species. A molecular phylogeny of the great and lesser apes and man was derived based on genetic distance of 383 different proteins resolved by 2-dimensional gel electrophoresis. A molecular phylogeny of 37 species of the Felidae family was constructed based on several molecular measures of evolutionary distance. Similarly, consensus phylogenies of the Ursidae (the giant panda, Ailuropoda, and the lesser panda, Ailurus) and the Canidae were derived from distance matrices derived from three distinct molecular measures of genetic distance. A correlative relationship was observed between the extent of genetic variation and the physiology of reproduction during studies of two subspecies of lions. Genetic variation and phylogenetic relationships of Mustela species were examined. A comparative analysis of cytological linkage maps of mammals has indicated a noncontinuous tempo of chromosomal evolution in certain lineages (e.g., primates, felids) that are highly conserved in their chromosomal presentation, while others (rodents, lesser apes, canids) are chromosomally shuffled as if rapid saltatory cytological rearrangements occurred during the speciation events. A reconstruction of cytological rearrangements which had occurred during carnivore evolution has been achieved.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05382-04 LVC

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 charecters or less. Title must fit on one line between the borders.): Genes Involved in Preneoplastic Progression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Nancy H. Colburn	Chief, Cell Biology Section	LVC	NCI
Others:	John Seed	Special Volunteer	LVC	NCI
	W. Karol Dowjat	Visiting Fellow	LVC	NCI
	Cao Ya	Guest Researcher	LVC	NCI
	Michael Antecol	Visiting Fellow	LVC	NCI
	Glenn A. Hegamyer	Health Science Officer	LVC	NCI

COOPERATING UNITS (if any)

Hunan Med. College, Hunan, China (K.-T. Yao); Cancer Res. Lab., Univ. W. Ontario, Canada (D. Denhardt); Dept. Radiation Oncology, Univ. Arizona Med. Sch., Tucson, AR (T. Bowden); PRI, Frederick, MD (R. Garrity)

LAB/BRANCH: Laboratory of Viral Carcinogenesis

SECTION: Cell Biology Section

INSTITUTE AND LOCATION: NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.7	4.0	1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this research is to identify and characterize genes that specify susceptibility to tumor promoter-induced neoplastic transformation in mice and humans. Evidence suggesting the involvement of such genes in animal and human systems has come from the observation that animals can be bred for sensitivity to tumor promotion. Two genes that specify sensitivity to promotion of neoplastic transformation by tumor promoters in mouse epidermal JB6 cells have been previously cloned. These putative genes, termed pro-1 and pro-2, have been sequenced and are being characterized with respect to mode of activation and regulation of expression. Unique pro-1-hybridizing transcripts have been identified in mouse cytoplasmic and poly(A)+ RNA. P- cells express lower levels of this transcript than do P+ or cells transformed by the tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA), suggesting overexpression as a possible mode of activation. Genomic DNA of a Chinese nasopharyngeal carcinoma cell line, CNE2, confers promotion sensitivity (P+) activity on resistant mouse cells. This activity is, at least in part, attributable to activated homologs of mouse pro-1, as shown by screening a CNE2 genomic library with a mouse pro-1 probe and testing the homologs for P+ activity after transfection into resistant mouse cells. Inactive pro-1 homologs isolated from a normal human library and from the CNE2 library are being compared with activated CNE2 pro-1 to ascertain the mode of activation. Heteroduplex analysis is being carried out to pinpoint nonhomologous sequences. Assay of chimeric constructs of sequences from human pro-1 that is P+ active or inactive is expected to elucidate sequences critical to biological activity. Two cDNA libraries, one from initiation-promotion induced skin papillomas and the other from a squamous carcinoma, have yielded clones homologous to pro-2, containing cDNA fragments of 2.1 and 0.9 kb. This finding is significant in that it not only facilitates intron-exon assignment in the genomic clone, but also opens up investigation of the role of pro-2 expression in carcinogenesis in vivo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05383-04 LVC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Membrane Signal Transduction in Tumor Promotion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Nancy H. Colburn	Chief, Cell Biology Section	LVC	NCI
Others:	Bonita M. Smith	Special Volunteer	LVC	NCI
	John Seed	Special Volunteer	LVC	NCI

COOPERATING UNITS *(if any)*

Inst. Med. Sci., Univ. of Tokyo, Tokyo, Japan (T. Kuroki); Swiss Inst. for Exp. Cancer Res., Lausanne, Switzerland (P. Cerutti)

LAB/BRANCH

Laboratory of Viral Carcinogenesis

SECTION

Cell Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	1.5	1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of these studies is to determine the required biochemical events that occur between tumor promoter-receptor interaction and the activation of effectors of neoplastic transformation. Candidate second messengers include protein phosphorylation, reactive oxygen generation, and calcium mobilization. Both activation of protein kinase C (PKC) and the subsequent loss of PKC activity may be on the signal transduction pathway for 12-O-tetradecanoylphorbol-13-acetate (TPA)-promoted transformation. A C-kinase substrate of 80 kDa has been found to be differentially phosphorylated in P-, P+, and neoplastically transformed JB6 cells, with little or no phosphorylated 80-kDa phosphoprotein (pp80) seen in transformed cells. This pp80 is postulated to be a tumor suppressor. Pharmacological analogs of calcium, the lanthanides, promote neoplastic transformation in JB6 cells by a PKC-independent pathway. The lanthanides, like phorbol esters, induce transformation in (activated) pro-1- or pro-2-transfected P- cells. This indicates that tumor promoters can collaborate with activated pro genes to bring about neoplastic transformation by either PKC-dependent or PKC-independent pathways. The synthesis of nuclear proteins of 15 and 16 kDa is TPA inducible in P+, but not in P- cells, an event that may account, in part, for the promotion sensitivity of P+ cells. Finally, P+ and P- cells differ in a transient, TPA-stimulated focus-associated expression of cellular P21 H-ras and an irreversible change in actin configuration, suggesting a possible collaboration of cytoskeletal, cytoplasmic and nuclear proteins with activated pro genes to bring about transformation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05384-04 LVC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Analysis of Human Cellular Genes in Neoplastic Transformation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Stephen J. O'Brien	Chief	LVC	NCI
Others:	Janice S. Martenson	Microbiologist	LVC	NCI
	Mary A. Eichelberger	Microbiologist	LVC	NCI
	Takis S. Papas	Chief	LMO	NCI
	Dennis K. Watson	Senior Staff Fellow	LMO	NCI

COOPERATING UNITS *(if any)* BRI,Fred.,MD(G.Vande Woude, M.Cohen, M.Barbacid, E.Brownell); USUHS, Beth.,MD(E.Chang); CHB,NHLBI,NIH,Beth.,MD(N.Anagnou, A.Nienhuis); LMM, NIAID,NIH,Beth.,MD(M.Martin); Johns Hopkins Hosp.,Balt.,MD(B.Vogelstein); H&W Cytogenet. Serv.,Sterling,VA(W.Nash); Meloy Labs.,Springfield,VA(M.Jaye)

LAB/BRANCH

Laboratory of Viral Carcinogenesis

SECTION

Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	0.7	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cumulative techniques of cell genetics, molecular biology, linkage analysis, and in situ hybridization have resulted in the identification and characterization of over 1500 human loci, a value which now exceeds the number of genes mapped in Drosophila. We have concentrated our efforts on somatic cell hybrid panels and on in situ hybridizations to genes related to neoplastic processes including (1) cellular proto-oncogenes, (2) growth factors, (3) growth factor receptors, (4) endogenous retroviral families, (5) integration sites for retroviruses, and (6) restriction genes that delimit retrovirus replication in mammals. Within the last few years, the human gene map has experienced a large increase in the number of neoplastic loci that have been mapped to specific chromosomal positions. The human gene map contains about 74 loci whose products have been related to cancer cause and progression, and of these, 45 are proto-oncogenes. We have genetically mapped 13 (29%) of these proto-oncogenes and 20 of the 74 (27%) neoplasia-related genes. This year we have concentrated on several new oncogenes (trk, tpr, ets, erg, gli), growth factors and receptors (endothelial cell growth factor, interleukin-3), and viral integration sites (HEPBI, MLVI1 and -2). Truncation of these cellular genes in a variety of human neoplasias, as well as in certain nonneoplastic pathologies (e.g., ets-2 in Down's syndrome or met in cystic fibrosis), which were suggested by their chromosomal positions, are under investigation. A previously unknown cluster of nine structural loci related to hematological development was discovered on human chromosome 5q and was found to be related to the 5q- anemia, a syndrome characterized by several abnormalities in blood cell production. The collaborative gene mapping studies have served as the basis for several ongoing projects which relate to the genetic events involved in neoplastic transformation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05385-04 LVC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetic Analysis of Feline Cellular Genes: A Comparative Approach

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Stephen J. O'Brien	Chief	LVC	NCI
Others:	David Derse	Senior Staff Fellow	LVC	NCI
	Naoya Yuhki	Visiting Fellow	LVC	NCI
	James W. Casey	Senior Staff Fellow	LVC	NCI
	Raoul E. Benveniste	Medical Officer	LVC	NCI
	Hector Seuanez	Visiting Scientist	LVC	NCI
	Janice S. Martenson	Microbiologist	LVC	NCI
	Mary A. Eichelberger	Microbiologist	LVC	NCI

COOPERATING UNITS *(if any)*

PRI, Frederick, MD (D. A. Gilbert, W. S. Modi); H&W Cytogenetics Services, Inc., Sterling, VA (W. G. Nash); Univ. of CA, San Diego, CA (J. S. O'Brien); NIAID, NIH, Bethesda, MD (C. Kozak); National Zoological Park, Washington, DC (D. E. Wildt)

LAB/BRANCH

Laboratory of Viral Carcinogenesis

SECTION

Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.4	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A genetic map of over 60 loci has been developed in the domestic cat. A remarkable extent of linkage homology between the feline and human maps was discovered which was three to four times more conserved than the mouse-to-human genetic synteny (linkage homology). Nearly 40% of the human cytological map can be aligned, band-for-band, with syntenically homologous feline chromosomes. This degree of linkage homology was used to estimate chromosomal location of the human albino locus and to test for transposition of the proto-oncogene family during the over 80 million years of evolution which has elapsed since man and cat shared a common ancestor. The organization of three distinct endogenous retroviral families was studied and found to resemble endogenous retroviral families in other mammalian species, including man. Genetic loci, which encode a series of lysosomal enzymes involved in feline models of human neurological storage diseases, have been localized. A molecular phylogeny of the Felidae family has been derived based upon three methodologies, and a cytogenetic description of Felidae evolution was developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05389-04 LVC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Reproductive-Endocrine-Genetic Strategies in Animal Species

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Stephen J. O'Brien	Chief	LVC	NCI
Others:	David E. Wildt	Special Volunteer	LVC	NCI
	Janice S. Martenson	Microbiologist	LVC	NCI

COOPERATING UNITS *(if any)*

Department of Animal Health, National Zoological Park, Washington, DC (M. Bush); Veterinary Resources Branch, DRS, NIH, Bethesda, MD (P. M. Schmidt, K. L. Goodrowe, M. C. Schiewe, J. G. Howard)

LAB/BRANCH

Laboratory of Viral Carcinogenesis

SECTION

Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.5	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The investigation of basic reproductive-endocrine-genetic factors in domestic animals which appear to be the most critical prerequisites to the application of artificial breeding strategies is the primary objective of this project. A multidisciplinary approach targeted toward female and male reproduction and genetics is employed. Areas of effort in the female include (1) ovulation induction combined with timed artificial inseminations; and (2) in vitro fertilization and embryo collection, culture, freezing and transfer as techniques for cryobanking genetic stock and for improving reproductive potential. The latter methods are being applied to the development of delivery techniques of molecularly-cloned genes which participate in transformation and inborn errors. Emphasis has been applied to the collection, in vitro culture, freezing, and micromanipulation of embryos of mouse, cat and miniature swine (animal models for both rare species and the study of human disease). Areas of effort in the male include (1) seminal evaluations to characterize ejaculate norms, correlating these findings to the level of genetic polymorphism in wildlife populations; (2) semen handling and cryopreservation to increase spermatozoal viability and to establish optimal methods for long-term storage of genetic material; and (3) hormonal evaluations to improve the understanding of pituitary-gonadal-adrenal relationships with particular emphasis on the marked differences in stress responses among taxonomically-related wildlife species. These reproductive procedures are being applied to a coordinate effort to develop embryo gene delivery in the cat.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05401-03 LVC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Transcriptional Regulation of the Bovine Leukemia Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James W. Casey	Senior Staff Fellow	LVC	NCI
Others:	David D. Derse	Senior Staff Fellow	LVC	NCI

COOPERATING UNITS *(if any)*
Program Resources, Inc., Frederick, MD (M. Gonda); University of California, Davis, CA (M. Thurmond); Colorado State University, Fort Collins, CO (G. Cockerell)

LAB/BRANCH
Laboratory of Viral Carcinogenesis

SECTION
Viral Leukemia and Lymphoma Section

INSTITUTE AND LOCATION
NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The structure and expression of deletion-type and full-length bovine leukemia virus (BLV) proviruses have been examined from tumors of infected cattle, sheep, and established cell lines. A deletion-type provirus present in a bovine lymphoid cell line (NBC-13) has been cloned, sequenced, and shown to arise by a recombination event between two 10 base-pair direct repeats. Different deletion-type proviruses were detected in both tumors and circulating lymphocytes from infected pre-tumor animals. These deletion-type proviruses are different in size, but maintain the pX region of the genome. One of these proviruses, from tumor 85 X 1007, has been molecularly cloned and is currently being analyzed. The transcriptional activity of the deletion-type provirus from NBC-13 is enhanced by growth in cell culture in the presence of horse serum, and is inhibited by factors present in fetal calf serum. Examination of lymphocytes from experimentally-infected sheep shows that the BLV provirus is monoclonal 3 to 6 months prior to the appearance of tumors. A spontaneous amplification of B-lymphocytes occurred in one animal and transcripts originating from the pX region of the provirus were detected.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05414-04 LVC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Retroviruses (Type-D and Lentiviruses) Isolated from Primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Raoul E. Benveniste	Medical Officer	LVC	NCI
Others:	Gisela Fanning-Heidecker	Staff Fellow	LVC	NCI
	David Derse	Senior Staff Fellow	LVC	NCI

COOPERATING UNITS *(if any)*

Univ. of Washington Primate Research Center, Seattle, WA (W. Morton, M. Thouless, C.-C. Tsai); Bionetics Research, Inc., Frederick, MD (L. Henderson, S. Oroszlan); Program Resources, Inc., Frederick, MD (P. Dorn-Williams, M. Gonda, L. Arthur)

LAB/BRANCH

Laboratory of Viral Carcinogenesis

SECTION

Viral Leukemia and Lymphoma Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	0.6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

At the University of Washington Primate Research Center, several macaque species show an acquired immunodeficiency syndrome (simian AIDS, SAIDS) characterized by lymphocytopenia, opportunistic infections, and a retroperitoneal fibromatosis (RF) tumor. Numerous type-D retroviruses, designated SAIDS-D/Washington (SAIDS-D/W), have been isolated by cocultivation of tissues and blood from animals with RF on lymphocyte and monolayer cultures. This virus has been molecularly cloned; the restriction enzyme pattern reveals that it can be distinguished from all other type-D retroviruses. Epidemiological studies reveal that over 90% of colony animals have antibodies that cross-react with SAIDS-D/W viral proteins. A survey of macaques bled in Indonesia reveals that many of these animals are already antibody positive in their natural habitat.

Another retrovirus has been isolated on lymphocyte cell lines after cocultivation of a lymph node from a Macaca nemestrina that had died with lymphoma in 1982 at the Washington Primate Center. This isolate, designated SIV/Mne (simian immunodeficiency virus, M. nemestrina), is partially related to human immunodeficiency virus (HIV, formerly HTLV-III/LAV) as evidenced by an immunological cross-reaction of the major gag protein. SIV/Mne is even more closely related to the west African AIDS isolate, HIV-2, with a 90% amino acid homology in the gag region of the virus. Nine independent molecular clones of SIV/Mne have been obtained and are being characterized. SAIDS-D/W and SIV/Mne have been inoculated into several primate species; the former virus causes RF tumors and the latter causes severe immunosuppression with absolute depletion of T4+ lymphocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05417-03 LVC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Characterization of raf Oncogenes in Normal and Tumor Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ulf R. Rapp	Chief, Viral Pathology Section	LVC	NCI
Others:	Thomas W. Beck	Biotechnology Fellow	LVC	NCI
	Gisela Fanning-Heidecker	Staff Fellow	LVC	NCI
	Walter Kolch	Guest Researcher	LVC	NCI
	John L. Cleveland	Senior Staff Fellow	LVC	NCI
	Takayasu Matsugi	Visiting Fellow	LVC	NCI
	Berton Zbar	Chief, Cellular Immunity Section	LI	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Viral Carcinogenesis

SECTION

Viral Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
1.8	1.5	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two active oncogenes related to v-raf have been identified in both mouse and man. c-raf-1 has been localized to mouse chromosome 6 and to human chromosome 3p25 near sites specifically altered in small cell lung carcinoma (SCLC), familial renal cell carcinoma, mixed parotid gland tumors, and ovarian cancer. The human c-raf-1 gene contains 16 coding exons and spans more than 40 Kbp. The human 3.4-Kb mRNA encodes a protein of 648 amino acids (73 Kd) and is expressed in most mouse tissues and cell lines (including SCLC cell lines) at various levels. c-raf-1 mRNA expression is unaffected by growth factors, growth inhibitors, and tumor promoters, suggesting that c-raf-1 performs basic cellular functions and regulation of its activity occurs at the translational or protein level.

A-raf-1 has been localized to the X chromosome in both mouse and man. It represents the first active human oncogene on a sex chromosome and is located between p21-q11, near the locus for testicular feminization syndrome and Menkes syndrome. Although no specific alterations involving the X chromosome have been described, a role in certain rare X-linked lymphoproliferative diseases seems possible. The

A-raf mRNA is 2.6 Kb in both mouse and man. It encodes a 606 amino acid protein (67.5 Kd) which shows 60% homology with c-raf-1, and it displays a more restricted pattern of tissue expression than c-raf-1, with highest levels in the epididymis and intestine, suggesting a cell type-specific function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05418-03 LVC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Role of raf and myc Oncogenes in Transformation In Vivo and In Vitro

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, leboratory, and institute affiliation)*

PI:	Ulf R. Rapp	Chief, Viral Pathology Section	LVC	NCI
Others:	John L. Cleveland	Senior Staff Fellow	LVC	NCI
	Mahmoud Huleihel	Visiting Fellow	LVC	NCI
	Robert Nalewaik	Microbiologist	LVC	NCI
	Michael Potter	Biologist	LG	NCI

COOPERATING UNITS *(if any)*

Program Resources, Inc., Frederick, MD (M. Dean and P. Lloyd); NCI, NIH, Bethesda, MD (J. Pierce); NIAID, NIH, Bethesda, MD (H.C. Morse); Bionetics Research, Inc., Frederick, MD (J.N. Ihle); NIDR, NIH, Bethesda, MD (W. Horton)

LAB/BRANCH

Laboratory of Viral Carcinogenesis

SECTION

Viral Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to evaluate the target cell range for transformation by v-raf, as well as to determine whether v-raf is capable of inducing transformation by itself or requires interaction with a second oncogene, myc, a series of recombinant viruses was constructed with either or both viral oncogenes on the 3611-murine sarcoma virus (MSV) background. A combination of both oncogenes in an infectious murine retrovirus (J-2) induces hematopoietic neoplasms, in addition to less prominent fibrosarcomas and pancreatic adenocarcinomas 1 to 3 weeks after inoculation. The hematologic neoplasms consist of immunoblastic lymphomas of T- and B-cell lineage, and erythroblastosis. In parallel to the synergistic action of both oncogenes on hematopoietic cells in vivo, we find that raf oncogene-induced transformation of bone marrow cells in culture is enhanced by the addition of myc, which by itself does not transform these cells when grown in standard media. We conclude that concomitant expression of raf and myc oncogenes in hematopoietic cells alters their respective transforming activities. The contribution of myc to this synergism was examined by using a series of recombinant murine retroviruses capable of expressing avian v-myc or mouse c-myc to study the effect of altered myc expression on hematopoietic/lymphoid cells. The v-myc-carrying virus, J-3, was shown to synergize with the mineral oil, pristane, in the induction of plasmacytomas, where it functionally replaces activation of c-myc by chromosomal translocation. With either interleukin-3 (IL-3)- or IL-2-dependent cell lines, introduction of the recombinant viruses abrogated the requirement for IL-3 or IL-2 for growth, and associated with this was the suppression of c-myc expression. The findings suggest that myc is a component in the signal transduction pathway for IL-3 and IL-2 and support an autoregulatory mechanism of c-myc expression. In contrast to v-myc, expression of v-raf in primary lymphoid/hematopoietic cells has an immortalizing function without abrogating the requirement for IL-3 for growth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05490-02 LVC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Basics of Lentiviral Transcriptional Trans-activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: James W. Casey Senior Staff Fellow LVC NCI

Others: None

COOPERATING UNITS *(if any)*

Program Resources, Inc., Frederick, MD (M. Gonda); Bionetics Research, Inc., Frederick, MD (N. Rice); Texas A & M University, College Station, TX (J. Edwards)

LAB/BRANCH

Laboratory of Viral Carcinogenesis

SECTION

Viral Leukemia and Lymphoma Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.5	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The lentivirus, equine infectious anemia virus (EIAV), displays a highly restricted cell type preference both in vitro and in vivo. Additionally, like other members of the lentivirus family, EIAV is subject to antigenic variation as evidenced by changes that occur in envelope glycoproteins during the course of infection. To further understand the restrictive host range and correlate proviral changes that occur during pathogenesis, we have molecularly cloned and sequenced an EIAV provirus. Comparison of the gag and pol genes of EIAV with the human immunodeficiency virus and the visna virus clearly establishes that EIAV is genetically related and equally divergent from these two distinct lentiviruses. Additionally, we have performed DNA-mediated transfection analysis and viral infectivity assays of DNA isolated from a productively infected dog cell line (EIAV cf-2). Results from these experiments indicate that some proviruses harbored in this cell are biologically active. We have molecularly cloned 23 of these proviruses using lambda vectors and are currently assaying each for infectivity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05491-02 LVC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Feedback Regulation of c-myc Transcription by myc Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John L. Cleveland	Senior Staff Fellow	LVC	NCI
Others:	Ulf R. Rapp	Chief, Viral Pathology Section	LVC	NCI
	Mahmoud Huleihel	Visiting Fellow	LVC	NCI

COOPERATING UNITS *(if any)* Laboratory of Immunoregulation, NIAID, NIH, Bethesda, MD (U. Siebenlist, P. Bressler); Bionetics Research, Inc., Frederick, MD (J. Ihle); Fred Hutchinson Cancer Research Center, Seattle, WA (R. Eisenman); Program Resources, Inc., Frederick, MD (P. Lloyd, M. Dean)

LAB/BRANCH

Laboratory of Viral Carcinogenesis

SECTION

Viral Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Infection of mouse cells from a variety of lineages with retroviruses expressing high levels of avian v-myc was found to be invariably associated with a lack of c-myc expression. To distinguish between v-myc-induced shutdown versus a cell-programmed down regulation of c-myc expression, we have analyzed this phenomenon in three different cell lines in culture which express various levels of c-myc prior to infection. Extreme levels of v-myc expression (10- to 100-fold excess over c-myc) were achieved in a myeloid (FDC-P1) and a T-lymphoid (CTB-6) cell line. In both lines, c-myc expression was absent in the infected cells and, in the case of FDC-P1 cells, occurred at the level of transcription initiation and could not be induced by growth factor (IL-3) or inhibitors of protein synthesis (to remove a labile repressor). Moreover, DNase I hypersensitive sites typical for active c-myc alleles were absent in FDC-P1 v-myc-infected cells. c-myc expression was also suppressed in FDC-P1 cells infected with a c-myc retrovirus. In NIH 3T3 fibroblast cells, v-myc was expressed at levels 5 to 10 times higher than those of c-myc present in uninfected cells. Suppression of c-myc in these cells was not due to clonal variation nor to changes in c-myc gene structure, and occurred at the level of transcription initiation. The suppression of c-myc expression was mediated directly by v-myc, since cells infected with constructs containing frameshifts and deletions in v-myc had levels of c-myc mRNA and protein comparable to uninfected cells. Suppression of c-myc expression was not associated with any gross changes in chromatin structure and could be reversed by treating infected cells with anisomycin or by stimulating growth factor-deprived cells with serum. Suppression of c-myc expression was also observed in fibroblasts transfected with an N-myc expression vector. These findings establish that myc proteins function in an auto-and cross-regulatory circuit which transcriptionally regulates myc family proto-oncogenes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05492-02 LVC

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Activation of raf Oncogenes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ulf R. Rapp	Chief, Viral Pathology Section	LVC	NCI
Others:	Mahmoud Huleihel	Visiting Fellow	LVC	NCI
	John L. Cleveland	Senior Staff Fellow	LVC	NCI
	Gisela Fanning-Heidecker	Staff Fellow	LVC	NCI
	Robert Nalewaik	Microbiologist	LVC	NCI
	Michael Potter	Biologist	LG	NCI

COOPERATING UNITS *(if any)*: NIAID, NIH, Bethesda, MD (H. C. Morse)

LAB/BRANCH: Laboratory of Viral Carcinogenesis

SECTION: Viral Pathology Section

INSTITUTE AND LOCATION: NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.1	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A 1.6-Kb cDNA (A-raf) has been isolated from a murine spleen cDNA library, which encodes part of a protein related to the v-raf oncogene. Its amino acid sequence has 85% homology to raf in a central protein of 100 amino acids. When incorporated into a retrovirus, the resulting gag-A-raf fusion gene causes transformation in vitro and induces tumors in newborn mice. Later on we isolated the complete 2453- nucleotide sequence of the human A-raf gene from a human T-cell cDNA library. When the 5' deleted fragment of the cDNA is incorporated into a murine retrovirus, the resulting gag-A-raf fusion gene causes transformation in vitro and in vivo. Whereas, the full-lengths of c-raf-1 and human A-raf were not transforming when they were constructed under the control of a murine leukemia virus promoter (long terminal repeat). Moreover, when we deleted 20 amino acids from the N-terminal of c-raf-1 and incorporated them into a murine retrovirus, the resulting gag-c-raf-1 fusion gene caused transformation of fibroblasts. In trying to define the minimal sequences of raf oncogenes required for their transforming ability, we found that deletions of 14 N-terminal and 13 C-terminal amino acids were dispensable, but deletion of 28 or more amino acids from v-raf at the carboxy terminal abolished all transforming activity. Furthermore, we made four different XHOI linker insertion mutants of c-raf-1. These mutants will be incorporated into retroviral vectors for expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05527-01 LVC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of HIV Mutants Defective in gag Gene Processing

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Raoul E. Benveniste	Medical Officer	LVC	NCI
Others:	Gisela Fanning-Heidecker	Staff Fellow	LVC	NCI

COOPERATING UNITS *(if any)*

Fairfax Hospital, Falls Church, VA (L. Eron); Bionetics Research, Inc., Frederick, MD (L. Henderson, R. Sowder, S. Oroszlan); Program Resources, Inc., Frederick, MD (M. A. Gonda)

LAB/BRANCH

Laboratory of Viral Carcinogenesis

SECTION

Viral Leukemia and Lymphoma Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.6	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An HIV isolate obtained from an HIV seropositive patient was shown to have a low titer of infectious particles. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western immunoblot analyses of proteins associated with this virus, designated HIV (FRE-3), showed that it contained large amounts of the gag viral protein precursor, Pr55. Electron microscopy (EM) of cells from the infected T-cell line, HuT 78, revealed a mixed population of lymphocytes; some cells were releasing only mature extracellular virus particles, while others produced aberrant "immature" virus particles. Individual cells were obtained by cloning HuT 78 on a feeder layer of primary sheep choroid plexus cells. Some of the clones are producing what appears to be "wild-type" HIV (reverse transcriptase-positive, mature gag proteins visualized on SDS-PAGE), which by EM appear normal in all stages of maturation. Other single-cell clones release noninfectious, structurally aberrant, immature virus particles. These latter clones do not have any detectable mature gag proteins and accumulate large amounts of the Pr55 gag precursor; some also lack reverse transcriptase activity. Purified and lysed whole virus preparations lack an intact protease; the addition of partially purified protease isolated from a "wild-type" virus results in the degradation of Pr55 to proteins that comigrate with mature HIV gag proteins. These results suggest that the genetic defect may reside in the protease gene itself.

This in vitro assay for HIV protease, using its natural substrate, Pr55, will be used to identify HIV protease-specific inhibitors that may have therapeutic applications in treating HIV-infected patients. The large amount of Pr55 gag precursor protein present in these viruses has been useful in detecting the passive acquisition of HIV antibodies in patients with primary immunodeficiency syndromes receiving large doses of intravenous IgG. Rabbit antisera raised against these viruses have also been useful for detecting the presence of HIV antigen by immunohistochemical staining of routinely fixed autopsy specimens from AIDS patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05528-01 LVC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Control of Retrovirus Gene Expression by Virus Proteins and Response Elements

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David Derse	Senior Staff Fellow	LVC NCI
Others:	Stephen J. O'Brien	Chief	LVC NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Viral Carcinogenesis

SECTION

Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.8	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

Bovine leukemia virus (BLV) and the human T-cell leukemia viruses (HTLV-I and HTLV-II) are lymphotropic retroviruses that have evolved similar strategies for regulating their expression. These viruses exhibit a highly restricted pattern of gene expression in vivo and in vitro that results from an interaction of cis-acting elements in the proviral long terminal repeats (LTRs) and trans-acting factors. Unlike other RNA tumor viruses, HTLV and BLV possess genes coding for nonstructural proteins that are likely to play a role in gene expression. To characterize the components of the system that interact to regulate virus expression, cis-trans experiments were performed. The LTRs from BLV, HTLV-I, and HTLV-II, as well as from the lentivirus equine infectious anemia virus, were coupled to a variety of bacterial or mammalian "reporter" genes including chloramphenicol acetyltransferase, aminoglycoside phosphotransferase (Neo), or rabbit beta-globin. The expression of these genes following transfection into mammalian cells was analyzed by enzymatic assays, RNA blot hybridization or quantitation of drug-resistant cell colonies. These experiments revealed that each of these LTRs was active only in cell lines producing the respective virus, i.e., the BLV LTR was active only in BLV-infected cells. To determine whether the viruses encode the factors that act in trans to regulate transcription, plasmids were constructed to express the BLV X-region genes. These pX expression plasmids were tested by cotransfection with the "reporter" plasmids into uninfected mammalian cells. It was found that BLV encodes a protein of 38 Kd (p38) that functions in trans to activate BLV transcription. These viruses produce a second protein encoded by a different reading frame within the X-region. The function of this protein (p18 in BLV) was examined in complementation experiments which revealed that p18 acts in trans to regulate virus expression by modulating viral mRNA processing events.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1CP05529-01 LVC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic and Molecular Organization of the MHC in the Domestic Cat

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Stephen J. O'Brien	Chief	LVC	NCI
Others:	Naoya Yuhki	Visiting Fellow	LVC	NCI
	Stanley J. Cevario	Biologist	LVC	NCI

COOPERATING UNITS *(if any)*

Program Resources, Inc., Frederick, MD (C. A. Winkler); Bionetics Research, Inc., Frederick, MD (A. Schultz)

LAB/BRANCH

Laboratory of Viral Carcinogenesis

SECTION

Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.3	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major histocompatibility complex (MHC) in the domestic cat was characterized using serological and molecular procedures. Reciprocal skin grafts were exchanged between unrelated cats; 75% of the skin grafts between the siblings of unrelated parents and 100% of those between the unrelated cats were acutely rejected within 14 days. Cytotoxic alloantisera were derived from 14 different individuals and were used in a population cluster analysis of unrelated feral cats to define overlapping immunogenetic specificities. In addition, pedigree analysis of the nine families in the NIH cat colony led to the description of allogeneic haplotypes which segregated from each other in family experiments. The data were used to derive the first feline MHC (termed FLA, feline leukocyte antigen) chart of detected haplotypes. Immunoprecipitation experiments using cytotoxic typing alloantisera identified both class I and class II type molecules. A molecular analysis of feline DNA using heterologous human or mouse molecular probes (class I and class II) revealed that the cat haploid genome contains approximately 20 class I loci and 2 class II genes. Class I genes of the domestic cat expressed limited restriction fragment length polymorphism (RFLP); approximately five times lower than the extent of RFLP observed in mice, rats, or pigs, and almost equivalent to the extent of MHC gene RFLP that is detected in humans and in the MHC-monomorphic Syrian hamster. Class I and class II genes were both genetically mapped to feline chromosome B2 using a panel of rodent x cat somatic cell hybrids. A partial cDNA class I gene (pFLA2) isolated from a cDNA library of a cat T-cell lymphoma cell line is presently under analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05530-01 LVC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Replication and XC-Fusion Deficiency of Endogenous Ecotropic C3H/He Provirus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ulf R. Rapp	Chief, Viral Pathology Section	LVC	NCI
Others:	Gunamani Sithanandam	Guest Researcher	LVC	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Viral Carcinogenesis

SECTION

Viral Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The molecular basis has been determined for differences in the infectivity and XC phenotype of the endogenous ecotropic murine leukemia virus (MuLV) of the low leukemia mouse strain, C3H/He; its relative in the high leukemia mouse strain AKR; and highly infectious, XC-positive C3H virus variants selected in vitro. Endogenous ecotropic type C virus induced by iododeoxyuridine from the non-transformed C3H/10T1/2 cell line is XC negative and replication deficient. In contrast, viruses produced late after iododeoxyuridine induction in chemically transformed C3H/10T1/2 cells (MCA5) are XC positive and infectious. XC-negative viruses can be converted to XC-positive viruses upon growth in certain transformed cell lines. We have cloned the endogenous ecotropic provirus of C3H/He from MCA5 cells, which is XC negative and replication deficient, as well as two XC-positive C3H proviruses derived by in vitro conversion. Nucleotide sequencing established that the XC-negative C3H p110 was integrated within the R region of an endogenous VL30 long terminal repeat in reverse orientation, and differed from the infectious AKR p623 provirus by a point mutation substituting Lys for Arg at the potential precursor cleavage site for gp70 and p15E. The in vitro-converted XC-positive C3H proviral clones, C1 3211 and 4211, have Arg at this site and the normal cleavage site is thus regenerated in these clones. We have altered the Lys residue to Arg at the proteolytic cleavage site of p110 by site-directed mutagenesis and we have reconstructed the provirus. DNA from this construct, upon transfection, gave rise to XC-positive, replication-competent provirus. Thus, we have established that a single point mutation at the processing site of the envelope precursor protein, gp85, is responsible for the difference in the infectivity and XC phenotype of endogenous ecotropic MuLV from C3H/He and AKR mice, and that the basis for in vitro conversion is a mutation at this site.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05531-01 LVC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Isolation and Molecular Characterization of Mammalian raf-Related Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ulf R. Rapp	Chief, Viral Pathology Section	LVC	NCI
Others:	Walter Kolch	Guest Researcher	LVC	NCI
	John L. Cleveland	Senior Staff Fellow	LVC	NCI
	Mahmoud Huleihel	Visiting Fellow	LVC	NCI
	Thomas Beck	Biotechnology Training Fellow	LVC	NCI
	G. Fanning-Heidecker	Staff Fellow	LVC	NCI

COOPERATING UNITS (if any)

Bionetics Research, Inc., Frederick, MD (D. Garfinkel); Physiologisch-Chemisches Institut der Universitaet Marburg, Marburg, Federal Republic of Germany (D. Gallwitz)

LAB/BRANCH

Laboratory of Viral Carcinogenesis

SECTION

Viral Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.6	1.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The raf gene is an evolutionarily old gene and well conserved throughout mammals. At present, four v-raf-related genes are known in man and mouse: c-raf-1, A-raf-1, and their inactive pseudogenes. Here we describe attempts to identify further raf-related genes. Using A-raf as the probe for screening human cDNA libraries led to the isolation of four candidate clones. Their molecular characterization is currently underway. One seems to be highly homologous to A-raf, yet deviates in restriction pattern and preliminary nucleotide sequence. As judged from Southern blot hybridizations, the other clones show moderate raf homology. Two of them show identical length, restriction pattern, and nucleotide sequence (partially obtained).

We then present evidence that S. cervisiae contains a raf-related gene(s). At present, we are cloning the gene(s) from yeast genomic and cDNA libraries. While, in general, yeast and mammalian proteins share extensive structural and functional similarities, the simplicity and experimental accessibility of yeast allow protein function studies which are severely impeded by the complex organization of mammalian cells. Thus, we want (1) to gain insight into functional properties of the raf gene products concerning interaction with ligands and regulation of kinase activity, and (2) to test whether the observation made in fibroblasts that ras function is dependent on raf for cellular growth control also applies to yeast. If so, a eukaryotic model system for studying transduction of growth-regulating signals may be developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05532-01 LVC

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Effect of raf Family Protein Kinases on Cell Physiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ulf R. Rapp	Chief, Viral Pathology Section	LVC	NCI
Others:	Thomas W. Beck	Biotechnology Training Fellow	LVC	NCI
	G. Fanning-Heidecker	Staff Fellow	LVC	NCI
	Walter Kolch	Guest Researcher	LVC	NCI
	John L. Cleveland	Senior Staff Fellow	LVC	NCI
	Mahmoud Huleihel	Visiting Fellow	LVC	NCI
	Robert Nalewaik	Microbiologist	LVC	NCI
	Robert Bassin	Senior Investigator	LTIB	NCI

COOPERATING UNITS *(if any)*: Laboratory of Biochemical Physiology, National Cancer Institute, Frederick, MD (H.-F. Kung)

LAB/BRANCH: Laboratory of Viral Carcinogenesis

SECTION: Viral Pathology Section

INSTITUTE AND LOCATION: NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Since the identification of v-raf as the oncogene of the acutely transforming retrovirus, 3611-murine sarcoma virus, significant progress has been made in the molecular and functional characterization of raf proteins and their effects on cell physiology. (1) Amino terminally truncated versions of c-raf-1 and A-raf-1 are transforming in vitro and in vivo. (2) raf proteins are cytoplasmically located protein kinases related to the src gene superfamily and truncated versions possess ser/thr-specific protein kinase activity. Moreover, c-raf and A-raf show homology to protein kinase C, not only in the C-terminal kinase domain, but also in the N-terminal putative regulatory domain. (3) raf-transformed fibroblasts release transforming growth factor(s) (TGF), express TGF-alpha mRNA in certain cases, and are inhibited in collagen synthesis. (4) Functional assays utilizing NIH 3T3 cells that are growth arrested by microinjection of ras monoclonal antibody or transformation of flat revertants of Kirsten sarcoma virus-transformed fibroblasts suggest that raf family oncogenes act independent of ras, either through a signal transduction pathway not involving ras or one in which raf has a position downstream of ras.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05533-01 LVC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

raf Protein Structures Involved in Transformation and Kinase Activity

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigetor.) (Neme, title, leboretory, end institute effilietion)*

PI:	Gisela Fanning-Heidecker	Staff Fellow	LVC	NCI
Others:	Ulf R. Rapp	Chief, Viral Pathology Section	LVC	NCI
	Mahmoud Huleihel	Visiting Fellow	LVC	NCI
	Thomas Beck	Biotechnology Training Fellow	LVC	NCI
	Robert Malewaik	Microbiologist	LVC	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Viral Carcinogenesis

SECTION

Viral Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.7	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The murine cellular homolog of the 3611-murine sarcoma virus (MSV) v-raf oncogene was isolated as cDNA clones and characterized. Comparisons at the DNA and protein levels showed that the gene is under strict selection, as only 5 of the 95 nucleotide exchanges in the 3' half of the mouse and human c-raf genes resulted in amino acid differences. Twelve nucleotide exchanges occurred during the conversion of mouse c-raf to 3611-MSV v-raf. Eight of these were in the coding sequence and resulted in four amino acid exchanges. None of the differences coincide with those observed in the activation of v-mil, the avian homolog of raf, indicating that truncation and/or protein fusion, rather than point mutations, is the major factor in oncogene activation. The importance of conserved amino acid motifs for the kinase function of the raf protein was investigated by comparing the transforming potential of mutants generated by site-directed mutagenesis. The conversion of the second lysine in the putative ATP-binding site, Gly-X-Gly-X2-Gly-X13-Lys-Ile-Leu-Lys, to either glutamine or glutamic acid did not significantly affect the transforming efficiency of the v-raf gene, while conversion of the first lysine to tryptophan eliminated the activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04629-22 LB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Stages of Carcinogenesis Induced by Chemical or Physical Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. A. DiPaolo	Chief	LB	NCI
Others:	J. Doniger	Senior Staff Fellow	LB	NCI
	L. A. Pirisi	Visiting Fellow	LB	NCI
	N. C. Popescu	Microbiologist	LB	NCI
	C. Woodworth	Senior Staff Fellow	LB	NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biology

SECTION

Somatic Cell Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	4.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To gain further understanding concerning the mechanism by which cells are converted from normal to malignant, animal and human cells have been examined after being subjected to environmental agents. Because human cells are refractory to conversion in vitro to malignancy, molecular changes observed in animal cells serve as prototypes for the human cell studies. N-ras activation correlates with acquisition of tumorgenicity for a series of transformed guinea pig lines obtained with diverse carcinogens. Comparison of sequences of guinea pig and human genomic N-ras clones reveals extensive conservation, greater than expected from drift at silent sites within coding regions. Thus, these regions probably have an important function in controlling N-ras expression. In fact, all the transformed lines exhibit a significant increase in N-ras expression compared to normal cells. A model for studying carcinogenesis, molecular biology and differentiation has been developed. Human neonatal kerotinocytes derived from foreskin have been converted into permanent lines by tranfection with recombinant human papilloma virus (HPV) 16 providing an opportunity to study the role of HPV 16 in human cancer. Chromosome analysis soon after transfection demonstrates drastic alterations: pulverization, endoreduplication, dicentrics, and double minutes; control human kerotinocytes were diploid. With further growth, the number of chromosome alterations and complexity evolved to a simpler state. After BamHI digestion, the majority of the HPV 16 DNA in the cells was detected as a 7.9 kbp band, indicating that most of the HPV 16 genome was intact. The pattern of additional bands, initially complex, became simpler and stabilized with time, suggesting a polyclonal population at low population doublings that became clonal. Subsequent digestion with EcoRV, a non-cut enzyme for the plasmid, reduced the size of the additional bands suggesting integration. Indefinite growth potential (immortality) may represent an important early event because the resulting cells can become vulnerable to carcinogenic insult.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04673-16 LB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Immunobiology of Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. H. Evans	Chief, Tumor Biology Section	LB	NCI
Others:	S.C. Barnett	Visiting Fellow	LB	NCI
	B.A. Gelleri	Visiting Fellow	LB	NCI
	P. Furbert-Harris	IRTA Fellow	LB	NCI
	P. D. Baker	Microbiologist	LB	NCI
	A. C. Wilson	Chemist	LB	NCI

COOPERATING UNITS *(if any)*

Laboratory of Neurophysiology, NINCDS, NIH (P. A. Sheehy, J.L. Barker)

LAB/BRANCH
Laboratory of Biology

SECTION
Tumor Biology

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	4.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lymphokines, interleukins, and other immunological hormones, i.e., the secretory bioregulatory macromolecules of lymphocytes, macrophages, and other leukocytes, are being studied to define their effective anticarcinogenic and tumor cell growth inhibitory activities. Leukoregulin, a lymphokine recently isolated during the course of this project, can prevent carcinogenesis and inhibit tumor cell growth. Anticarcinogenic action is direct, irreversible and occurs without cytotoxicity. Inhibition of tumor cell growth is primarily reversible but can become irreversible due to increased susceptibility of preneoplastic and neoplastic cells to cytolytic destruction by natural killer cells resulting from leukoregulin target cell interaction. Leukoregulin at very high concentrations is also directly cytolytic for tumor cells. The direct-acting anticarcinogenic activity of leukoregulin is more potent than the tumor cell inhibitory activity; but by also being able to increase target cell sensitivity to the cytoreductive action of naturally cytotoxic lymphocytes, leukoregulin can be an effective homeostatic mechanism for control of carcinogenesis at its later stages of development. Leukoregulin-induced changes in plasma membrane permeability are partially dependent upon extracellular ionic calcium and are accompanied by increased calcium flux, the rapid opening and closing of plasma membrane single ion channels and translocation of protein kinase C from the cytosol to the plasma membrane which may be important events in the molecular pathway resulting in inhibition of tumor and other abnormal cell proliferation by this immunologic hormone. Leukoregulin induces identical changes in target cell plasma membrane permeability as occur during natural killer lymphocyte cytotoxicity providing strong evidence that it is an intrinsic mediator or element of the natural cytotoxicity reaction and possibly signifying its central role in immunological homeostasis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05499-01 LB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chromosome Alterations and Proto-Oncogenes Transposition in Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. C. Popescu	Microbiologist	LB	NCI
Others:	S. Amsbaugh	Microbiologist	LB	NCI
	J. A. DiPaolo	Chief	LB	NCI
	M. Kraus	Visiting Associate	LCMB	NCI
	G. Kruh	Medical Staff Fellow	LCMB	NCI
	R. C. King	Senior Staff Fellow	LCMB	NCI

COOPERATING UNITS *(if any)*

Duke University Medical Center (Y.T. Chen)

LAB/BRANCH
Laboratory of Biology

SECTION
Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	0.9	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two newly isolated proto-oncogenes, erbB-2 and arg have been localized by in situ hybridization to chromosomes 17 and 1, respectively. The erbB-2 gene is frequently overexpressed in mammary cancer and the long arm of chromosome 1, where arg gene is located, is involved in structural rearrangements or duplications in the majority of the solid tumors. Human cytochrome P1-450 was localized on chromosome 15 at the site of breakpoint of the translocation 15;17 characteristic for acute promyelocytic leukemia. A North American Burkitt's lymphoma cell line exhibits, in addition to a common 8;22 reciprocal translocation, two other translocations occurring near proto-oncogene sites; however, only myc mRNA is highly elevated, indicating that myc gene was implicated in neoplastic development of this B-cell malignancy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04504-15 CCTP

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Model Systems for the Study of Chemical Carcinogenesis at the Cellular Level

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. H. Yuspa	Chief	LCCTP	NCI
Others:	H. Hennings	Research Chemist	LCCTP	NCI
	M. Poirier	Research Chemist	LCCTP	NCI
	D. Roop	Microbiologist	LCCTP	NCI
	J. Strickland	Research Chemist	LCCTP	NCI
	D. Greenhalgh	Visiting Fellow	LCCTP	NCI
	U. Lichti	Guest Researcher	LCCTP	NCI

COOPERATING UNITS *(if any)*
ImmuQuest Laboratories, Rockville, MD (E. F. Spangler); Johns Hopkins Oncology Center, Baltimore, MD (R. Tucker); University of Arizona, Tucson, AZ (G. T. Bowden); Alton Jones Cell Science Center, Lake Placid, NY (S. Jaken).

LAB/BRANCH
Laboratory of Cellular Carcinogenesis and Tumor Promotion

SECTION
In Vitro Pathogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9.0	6.0	3.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cellular and molecular aspects of chemical carcinogenesis in lining epithelia are studied in mouse epidermis by in vivo and in vitro techniques. The initiation event in skin carcinogenesis is highly correlated to an alteration in the program of terminal differentiation of epidermal cells. In cell culture, epidermal differentiation is regulated by the concentration of extracellular calcium. Induction of epidermal differentiation by increasing the calcium concentration in the culture medium causes a 5- to 10-fold increase in the level of intracellular free calcium. The most effective induction of terminal differentiation was found when treatment with the ionophore ionomycin was combined with activation of protein kinase C by phorbol esters. The ras oncogene is highly correlated to the initiated phenotype in epidermis. Introduction of an activated ras gene into normal keratinocytes leads to their conversion into papilloma cells. Chemically induced papillomas yield an activated ras oncogene with a mutation at codon 61. Papilloma cells and initiated cells are resistant to the differentiation-inducing effects of phorbol ester tumor promoters. Since phorbol esters induce differentiation in normal cells, papilloma cells can be selected among an excess of normal cells in culture by their ability to continue to proliferate in culture medium containing phorbol esters. In vivo, several classes of benign tumors can be induced by initiation and promotion. Papillomas with a high risk for spontaneous conversion to carcinomas are also most responsive to chemical converting agents. Malignant conversion can be accomplished by a single injection of cis-diamminedichloroplatinum II. Bryostatin 1, an activator of protein kinase C, inhibits phorbol ester tumor promotion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04798-17 CCTP

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less Title must fit on one line between the borders.)*: Metabolism and Mode of Action of Vitamin A

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	L. M. De Luca	Research Chemist	LCCTP NCI
Others:	K. E. Creek	Staff Fellow	LCCTP NCI
	S. Kato	Visiting Fellow	LCCTP NCI
	E. M. McDowell	IPA Appointee	LCCTP NCI
	D. Joel	IRTA Appointee	LCCTP NCI
	R. Sinha	Volunteer	LCCTP NCI

COOPERATING UNITS *(if any)*: ImmuQuest, Rockville, MD (R. Shores and E. F. Spangler)

LAB/BRANCH: Laboratory of Cellular Carcinogenesis and Tumor Promotion

SECTION: Differentiation Control Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	5.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Vitamin A deficiency causes apparent hyperplasia of basal cells and squamous metaplasia of the hamster tracheal epithelium in vivo and in organ culture. Using a system of hamster tracheal epithelial cells cultured on a collagen gel substrate, it was shown that the target cell for retinoid action in the hamster tracheal epithelium is the secretory (mucous) cell. In the absence of retinoic acid, the secretory cells flattened and their capacity to divide was greatly diminished. Since the basal cells continued to replicate, when the secretory cells did not, the population density of the basal cells increased, giving the appearance of "basal cell hyperplasia." In addition to effects on the maintenance of epithelial cell differentiation, retinoic acid (10E-6 to 3x 10E-8 M) was shown to profoundly and reversibly enhance cell to substratum adhesion of mouse fibroblasts, NIH-3T3 cells, maintained in culture for 6 hr to 2 days in the presence of the retinoid. Both trypsinized retinoid-pretreated and control cells attached efficiently to fibronectin or gelatin substrates in a short term (90 min.) attachment assay. In contrast, only retinoic acid-treated cells were able to adhere to laminin and type IV collagen substrates, while control cells showed little or no attachment. Other mouse fibroblast lines (3T3-Swiss, 3T6-Swiss, Balb 3T3, and Balb/3T12-3) responded to retinoid treatment in a similar way. However, the virus-transformed Balb/3T3 lines, SV-T2 and M-MSV, showed significant attachment to laminin substrates without retinoid treatment and retinoic acid either did not affect or slightly decreased the cell attachment to laminin substrates. The retinoic acid also caused a 50-60% decrease in the uptake of tritiated myoinositol by NIH-3T3 cells. Concentration and time dependency of this effect were similar to those measured for the enhanced attachment to laminin. Moreover, uptake of radiolabeled mannose, glucose, or galactose was not affected, thus suggesting inositol transport is specifically inhibited by retinoic acid.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05177-06 CCTP

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Use of Immunological Techniques to Study Interaction of Carcinogens with DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. C. Poirier	Research Chemist	LCCTP	NCI
Others:	S. H. Yuspa	Chief	LCCTP	NCI
	O. Olivero	Fogarty Fellow	LCCTP	NCI
	E. Reed	Special Assistant for Science	DCT	NCI
	C. Litterst	Research Chemist	LMCP	NCI
	R. Ozols	Chief	MB	NCI

COOPERATING UNITS *(if any)* MIT, Boston,MA (S. Lippard); Univ. of Texas Med. School, Houston, TX (J. M. Hunt); NCTR, Jefferson,AR (F. A. Beland); National Hosp.,Oslo,Norway (H. Huitfeldt); CIIT, Res. Triangle Park,NC (J. Swenberg); U. of Iowa, Iowa City,IA (J. Baron); Mt.Sinai Med. Ctr., N.Y.,NY (T. Fasy); McArdle, Madison,WI (H. Pitot).

LAB/BRANCH
Laboratory of Cellular Carcinogenesis and Tumor Promotion

SECTION
In Vitro Pathogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.25	1.75	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Antibodies specific for carcinogen-DNA adducts have probed the nature, extent, and consequences of in vitro and in vivo DNA modification. Biological samples substituted with 2-acetylaminofluorene (AAF) and cis-diamminedichloroplatinum II (cis-DDP) have been analyzed by quantitative immunoassays and by immunohistochemical procedures developed to localize adducts in situ. In hepatic DNA of rats fed a carcinogenic dose of AAF for 4 weeks, adduct accumulation reached a plateau at 2-3 weeks and adduct removal was biphasic during 4 subsequent weeks on control diet. A computer-derived pharmacokinetic model proposed two genomic compartments, one from which adducts are removed rapidly and another from which they are removed slowly. Studies initiated to identify the two compartments have investigated different cell types within the liver and DNA associated with more or less tightly-bound chromatin regions including the nuclear matrix. Immunohistochemical localization of AF-DNA adducts in livers of rats fed AAF demonstrated high adduct concentrations in periportal regions and no adducts detectable in preneoplastic foci induced by several different protocols. Cis-DDP-DNA adducts were measured in 231 nucleated peripheral blood cell DNA samples from cancer patients at multiple times during courses of cis-DDP therapy. Adduct accumulation, in positive samples (43% of the total), occurred as a function of total cumulative dose and suggested relatively slow adduct removal. Disease response data on 55 ovarian cancer patients indicated that individuals with high adduct levels have a high rate of complete response to therapy, and many individuals who do not respond also do not form adducts. Adduct persistence was demonstrated in many tissues obtained at autopsy from patients who received their last therapy several weeks or months prior to expiration. Mechanisms of cis-DDP efficacy are also being investigated in animal models.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05178-06 CCTP

PERIOD COVERED

October 1, 1986, to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular and Tissue Determinants of Susceptibility to Chemical Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. E. Strickland	Research Chemist	LCCTP	NCI
Others:	S. H. Yuspa	Chief	LCCTP	NCI
	H. Hennings	Research Chemist	LCCTP	NCI
	A. Koceva-Chyla	Guest Researcher	LCCTP	NCI
	D. Greenhalgh	Visiting Fellow	LCCTP	NCI

COOPERATING UNITS *(if any)*

ImmuQuest Laboratories, Inc., Rockville, MD (E. F. Spangler)

LAB/BRANCH

Laboratory of Cellular Carcinogenesis and Tumor Promotion

SECTION

In Vitro Pathogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.7	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In vivo studies show that the SENCAR mouse is unusually sensitive to skin carcinogenesis by initiation and promotion, while the BALB/c mouse is resistant. We have developed a technique of grafting epidermal and dermal cells to athymic nude mice to form a reconstituted skin. Four cell lines, derived from either papillomas or chemically initiated skin of BALB/c and SENCAR mice have been developed and characterized. Each forms benign squamous papillomas in grafts and has an activated ras oncogene. Since malignant conversion has occurred within some papillomas produced by grafting each of the lines, they are all on the pathway to malignancy. However, neither culture for 8 weeks at confluence nor culture in the presence of epidermal growth factor or the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) influences malignant conversion of the grafted cells. Suppression of papilloma size occurs when normal primary epidermal cells are grafted along with small numbers of cells from these lines. Both BALB/c and SENCAR primaries suppress regardless of the strain of origin of the cell line used. These cell lines allow us to reconstruct an "initiated" skin using mixtures of papilloma-forming cells with primary epidermal cells. Clonal growth studies in culture have shown a variety of growth responses to TPA. The proliferation of two lines is enhanced by TPA while that of the others is suppressed. Calcium levels further modulate growth. We therefore expect to find a variety of biological responses, depending upon cell line, when such reconstructions are treated with TPA. These cell lines also provide excellent model systems for studying the mechanism of conversion of benign tumor cells to malignancy. The ability to create mixtures of normal BALB/c with papilloma-forming SENCAR cells and vice-versa should be helpful in elucidating mechanisms of sensitivity to promotion.

PHS 6040 (Rev 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05270-06 CCTP

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Molecular Mechanism of Action of Phorbol Ester Tumor Promoters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter M. Blumberg	Research Chemist	LCCTP	NCI
Others:	M. Dell'Aquila	Staff Fellow	LCCTP	NCI
	B. Warren	Guest Researcher	LCCTP	NCI
	T. Nakadate	Visiting Fellow	LCCTP	NCI
	H. Nakakuma	Visiting Fellow	LCCTP	NCI
	T. Sako	Visiting Fellow	LCCTP	NCI
	D. deVries	Visiting Fellow	LCCTP	NCI
	E. Rivedal	Fogarty International Fellow	LCCTP	NCI

COOPERATING UNITS *(if any)* Boston Univ. School of Med., Boston, MA (A. I. Tauber, J. Cox); Stanford Univ., Palo Alto, CA (P. Wender, C. Cribbs); Arizona State Univ., Tempe, AZ (G. R. Pettit, C. L. Herald, Y. Komano); Ciba-Geigy, Summit, NJ (A. Y. Jeng); Upjohn Co., Kalamazoo, MI (K. L. Leach)

LAB/BRANCH
Laboratory of Cellular Carcinogenesis and Tumor Promotion

SECTION
Molecular Mechanisms of Tumor Promotion Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10	7.5	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The efforts of the Molecular Mechanisms of Tumor Promotion Section are directed toward understanding the early events in the interaction of phorbol ester tumor promoters with cells and tissues. Particular attention is being devoted to the analysis of the major phorbol ester receptor, protein kinase C. The bryostatins, macrocyclic lactones, activate protein kinase C and compete for phorbol ester binding. They only induce a subset of the typical phorbol ester responses, however. In Friend erythroleukemia cells, they restore differentiation inhibited by the phorbol esters. In primary mouse epidermal cells, they induce markers of the proliferative response but block phorbol ester induction of markers of differentiation. Part of the difference in response pattern can be explained by the bryostatins acting to activate protein kinase C transiently followed by suppression of the pathway. Thus, both for cell-cell communication and epidermal growth factor binding, the bryostatins initially act like the phorbol esters but subsequently block phorbol ester responsiveness. In addition, the bryostatins intrinsically differ from the phorbol esters in their stimulatory activity for some responses; for example, they fail to induce arachidonic acid release in C3H10T1/2 cells even at very early times. The biochemical mechanisms for these differences are being explored through immunoblotting of protein kinase C, comparison of phosphorylation patterns, and tritiated bryostatin binding analysis. Protein kinase C has been implicated in the actions of several oncogenes. The mitogenic response of Swiss 3T3 cells to ras was shown to be inhibited by protein kinase C depletion and restored by microinjection of purified protein kinase C. The mechanisms of action of protein kinase C inhibitors were clarified by comparison of their effects on the functional domains of protein kinase C.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05445-03 CCTP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Regulation of Epidermal Specific Differentiation Products

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dennis R. Roop	Microbiologist	LCCTP	NCI
Others:	S. H. Yuspa	Chief	LCCTP	NCI
	H. Nakazawa	Visiting Fellow	LCCTP	NCI
	T. Mehrel	Visiting Fellow	LCCTP	NCI
	D. Rosenthal	Biotechnology Fellow	LCCTP	NCI
	L. De Luca	Research Chemist	LCCTP	NCI
	P. Steinert	Visiting Scientist	DB	NCI
	S. Chung	Senior Staff Fellow	LEC	NCI

COOPERATING UNITS *(if any)* Microbiological Assoc., Bethesda, MD (E. F. Spangler); Univ. of Munich (Thomas Krieg); USUHS, Bethesda, MD (E. Chang); Center for Drugs and Biologics, Bethesda, MD (J. Ridge); Baylor College of Med., Houston, TX (J. Clark); U. of Wash., Seattle, WA (C. Fisher); Univ. of Oslo, Oslo, Norway, (H. Huitfeldt)

LAB/BRANCH

Laboratory of Cellular Carcinogenesis and Tumor Promotion

SECTION

In Vitro Pathogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

cDNA clones corresponding to the major proteins expressed in mouse epidermis have been isolated and characterized. Using a combination of in situ hybridization with RNA probes (which are specific for individual mRNAs) and indirect immunofluorescence with monospecific antisera (which were elicited with synthetic peptides corresponding to unique sequences within each protein), it is possible to show that these genes belong to at least four subsets: those expressed predominantly in the proliferating basal layer of the epidermis; those expressed predominantly in the differentiated suprabasal spinous layers and, to a less extent, in the granular layer; those only expressed in the granular layer; and those only expressed under hyperproliferative conditions. Genes representing each subset have been isolated and sequenced. Various strategies have been employed to identify sequences regulating the expression of these genes, including vector constructs using different regions of the genomic clones to drive expression of the chloramphenicol acetyl transferase gene and the production of transgenic mice which express a human differentiation-associated keratin gene in a tissue- and differentiation-specific pattern. A gene encoding a cysteine-rich protein, which is a major component of the cornified envelope, has been isolated and shown by in situ hybridization experiments to be expressed in the granular layer of the epidermis. A monospecific antiserum has been used to demonstrate that the C-terminal portion of this protein is only detectable on the inner surface of mature envelopes. Monospecific antisera that have been produced against mouse and human keratins and other epidermal-specific differentiation products have been used to study various stages of carcinogenesis, gene expression in mutant mice exhibiting developmental defects in epidermal differentiation, the induction of terminal differentiation in malignant cell lines by pharmacological agents, the in vivo kinetics of expression of the differentiation-associated keratins with respect to cell division, and requirements for the induction of terminal differentiation products in vitro.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05051-09 LC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biology and Molecular Biology of Transforming Growth Factor-beta

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A.B. Roberts Staff Scientist LC NCI

Others:

S.B. Jakowlew	Sr. Staff Fellow	LC NCI	P. Kondaiah	Visiting Fellow	LC NCI
K.C. Flanders	Sr. Staff Fellow	LC NCI	J.M. Smith	Biologist	LC NCI
N.B. Roche	Biologist	LC NCI	P.J. Dillard	Chemist	LC NCI
U. Heine	Staff Scientist	LCC NCI			
B. de Crombrugghe	Staff Scientist	LMB NCI			

COOPERATING UNITS (if any)

Pamela Robey, Marian Young, John Termine, Bone Research Branch, NIDR

LAB/BRANCH
Laboratory of Chemoprevention

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	3.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of the project is twofold: to study the biology of transforming growth factor-beta (TGF-beta), particularly in terms of its effects on cell function, and to investigate the molecular biology of TGF-beta with emphasis on evaluating the degree of conservation between species of both the precursor and processed TGF-beta 1 peptide, as well as conservation between TGF-betas types 1 and 2. With respect to the biology of TGF-beta, one of the principal effects of the peptide on cells of mesenchymal origin is to control synthesis of matrix proteins. Control is exerted both at the level of synthesis and at the level of degradation. Effects on collagen synthesis have been previously reported by our laboratory and recent investigations are focused on the ability of TGF-beta to increase mRNA for collagen types I, III, and V. TGF-beta induced increases in collagen mRNA derive, at least in part, from direct effects on type I and III collagen promoters, as determined by collaborative studies in which the promoter was linked to a reported gene for chloramphenicol acetyltransferase. Use of deletion constructs of the promoter have identified specific sites required for TGF-beta control of expression. With respect to the molecular biology of TGF-beta, cloning of porcine and chicken genes has resulted in identification of alternate splicing patterns which may be important in control of TGF-beta expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05396-04 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Analogs for Study of Oncogenesis and Development of the Rat

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Shinichi Watanabe	Sr. Staff Fellow	LC	NCI
Others:	Eliane Lazar	Visiting Fellow	LC	NCI
	Elisa Vicenzi	Guest Researcher	LC	NCI
	Linda Durham	Guest Researcher	LC	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Chemoprevention

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several mutations have been introduced into a cloned human TGF-alpha gene by site-directed mutagenesis. These mutant forms of TGF-alpha were expressed in a yeast expression vector. Some of them show altered characteristics compared to normal (wild type) TGF-alpha. The rat TGF-alpha gene was chemically synthesized and expressed in a retrovirus vector. Infectious recombinant retrovirus carrying the rat TGF-alpha gene makes normal rat kidney (NRK) cells secrete rat TGF-alpha at a higher level than most transformed cells. The rat TGF-alpha gene has been inserted into an E. coli plasmid which has a strong promoter. The rat TGF-alpha gene has also been fused with the hepatitis B surface antigen (HBsAg) gene to express it as a fusion protein in a eukaryote expression vector.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05398-04 LC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Characterization of Latent Transforming Growth Factor Beta and its Receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael B. Sporn	Chief	LC	NCI
Others:	Lalage M. Wakefield	Visiting Associate	LC	NCI
	Diane M. Smith	Biologist	LC	NCI
	Cornelius Knabbe	BCSG Fellow	MB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Chemoprevention

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transforming growth factor-beta (TGF-beta) is a multifunctional peptide that regulates growth and differentiation of a wide variety of cell types. The purpose of this project is to determine the role that endogenously-produced TGF-beta may play in the control of growth of normal and transformed cells, and to study the regulation of TGF-beta action in this context. To this end, polyclonal antisera have been raised against TGF-beta and synthetic peptides corresponding to regions of the putative precursor. The effects of these antibodies on the anchorage-dependent and -independent growth of normal and transformed cells are being investigated and the antisera are also being used as tools in the immunochemical characterization of the latent forms of TGF-beta. Extensive analysis of the distribution and modulation of the cellular receptor for TGF-beta has shown that binding of TGF-beta to its receptor is not a major control point in TGF-beta action. However, normal and transformed cells have been shown to secrete TGF-beta in a biologically inactive form that is unable to bind to the receptor, and it is anticipated that activation of this latent form will be a critical regulatory step in TGF-beta action. Using immunochemical techniques, the latent form of TGF-beta secreted by human platelets has been shown to be a high molecular weight complex in which mature TGF-beta is non-covalently associated with precursor sequences and a further unidentified component; this probably represents a delivery complex. The complex is being purified to homogeneity for sequencing and identification. A second latent form of TGF-beta, found in serum, has been identified as TGF-beta bound to alpha-2-macroglobulin; this probably represents a clearance complex. Further characterization of the nature and regulation of endogenous forms of TGF-beta should help elucidate the role these molecules may play in the process of carcinogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05525-01 LC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
cDNA Cloning and Functional Analysis of Transforming Growth Factor Beta

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ellen E. Van Obberghen	Staff Fellow	LC NCI
Others:	Carl Baker	Senior Investigator	LTVB NCI

COOPERATING UNITS (if any)

Monique Dubois-Dalcq, Section Chief, Laboratory of Molecular Genetics, National Institute of Neurological and Communicative Disorders and Stroke

LAB/BRANCH
Laboratory of Chemoprevention

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transforming growth factor beta (TGF-beta) is a multifunctional polypeptide, which was the first in an emerging super-family of regulatory polypeptides to be identified and purified to homogeneity. In our laboratory, TGF-beta is currently being isolated from human platelets. An alternate and abundant source of TGF-beta used by Collagen Corp. (Palo Alto, CA) is bovine bone. The amino acid sequence of human TGF-beta has been deduced from its cDNA sequence; however, only the first 30 N-terminal residues of the bovine homolog have been sequenced. One aim of the present study was to determine the complete amino acid sequence of bovine TGF-beta by cDNA cloning and sequencing. Interestingly, a second molecule, which shares about 70% amino acid sequence homology in the N-terminus with TGF-beta, has been isolated from bovine bone; this second form of TGF-beta is the most closely related member of the above-mentioned gene family. Isolation of a cDNA clone specific for the second form of TGF-beta would allow further characterization of its molecular nature and functional role.

About five years have passed since the isolation of TGF-beta and its designation as a "transforming growth factor." However, the study of TGF-beta is no longer limited to that of its role in malignant transformation. Rather, its has become an increasingly expanding field which now encompasses growth modulation, differentiation, embryogenesis and wound repair. A second aspect of my work on TGF-beta involves a collaboration with Dr. Monique Dubois-Dalcq in the Laboratory of Molecular Genetics (NINCDS) designed to investigate the role of TGF-beta on growth and differentiation of glial cells of the vetebrate central nervous system (CNS).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04542-15 LCC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemistry of Nitroso Compounds & Other Substances of Interest in Cancer Research

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. K. Keefer	Chief, Chemistry Section	LCC	NCI
Others:	Y.-H. Heur	Visiting Fellow	LCC	NCI
	M. Stershic	Staff Fellow	LCC	NCI
	A. J. Streeter	Visiting Associate	LCC	NCI
	R. Nims	Chemist	LCC	NCI
	W. Blot	Chief	BB	NCI
	G.-Y. Li	Visiting Fellow	BB	NCI

COOPERATING UNITS *(if any)* PRI, Frederick, MD (J. Hrabie, L. Ohannesian, D. Williams); SK&F Labs., Philadelphia, PA (B. Mico); NJ Med. Sch., Newark, NJ (C. Yang); U. of Wash., Seattle, WA (S. Nelson); American Chem. Soc. (J. Malin); Cancer Res. Centre, Moscow, USSR (V. Turusov); Clemson Univ., Clemson, SC (J. Fanning)

LAB/BRANCH

Laboratory of Comparative Carcinogenesis

SECTION

Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, MD 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	3.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mechanisms potentially responsible for formation, activation, and detoxication of carcinogenic N-nitroso compounds in the human body are under intense investigation. Evidence implicating the alpha-nitrosamino radical as the critical intermediate in both activation and inactivation of the potent carcinogen, N-nitrosodimethylamine, has been obtained through deuterium isotope effect studies. Certain iron species have been found to convert amines to their carcinogenic N-nitroso derivatives in nonacidic media modeling those potentially found in vivo or in the environment; the rate law is zero order in nitrite, suggesting that the reaction may be as fast in the limit of very low nitrite concentrations (such as those found in the body or in the environment) as it is under the laboratory conditions used. The deuterium isotope effect on the carcinogenicity of 1,2-dimethylhydrazine suggests that at least three different mechanisms of tumor induction are simultaneously operative in dimethylhydrazine-treated mice. Urine specimens from a region of China having a very high incidence of esophageal cancer are being analyzed in a search for correlations with the degree of progression toward malignancy in the individual donors. The chemistry of a powerful mutagen isolated from human feces is being investigated with the aim of developing means of verifying its integrity, as well as stabilizing and solubilizing it during studies of its biological properties in mammals. The first O-trimethylsilylated nitrosamine salts have been prepared. Nitrite ion has been found to react with the common solvent, methylene chloride, to generate a powerful nitrosamine-forming intermediate.

PHS 6040 (Rev 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP04580-13 LCC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Role of Lipotropes in Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L. A. Poirier Supervisory Research Chemist LCC NCI

Others: P. T. Allen Microbiologist LCC NCI

COOPERATING UNITS *(if any)*
McArdle Laboratory, Madison, WI (H. Pitot)

LAB/BRANCH
Laboratory of Comparative Carcinogenesis

SECTION
Office of the Chief, Biomethylation Working Group

INSTITUTE AND LOCATION
NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanisms responsible for the alteration of chemical carcinogenesis by the dietary lipotropes, choline, methionine, folic acid and vitamin B-12, have been studied. The metabolism and carcinogenic activity of ethionine in different species is being compared. Correlations between the tissue levels of the physiological methyl donor S-adenosylmethionine, its chief metabolic inhibitor, S-adenosylhomocysteine, and 5-methylcytosine in animals treated with carcinogens, liver tumor promoters and methyl-deficient diets are being determined. Using standard bioassays, the effects of (1) the length of time of dietary methyl deprivation, (2) the interaction between methyl deprivation and hepatocarcinogens, and (3) deficiencies of other essential nutrients on hepatocarcinogenesis are under investigation. The effects of carcinogens and methylase inhibitors on the general and specific gene hypomethylation in target tissues are examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04582-12 LCC

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Mechanisms of Inorganic Carcinogenesis: Nickel

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	K.S. Kasprzak	Visiting Scientist	LCC	NCI
Others:	M.P. Waalkes	Senior Staff Fellow	LCC	NCI
	J.M. Ward	Chief, Tumor Pathol. & Pathogen. Section	LCC	NCI
	U.I. Heine	Chief, Ultrastructural Studies Section	LCC	NCI
	H. Miki	Visiting Fellow	LCC	NCI
	C.W. Reynolds	Chief, Cell. & Mol. Immunol. Section	LEI	NCI

COOPERATING UNITS (if any): Program Resources, Inc., Frederick, MD (O. Weislow, H. Issaq, R. Kovatch, B. Diwan, C. Riggs)

LAB/BRANCH: Laboratory of Comparative Carcinogenesis

SECTION: Office of the Chief, Inorganic Carcinogenesis Working Group

INSTITUTE AND LOCATION: NCI, NIH, Frederick, MD 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Investigations of the effects of essential divalent metals, magnesium, zinc and iron, on the carcinogenicity of nickel have been continued in bioassay and biochemical studies. Immunohistochemical investigations over the first month after injection of nickel revealed that this metal transiently inhibited activity of natural killer cells in the injected muscle, while magnesium reversed this effect. In an in vitro study, nickel diminished a mitogen-stimulated incorporation of tritiated thymidine into murine T-lymphocytes, while magnesium antagonized nickel action. Thus, magnesium appears to inhibit cytotoxicity of nickel and stimulate the natural cellular defenses against nickel-transformed cells. In yet another in vitro study, nickel was found for the first time to disrupt cell-cell communication which indicated its tumor-promotional activity; magnesium partially reversed this effect. Zinc, another antagonist of nickel carcinogenesis, is much less active than magnesium. It prolonged the latency of tumors without any significant influence on their final incidence in a 1.5-yr study. Zinc does not affect nickel retention in the injected muscle and has no detectable influence on the early local necrotic/inflammatory response to nickel. In a bioassay currently underway, iron, which is chemically closer to nickel than zinc and magnesium, appears to be a much stronger inhibitor of nickel carcinogenesis than the latter two metals. A new original hypothesis on the mechanism of nickel carcinogenesis has been formulated based on the known catalytic effects of the nickel(II)/nickel(III) couple on the oxidation of some polypeptides and proteins involving free-radical reactions. Experiments performed to test this hypothesis showed interstrand-DNA, DNA-histone, and histone-histone cross-linking when the substrates were incubated in vitro with nickel(II) in the presence of tetraglycine. Interactions of this type, in vivo, may damage the cellular genetic material and lead to neoplastic transformation of the cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04680-17 LCC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Application of In Vitro Systems to Study Perturbations of Methyl Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. A. Poirier	Supervisory Research Chemist	LCC NCI
	D. G. Blair	Chief, Microbiology Section	LMO NCI
	M. Bhave	Visiting Fellow	LCC NCI
	T. Flammang	Guest Researcher	LMO NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Carcinogenesis

SECTION

Office of the Chief, Biomethylation Working Group

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cultured eithelial cells derived from the livers of 10-day-old Fischer 344 rats are used as a model system for studying the mechanism of carcinogenesis resulting from an insufficiency of methyl donors. Transformation of liver cells has been achieved following treatment with 3-deazaadenosine (DAA). This compound is metabolized to 3-deazaadenosylhomocysteine, a potent inhibitor of S-adenosylhomocysteine (AdoHcy) hydrolase, and results in an accumulation of AdoHcy, a competitive inhibitor of most physiological methylation reactions. DNA has been isolated from tumors induced in rats initiated with N-nitrosodiethylamine and fed a diet deficient in methionine and choline and used in the NIH 3T3 cell transfection assay. Results indicate that activation of the c-Ha-ras oncogene appears to be involved in the development of hepatocellular carcinomas in methyl-deficient rats. This gene is hypomethylated in the liver tumors of rats fed the methyl-deficient diets.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04812-19 LCC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cell Interactions During Transformation of Epithelial Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	U. I. Heine	Chief, Ultrastructural Studies Section	LCC	NCI
Others:	H. Miki	Visiting Fellow	LCC	NCI
	K. S. Kasprzak	Visiting Scientist	LCC	NCI

COOPERATING UNITS *(if any)*

Program Resources, Inc., Frederick, MD (E. F. Munoz)

LAB/BRANCH

Laboratory of Comparative Carcinogenesis

SECTION

Ultrastructural Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To define the role of gap-junctional communication in tumor promotion, nonpromotable, promotable, and tumorigenic transformed epidermis-derived cells of line JB6 as well as NIH 3T3, cells were subjected to the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). Cell-cell communication was measured either by the radioisotope transfer technique or by microinjection of fluorescent dye. Our results give evidence for the importance of blocked cell-cell communication in focus-formation during the process of tumor promotion; however, reduced intercellular communication is not a decisive factor in maintaining malignancy. Interruption of gap-junctional intercellular communication was used as indicator in a short-term test model to uncover tumor-promoting properties in chemical agents, such as Ni-(II)-salts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05092-09 LCC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transplacental Carcinogenesis and Tumor Promotion in Nonhuman Primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. E. Palmer	Research Veterinarian	LCC	NCI
Others:	J. M. Rice	Chief	LCC	NCI
	J. M. Ward	Chief, Tumor Pathol. and Pathogen. Section	LCC	NCI
	L. M. Anderson	Expert	LCC	NCI
	P. J. Donovan	Chemist	LCC	NCI
	A. O. Perantoni	Microbiologist	LCC	NCI

COOPERATING UNITS *(if any)*

SEMA, Inc., Rockville, MD (J. Phillips); Baylor College of Medicine, Houston, TX (L. J. Lu); Oak Ridge Associated Universities, Oak Ridge, TN (N. Clapp)

LAB/BRANCH

Laboratory of Comparative Carcinogenesis

SECTION

Office of the Chief, Primate Research Working Group

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	2.0	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Nonhuman primates of the species Erythrocebus patas (patas) and Macaca fascicularis (cynomolgus) are subjected to direct-acting or metabolism-dependent chemical carcinogens by transplacental or direct exposure. In some cases the carcinogen-treated animals are subsequently exposed to chemicals that promote the development of neoplasms in rodents. Mechanisms of organ and species differences in the effects of chemical carcinogens and tumor promoters among rodent and nonhuman primate species are investigated. Induced tumors are evaluated by light microscopy using standard staining procedures, histochemical techniques and electron microscopy and are assayed for in vitro cultivability and transplantability to rodents. Selected tumors are subjected to DNA extraction and attempts are made to transfect NIH 3T3 cells with their DNA. These studies have shown that intrinsic susceptibility to transplacental carcinogenesis is greatest in nonhuman primates early in gestation and have provided the only animal model of chemically inducible gestational choriocarcinoma. The association of chronic ulcerative colitis and multifocal colonic carcinoma in the cotton-top tamarin (Saguinus oedipus) is being investigated in collaboration with Oak Ridge Associated Universities, with primary attention being given to a search for direct or indirect evidence for a fecal mutagen/carcinogen in this species.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05093-09 LCC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In Vitro Studies on Organ Specificity in Transplacental Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. M. Rice	Chief	LCC	NCI
Others:	P. Donovan	Chemist	LCC	NCI
	A. Perantoni	Microbiologist	LCC	NCI
	T. Enomoto	Visiting Fellow	LCC	NCI

COOPERATING UNITS *(if any)*

Microbiological Associates, Inc., Bethesda, MD (M.L. Wenk); Program Resources, Inc., Frederick, MD (B. Diwan)

LAB/BRANCH

Laboratory of Comparative Carcinogenesis

SECTION

Perinatal Carcinogenesis Section, Developmental Biology Working Group

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The roles of morphogenetic differentiation in controlling the phenotypic expression of neoplastic transformation, the degree of malignancy of tumors, and the susceptibility of developing organs to carcinogenesis are studied using organ culture and tissue transplantation techniques, with current emphasis on the kidney. A defined medium for growth of rat and mouse ureteric bud epithelium in monolayer culture has been developed in which epidermal growth factor and selenium have proved essential and insulin, hydrocortisone, and transferrin have proved highly beneficial. Cell lines were successfully established from fetal rat renal mesenchyme in serum-containing media, but such lines showed karyotypic abnormalities and could not be induced to form tubular epithelium. Serum-free media containing endothelial cell growth supplement appears to offer a solution to this problem. The ability of transplacentally administered carcinogens to induce genotoxic damage in cells of embryos or fetuses exposed at different stages of gestation was determined for rat, mouse, and Syrian hamster. Cells were isolated from exposed embryos and gene mutations at two to three loci (resistance to ouabain and 6-thioguanine and to diphtheria toxin in the hamster) were assayed in vitro with simultaneous determination of survival ability. Organ specificity of induced gene mutation is being determined in embryonal cells isolated from organs of various species exposed in utero at comparable stages of gestation. A maximum level of mutation induction was found to be induced by N-nitrosoethylurea at day 9 of gestation from mesenchymal cells of the Syrian hamster fetus, with a further, much higher sensitivity very early in gestation in the immediate post-implantation period. Cells derived from the brain of fetuses treated at different times of gestation also demonstrate a similar sensitivity. Evidence indicates that cells derived from other tissues have maximum levels of mutation induction at 6 to 7 days of gestation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05288-06 LCC

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 charecters or less. Title must fit on one line between the borders.): Signal Transduction and the Control of Developmental Gene Expression

PRINCIPAL INVESTIGATOR (List other professionel personnel below the Principel Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. D. Blumberg	Senior Staff Fellow	LCC NCI
Others:	J. F. Comer	Microbiologist	LCC NCI
	R. Das	Visiting Fellow	LCC NCI

COOPERATING UNITS (if any): None

LAB/BRANCH: Laboratory of Comparative Carcinogenesis

SECTION: Office of the Chief, Molecular Biology Working Group

INSTITUTE AND LOCATION: NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.25	1.25	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standerd unreduced type. Do not exceed the space provided.)

A very simple model system, the cellular slime mold Dictyostelium discoideum, is being used to study mechanisms which control developmental gene activation during normal differentiation. Postaggregation Dictyostelium cells transcribe an additional 26% of their genome which is not expressed in earlier pre-aggregation stage cells. Cell-cell interaction is a necessary prerequisite for the synthesis and stability of these new differentiation-specific messenger RNAs. Additionally, the transciption rate and stability of these messenger RNAs are further regulated by a cyclic AMP-mediated process. We have demonstrated that 1) lyzosomatrophic agents such as $(NH_4)_2SO_4$ can replace the need for cell-cell interaction for postaggregation gene expression; 2) cAMP acts to regulate post aggregation gene expression through the cell surface cAMP receptor; 3) accumulation of mRNA for differentiation-specific genes expressed in prestalk cells is regulated through a different kinetic form of the cell surface receptor than those expressed in prespore cells; 4) activation of the cAMP receptor-associated adenylate cyclase does not play a role in the second messenger signal transduction system utilized for activation of expression of either the prespore or the prestalk genes; 5) prespore genes but not prestalk genes utilize a Ca^{++}/Calmodulin-dependent second messenger signal transduction system for their activation; finally, 6) pathways that induce the expression of differentiation-specific genes in prespore cells suppress the expression of genes transcribed during growth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05299-06 LCC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 chereters or less. Title must fit on one line between the borders.)*

Interspecies Differences in Transplacental Carcinogenesis and Tumor Promotion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. M. Ward	Chief, Tumor Pathology and Pathogenesis Sect.	LCC	NCI
Others:	J. M. Rice	Chief	LCC	NCI
	L. M. Anderson	Expert	LCC	NCI
	L. K. Keefer	Chief, Chemistry Section	LCC	NCI
	A. Hagiwara	Guest Researcher	LCC	NCI

COOPERATING UNITS *(if any)*

Microbiological Associates, Inc., Bethesda, MD (M. L. Wenk); Program Resources, Inc., Frederick, MD (B. Diwan)

LAB/BRANCH

Laboratory of Comparative Carcinogenesis

SECTION

Tumor Pathology and Pathogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.6	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

Tumor promotion phenomena in two-stage carcinogenesis were systematically explored in various rodent species in conjunction with transplacental carcinogenesis. Structure-promoting activity relationships of various barbiturates, hydantoins and benzodiazepine tranquilizers were investigated by sequential administration to animals of a transient, low level exposure to a genotoxic carcinogen followed by the test agent under study. Two long-acting hypnotic barbiturates, allobarbital and aprobarbital, and one intermediate-acting compound, pentobarbital, were found to promote liver carcinogenesis in male rats, while two monosubstituted nonhypnotic barbiturates and an intermediate-acting barbiturate, secobarbital, lacked such activity. A long-acting sedative anticonvulsive agent, nirvanol (5-ethyl-5-phenylhydantoin), promoted the development of hepatocellular tumors while a nonhypnotic hydantoin, 5,5-diethylhydantoin, was ineffective. A close relationship was found to exist between the induction of certain cytochrome P-450 species and tumor promoting abilities of barbiturates and hydantoins. Unlike the rat and mouse, in the Syrian golden hamster liver parenchymal cells were resistant to tumor promotion by phenobarbital. Phenobarbital increased liver weight and enhanced hepatic alkoxyresorufin O-dealkylase and aminopyrine N-demethylase activities in rats and mice susceptible to liver tumors but failed to induce any of these parameters in hamster liver to a significant extent.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05301-06 LCC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biology and Pathology of Natural and Experimentally Induced Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. M. Ward	Chief, Tumor Pathology and Pathogenesis Section	LCC	NCI
Others:	S. Rehm	Visiting Associate	LCC	NCI
	A. Hagiwara	Guest Researcher	LCC	NCI
	P. Nara	Staff Fellow	OD	NCI
	R. Benveniste	Medical Officer	LVC	NCI
	E. Santos	Visiting Associate	LMM	NIAID

COOPERATING UNITS *(if any)* VA Hosp., Pittsburgh, PA (G. Singh); Delta Regional Primate Research Center, Covington, LA (G. Baskin); Faculty of Medicine, University of Leiden, The Netherlands (A. Ten Have-Opbroek); Natl. Inst. of Hygienic Sciences, Tokyo (K. Takahashi); Program Resources, Inc., Frederick, MD (C. Thompson)

LAB/BRANCH

Laboratory of Comparative Carcinogenesis

SECTION

Tumor Pathology and Pathogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The pathology and biology of selected experimentally-induced and naturally-occurring neoplasms and neoplastic-related diseases of rodents were studied in order to elucidate their pathogenesis, including mechanisms of disease. The origin and pathology of mouse lung tumors, in particular so-called Clara cell papillary tumors, were studied with serial sections, immunocytochemistry, histochemistry and electron microscopy. After a detailed analysis in two strains of mice, conclusive evidence was presented that virtually all N-nitrosoethylurea (ENU)-induced lung tumors in mice were of alveolar Type II cell origin; none were of Clara cell origin. These findings have great implications for mouse lung tumor classification since many recent authors have inadvertently joined the bandwagon of Clara cell tumor terminology without conclusive evidence of the origin of these papillary tumors. Retroviral antigens were localized in human, simian and murine fixed tissue sections from cases of AIDS or leukemia using polyclonal and monoclonal antibodies. This technique has allowed a major advance in understanding the neurologic complications of acquired immune deficiency syndrome (AIDS) by identifying specific central nervous system (CNS) cell types infected with human immunodeficiency virus (HIV).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05303-06 LCC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis and Promotion of Natural and Induced Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. M. Ward	Chief, Tumor Pathology and Pathogenesis Section	LCC	NCI
Others:	A. Hagiwara	Guest Researcher	LCC	NCI
	P. Donovan	Chemist	LCC	NCI
	D. Devor	Biologist	LCC	NCI
	R. Cantor	Staff Fellow	LEI	NCI

COOPERATING UNITS *(if any)* Program Resources, Inc., Frederick, MD (B. Diwan, J. Henneman); Nagoya City University Medical School, Nagoya, Japan (N. Ito); Pathology Institute, Holback, Denmark (K. Ostergaard)

LAB/BRANCH

Laboratory of Comparative Carcinogenesis

SECTION

Tumor Pathology and Pathogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanisms of action of nongenotoxic carcinogens or tumor promoters have been studied using in vivo models of mouse and rat liver, rat kidney and bladder carcinogenesis and tumor promotion. A mouse liver system with initiation by N-nitrosodiethylamine at 4 weeks of age and exposure to the test agent 1-2 weeks later revealed that tumor promoters could be detected in as short a period as 12 weeks. Butylated hydroxyanisole was shown for the first time to be a potent mouse liver tumor promoter. Acetaminophen, a known human and rodent hepatotoxin, was found not to be carcinogenic for mouse liver but was a weak tumor promoter. In order to understand the role of chronic toxicity and hyperplasia, models were developed to study the role of hyperplasia in carcinogenesis and tumor promotion by nongenotoxic agents. Tritiated thymidine autoradiography and bromodeoxyuridine (BrDU) immunohistochemistry were used to evaluate levels of DNA synthesis in mice exposed to chronic hepatic and renal toxins. The new BrDU method was applied to our studies and was highly successful. Although several nongenotoxic carcinogens or promoters produced a chronic increase in levels of DNA synthesis in target organs, some of these chemicals produced chronic hyperplasia without tumor promotion or carcinogenesis. In vitro models for rat bladder urothelium and renal epithelium are being developed. Rat urothelium responded to urothelial tumor promoters but cyclamate, a noncarcinogen and not a tumor promoter for bladder in vivo, was the most effective hyperplastic agent in vitro. Continuing studies will attempt to define the role of chronic hyperplasia in carcinogenesis and tumor promotion by nongenotoxic agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05352-05 LCC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Metabolic and Pharmacological Determinants in Perinatal Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. M. Anderson	Expert	LCC	NCI
Others:	J. M. Rice	Chief, Perinatal Carcinogenesis Section	LCC	NCI
	M. S. Miller	Senior Staff Fellow	LCC	NCI
	J. M. Ward	Chief, Tumor Pathology and Pathogenesis Sect.	LCC	NCI
	A. Hagiwara	Guest Researcher	LCC	NCI
	A. Perantoni	Microbiologist	LCC	NCI
	T. Enomoto	Visiting Fellow	LCC	NCI

COOPERATING UNITS *(if any)*

Program Resources, Inc., Frederick, MD (H. Issaq, R. Kovatch); American Health Foundation, Valhalla, NY (S. Hecht); and Baylor University, Houston, TX (L.J. Lu)

LAB/BRANCH

Laboratory of Comparative Carcinogenesis

SECTION

Perinatal Carcinogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	0.5	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project addresses carcinogenesis during the perinatal period with regard both to mechanisms underlying susceptibility and to assessment of public health related phenomena. A pharmacogenetic transplacental carcinogenesis experiment has been completed in mice, confirming that both fetal and maternal genotype, with regard to inducibility of metabolism of methylcholanthrene (MC), are critical determinants of susceptibility to tumorigenesis, and showing that pretreatment with a noncarcinogenic inducer can provide some protection of fetuses of inducible phenotype. Also, exposure of the fetuses to a high dose of xenobiotic results in a significant alteration in amount of metabolic products formed by the livers of the mice as adults. Studies of transplacental pharmacokinetics and of induction of the relevant enzymes in individual fetuses are in progress or planned, employing sensitive biochemical assays, monoclonal antibodies as biochemical probes, and DNA-RNA molecular hybridization techniques. Another recently-completed project has involved transplacental exposure of mice to a series of N-nitroso compounds, including N-nitrosodimethylamine, N-nitrosodiethylamine, N-nitrosoethylurea, and N-nitrosocimetidine. This study has yielded interesting information on the comparative actions of these chemicals at different stages of ontogeny. Neurogenic and hepatic tumors from these mice are being analyzed for oncogenes by the Developmental Biology Working Group. An investigation of the transplacental effects of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in the mouse is ongoing; although NNK does not appear to be effective in transplacental initiation of lung tumors, some lymphoid neoplasms have appeared. Other studies in progress of potential public health importance include assessment of polychlorinated biphenyls as promoters and enhancers of tumor initiated by a nitrosamine in infant mice and investigation of the effects of the human transplacental carcinogen diethylstilbestrol, in the infant rat, with and without pretreatment with modifiers of metabolism.

PHS 6040 (Rev 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05353-05 LCC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Sensitivity Factors in Special Carcinogenesis Models

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. M. Anderson	Expert	LCC	NCI
Others:	J. M. Rice	Chief, Perinatal Carcinogenesis Section	LCC	NCI
	J. M. Ward	Chief, Tumor Pathology & Pathogenesis Section	LCC	NCI
	A. Hagiwara	Guest Researcher	LCC	NCI
	S. S. Park	Expert	LMC	NCI
	H. V. Gelboin	Chief	LMC	NCI

COOPERATING UNITS *(if any)*

Temple University, Philadelphia, PA (G. Harrington, H. Pylypiw, and P. N. Magee); University of South Florida, Tampa, FL (A. Giner-Sorolla)

LAB/BRANCH

Laboratory of Comparative Carcinogenesis

SECTION

Perinatal Carcinogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.0	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Determination of the factors which influence susceptibility to chemical carcinogens and of means of modulating this susceptibility are important goals. Such factors have been studied in several animal model systems, with particular emphasis on metabolism of carcinogens and on tumor initiation and promotion. (1) The metabolism, distribution, toxicity, and carcinogenic effects of the environmental agent, N-nitrosodimethylamine (NDMA), in the mouse have been found to be significantly altered by co-administration of ethanol in the drinking water. The presence of ethanol resulted in reduced toxicity in liver but increased circulating levels of NDMA and, importantly, led to an increase in lung tumors. Experiments are in progress to distinguish between pharmacokinetic (dose delivery), cellular (repair of DNA damage), and tumor promotion mechanisms of this effect. (2) N-nitrosocimetidine (NMC), a derivative of a commonly-used pharmaceutical, though not a complete carcinogen, has been found to be a tumor initiator on mouse skin, giving rise to papillomas and carcinomas on about half of mice for which skin treatment with NCM has been followed by the tumor promoter, tetradecanoylphorbol acetate (TPA). (3) An immunohistochemical study with a specific monoclonal antibody to isozymes of cytochrome P450 induced by polycyclic aromatic hydrocarbons (PAH) has revealed that this procedure can be used for semiquantitative determination of metabolic phenotype of liver, and that in extrahepatic tissues, staining is especially intense in, and perhaps limited to, the endothelium of the capillaries, a finding of considerable potential importance in the context of vascular disease, as well as cancer etiology. (4) A related project is extending investigations of protection against carcinogenesis by enzyme induction and employs the environmental carcinogen, benzo[a]pyrene, with the direct-acting carcinogen, N-nitrosoethylurea, as control. (5) Measurements are in progress of the metabolism of NDMA by several murine tissues as a function of inducer and age and include kinetic analysis and use of monoclonal antibodies as biochemical probes to distinguish different isozymes of cytochrome P-450.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05399-04 LCC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Oncogene Expression in Chemically Induced Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. M. Rice	Chief	LCC	NCI
Others:	A. O. Perantoni	Microbiologist	LCC	NCI
	M. Watatani	Visiting Fellow	LCC	NCI
	C. D. Reed	Senior Health Services Officer	LCC	NCI
	J. M. Ward	Chief, Tumor Pathology & Pathogenesis Section	LCC	NCI

COOPERATING UNITS *(if any)*

Microbiological Associates, Inc., Bethesda, MD (M. L. Wenk)

LAB/BRANCH

Laboratory of Comparative Carcinogenesis

SECTION

Perinatal Carcinogenesis Section, Developmental Biology Working Group

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The expression of activated cellular oncogenes in chemically induced rat tumors and the relationship of oncogene expression to progression from the normal to the neoplastic phenotype are studied using 3T3 transfection and hybridization techniques and monoclonal antibodies directed against the specific oncogene products. Five types of tumors have been generated by single injection of F344 rats using various alkylating agents: renal mesenchymal tumors induced by methyl(methoxymethyl)nitrosamine (DMN-OMe), intestinal adenocarcinomas induced by methyl-(acetoxymethyl)nitrosamine (DMN-OAc), hepatocellular carcinomas induced by intraportal injection of DMN-OAc followed by phenobarbital promotion, and gliomas and schwannomas induced by transplacental exposure to nitrosoethylurea (ENU). DNA purified from these tumors is utilized for 3T3 transfection assays and in Southern blot hybridizations with available oncogene probes. Selective activation of neu, proved to result from a single base T → A transversion mutation at one specific site, was observed in 3T3 transformants and in DNA from primary tumors and was shown in 11 of 12 schwannomas tested, but in no other kinds of tumors. K-ras was selectively activated in renal mesenchymal tumors, but no specific and consistent association with a specific activated oncogene was seen in central nervous system gliomas, intestinal adenomas and carcinomas, or hepatocellular tumors. Using monoclonal and polyclonal antibodies, H-ras p21 was found in normal and neoplastic tissues dependent on the fixative and antisera used. Patterns of specific and nonspecific staining were characterized and applications of these antisera were developed. With one monoclonal antibody, granules (probably mitochondria) were immunostained in many normal tissues including renal tubules, muscle and brain.

PHS 6040 (Rev 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05465-03 LCC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Regulatory Role of Retinoids and Growth Factors in Tissue Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	U. I. Heine	Chief, Ultrastructural Studies Section	LCC	NCI
Others:	A. B. Roberts	Research Chemist	LC	NCI
	M. B. Sporn	Chief	LC	NCI

COOPERATING UNITS *(if any)*

Program Resources, Inc., FCRF, Frederick, MD (E. F. Munoz)

LAB/BRANCH

Laboratory of Comparative Carcinogenesis

SECTION

Ultrastructural Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The involvement of tumor growth factor-beta (TGF-β) in embryonal development of the mouse was investigated in embryos of 10 to 18 days of gestation, using antibodies raised against synthetic peptides of the TGF-β monomer to localize the growth factor. TGF-β was found in a variety of tissues of ectodermal and mesenchymal origin, predominantly around day 15 when organogenesis is most intense. The wide distribution of TGF-β indicates its involvement as a regulator in major events of cytodifferentiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05487-02 LCC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 chererters or less. Title must fit on one line between the borders.)*

Carcinogenesis and Mutagenesis by Fecapentaenes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. M. Ward	Chief, Tumor Pathology and Pathogenesis Sect.	LCC	NCI
Others:	L. K. Keefer	Chief, Chemistry Section	LCC	NCI
	P. J. Donovan	Chemist	LCC	NCI
	J. M. Rice	Chief	LCC	NCI

COOPERATING UNITS *(if any)*

Stanford Research Institute, Palo Alto, CA (W. Bradford); Program Resources, Inc., Frederick, MD (A. W. Andrews, L. Channesian)

LAB/BRANCH

Laboratory of Comparative Carcinogenesis

SECTION

Tumor Pathology and Pathogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

Fecapentaenes from human feces have been found to be direct-acting mutagens and are therefore prime candidates as human carcinogens, especially for the large bowel. A variety of in vitro studies by other investigators have demonstrated potent mutagenic effects of fecapentaene-12 (FP-12) in bacterial cells, although cell transforming activity in vitro is low and mutagenic activity for mammalian cells is weak. Thus, animal experiments have become necessary to characterize the in vivo toxic and carcinogenic effects. We first studied the purity and stability of FP-12 to determine the most effective handling procedures during animal exposure. The chemical was moderately stable under argon but quickly decomposed after exposure to air. Vitamin E has shown promise for stabilizing fecapentaene solutions for use in carcinogenesis studies. Several types of animal experiments were performed. Skin painting studies in SENCAR mice revealed neither initiating activity nor complete carcinogenesis to the skin by repeated exposure. Intrarectal and subcutaneous administration to mice and rats have not provided convincing evidence of the carcinogenesis of FP-12, although most studies are still in progress. In a preliminary but small intrarectal mouse study, 1/15 mice had a small colonic carcinoma and 3 had foci of atypical colonic hyperplasia. Primary tumors of the colonic mucosa, confirmed histologically as polypoid adenomas, occurred in 2 of 25 rats given repeated intrarectal instillations of FP-12 in ethanol and killed 72 weeks after the first instillation. Transplacental mutagenesis by FP-12 in hamsters was not convincingly demonstrable, but mutagenesis in vivo in rats by the granuloma pouch assay was unequivocal. Carcinogenesis studies by means of the granuloma pouch assay are in progress. One N-nitrosomethylnitroguanidine (MNNG)-induced tumor has occurred. From previous reports, the bulk of MNNG tumors should start being detected in the next few months and FP-12 tumors, if they occur, should develop during the next 6 months.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOICP05488-02 LCC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms of Inorganic Carcinogenesis: Cadmium

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. P. Waalkes	Senior Staff Fellow	LCC	NCI
Others:	M. Bhave	Visiting Fellow	LCC	NCI
	M. Miller	Senior Staff Fellow	LCC	NCI
	A. O. Perantoni	Microbiologist	LCC	NCI
	K. S. Kasprzak	Visiting Scientist	LCC	NCI
	T. Koizumi	Visiting Fellow	LCC	NCI
	S. Rehm	Visiting Scientist	LCC	NCI

COOPERATING UNITS *(if any)*
Program Resources, Inc., Frederick, MD (C. Riggs, H. Issaq); Microbiological Associates, Inc., Bethesda, MD (M. Wenk); Department of Pathology, University of Western Ontario (M. G. Cherian)

LAB/BRANCH
Laboratory of Comparative Carcinogenesis

SECTION
Office of the Chief, Inorganic Carcinogenesis Working Group

INSTITUTE AND LOCATION
NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanisms of cadmium carcinogenesis are under active investigation. In rats, subcutaneous injection of cadmium induced injection-site tumors in a dose-related fashion and testicular tumors that appeared to depend on the extent of chronic degeneration of the testes. Zinc pretreatment reduced cadmium carcinogenesis in a site-specific, route-specific and dose-dependent manner. A clear association of cadmium treatment with neoplastic and hyperplastic foci of the prostate was also recorded. Genetic mechanisms of susceptibility or resistance to cadmium were further explored, and several agents known to hypomethylate DNA were shown to confer tolerance to cadmium cytotoxicity. This resistance correlated with increased synthetic capacity for metallothionein, an inducible protein that confers tolerance to cadmium by high affinity sequestration and a reduction in the methylation of the metallothionein gene. Investigations into the nature of cadmium-binding proteins in target tissues of cadmium carcinogenesis showed an absence of metallothionein in the rat, mouse, and monkey testes and in the rat prostate, while the mouse testes were also shown to contain a highly methylated metallothionein gene when compared to non-target tissue such as liver. These results indicate that the capacity for production of this protein is a key determinant of tissue specificity in cadmium carcinogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05524-01 LCC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Chemical Carcinogens on Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. S. Miller	Senior Staff Fellow	LCC	NCI
Others:	J. M. Rice	Chief, Perinatal Carcinogenesis Section	LCC	NCI
	L. M. Anderson	Expert	LCC	NCI
	M. P. Waalkes	Senior Staff Fellow	LCC	NCI
	J. S. Rhim	Research Microbiologist	LCMB	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Carcinogenesis

SECTION

Perinatal Carcinogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Steroid interactions with target cells have proven to be quite amenable to mechanistic studies at the molecular level and are probably the best understood eukaryotic gene regulatory system. Thus, cellular responses to steroids constitute an ideal system in which to study the mechanism(s) by which carcinogens can alter the levels of expression of various genes. Previous studies have demonstrated that chemical carcinogens can inhibit steroid-inducible gene expression by at least two distinct mechanisms. Treatment of a rat hepatoma cell line with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) caused concurrent decreases of the levels of both tyrosine aminotransferase (TAT) enzyme activity and TAT-specific total RNA. MNNG thus inhibited the accumulation of total TAT RNA by acting at a pretranslational step, either by preventing the increase in transcription rate mediated by glucocorticoids or by decreasing RNA stability, or both. We have also demonstrated that administration of MNNG or benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE) to rat mT-1 cells resulted in an inhibition of the level of steroid-induced polyoma virus middle-T antigen, while the level of total middle-T RNA remained unchanged. This suggests that carcinogens may inhibit steroid-inducible gene expression through a post-transcriptional mechanism as well. Current studies are focusing on the exact mechanism(s) by which carcinogens mediate their effects. Also being studied is the possible role gene amplification may play in the response of tissues to chemical injury. Cells that have been neoplastically transformed by MNNG treatment have been developed and will be examined for amplification of cellular DNA sequences that may play a role in inducing the transformed phenotype.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04986-10 LEC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Basis of Steroid Hormone Action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael G. Cordingley	Visiting Associate	LEC NCI
Others:	Gordon L. Hager	Head, Hormone Action & Oncogenesis Section	LEC NCI
	Anna Riegel	Visiting Fellow	LEC NCI
	Ronald G. Wolford	Microbiologist	LEC NCI
	Diana S. Berard	Microbiologist	LEC NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Experimental Carcinogenesis

SECTION

Hormone Action and Oncogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mouse mammary tumor virus (MMTV) has emerged as the leading model system for the study of gene regulation by steroids at the transcriptional level. Hormone activation of transcription from the MMTV long terminal repeat (LTR) is contingent upon binding of the activated glucocorticoid receptor at the glucocorticoid response element (GRE) located upstream from the promoter. We sought to elucidate the molecular events at the promoter which occur on hormone activation. We have utilized a series of cell lines in which MMTV LTR fusion genes are amplified on extrachromosomally replicating bovine papilloma virus (BPV) "minichromosomes." Using an exonuclease protection assay on chromatin in isolated nuclei, we detected high resolution binding of factors to the steroid-activated MMTV promoter. No factors are bound with high affinity to the inactive promoter. In addition we determined that the factors responsible for the exonuclease-resistant complex established at the hormone-activated promoter are apparently equal in abundance and DNA-binding affinity in crude extracts from non-stimulated cell nuclei. Activation of transcription at the MMTV promoter therefore appears to result from recruitment of preformed transcription factors to the promoter by the steroid receptor. In experiments in which the accessibility of promoter chromatin was probed with restriction endonucleases, we demonstrated a hormone-dependent increase in accessibility of the sequences closely associated with transcription factor binding sites. These results suggest that transcription factor binding sites are sequestered by nucleoprotein structure in the inactive promoter and that activation occurs, in part, by receptor-mediated alterations in local nucleoprotein structure. Finally, mutations which result in increased activity of the non-stimulated promoter in the presence of an upstream enhancer element were found to cause increased restriction enzyme sensitivity of the promoter and increased transcription factor binding in the absence of hormone.

PHS 6040 (Rev 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05262-06 LEC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular Evolution of Chemically Induced Rat Hepatomas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ritva P. Evarts	Veterinary Medical Officer	LEC NCI
Others:	Snorri S. Thorgeirsson	Chief	LEC NCI
	Peter Nagy	Visiting Fellow	LEC NCI
	Elizabeth R. Marsden	Biologist	LEC NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Experimental Carcinogenesis

SECTION

Chemical Carcinogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are: (1) to study the possible role of oval cells as stem cells for hepatocytes and (2) to examine if these cells are the targets for carcinogens during chemical hepatocarcinogenesis. Administration of a small amount of 2-acetylaminofluorene (AAF) for two weeks, combined with partial hepatectomy, prevented the proliferation of hepatocytes, especially in the caudate lobe, whereas oval cells were resistant to the cytotoxic and cytostatic effect of AAF. At day 7, after partial hepatectomy, these cells occupied one-half of the area of the liver acinus. At day 9 small basophilic cells with vesicular round nuclei appeared on the area of oval cells. Only oval cells and islands of basophilic cells had mRNA for albumin and alpha-fetoprotein. Oval cells were gamma-glutamyltranspeptidase (GGT) positive and glucose-6-phosphatase negative and did not have surface receptor of asialoglycoprotein. The preneoplastic lesions produced by the Solt-Farber protocol included basophilic periportal "nodules" that were similar to those obtained without initiation; positive for albumin, alpha-fetoprotein and glutathione-S-transferase P and negative for glucose-6-phosphatase and asialoglycoprotein receptor. However, some of the "nodules" in initiated livers were positive for GGT. These findings suggest that basophilic GGT positive "nodules" (enzyme altered foci) and GGT negative "nodules" (regenerating hepatic nodules) are derived from oval cells. C-myc oncogene was expressed in oval cells and in basophilic cells. TGF-beta induced differentiation of rat liver epithelial cells in vitro towards an adult hepatocyte phenotype, indicating a possible role of TGF-beta in the maturation of hepatocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05263-06 LEC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Computer Analysis of Carcinogenesis by Two-Dimensional Gel Electrophoresis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mark J. Miller	Senior Staff Fellow	LEC NCI
Others:	Arthur David Olson	Computer Programmer	LEC NCI
	Snorri S. Thorgeirsson	Chief	LEC NCI
	Peter J. Wirth	Expert	LEC NCI
	Lori Hampton	Biologist	LEC NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Experimental Carcinogenesis

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.2	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to study the mechanism of carcinogenesis using quantitative two-dimensional gel electrophoresis. This technique allows the separation of total cellular polypeptides on a single gel and lets us examine both qualitative and quantitative changes in the pattern of protein synthesis as the cell undergoes malignant transformation. Research is focused on: (1) continued development of the computer system (dubbed ELSIE 4) used to automatically analyze gels and (2) use of ELSIE 4 to analyze experiments requiring computerized analysis of two-dimensional gels. In the past year we have continued developing software tools to aid the investigator in identifying interesting spots. Statistical tests have been included in programs to help search for spots that may vary over the course of an experiment. Once such spots are flagged, a computer-coupled image processor is used to examine the spots. Among other things, we are using ELSIE 4 to study modulation in the rates of protein synthesis in the rat hepatoma cell line, H4-II-E. Single-cell-derived cultures of H4-II-E were isolated. Cells were labeled and two-dimensional gels run. About 10% of the proteins were synthesized at variable rates. These differences were small, generally about 50%, although changes of as much as 400% were detected. Time course experiments, where cultures were labeled under identical conditions once a week for 12 weeks, showed similar modulation. There appear to be two major causes for this variability: (1) environmental factors, such as the age of the media, and (2) random drift caused by minor differences in the handling of cultures that affect the synthesis of a series of polypeptides in a cascading manner. We conclude that the rates of synthesis of many polypeptides can vary slightly, but significantly, in culture and that ELSIE 4 is capable of detecting these changes. These changes reflect the cell's ability to adjust to minor changes in its environment and are thus part of the normal biology of cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05283-05 LEC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Conditional Expression of Mammalian Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Gordon L. Hager	Head, Hormone Action & Oncogenesis Section	LEC NCI
Others:	Diana S. Berard	Microbiologist	LEC NCI
	Michael G. Cordingley	Visiting Associate	LEC NCI

COOPERATING UNITS *(if any)*

Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Manitoba, Canada (Arnold H. Greenberg)

LAB/BRANCH

Laboratory of Experimental Carcinogenesis

SECTION

Hormone Action and Oncogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	0.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The controlled expression of genetic information in cells in culture and in transgenic animals is an essential tool in the study of gene function in vitro, and eventually will prove central to the treatment of disease by introduced genetic material. We showed previously that conditional expression of the v-ras-H oncogene from the glucocorticoid-responsive MMTV promoter could result in a regulated cell phenotype; cells were transformed in the presence of hormone, and reverted when hormone was withdrawn. We have now shown that regulated "phenotype-switching" can be employed to study the oncogenic process in whole animals. The metastatic potential of NIH-3T3 fibroblasts carrying the hormone-inducible v-ras-H oncogene was markedly enhanced when cells were induced prior to inoculation into the animal. These experiments underscore the potential applications of this technology in studying various processes in the intact animal.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05313-05 LEC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Early Events in Chemically Induced Rat Hepatocarcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter J. Wirth	Expert	LEC NCI
Others:	Ritva P. Evarts	Veterinary Medical Officer	LEC NCI
	Lori L. Hampton	Biologist	LEC NCI
	Snorri S. Thorgeirsson	Chief	LEC NCI

COOPERATING UNITS *(if any)*

University of Toronto, Canada (Dr. M. Waheed Roomi)

LAB/BRANCH

Laboratory of Experimental Carcinogenesis

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	0.9	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was initiated to study the sequence of events during chemically induced neoplasia using the rodent hepatoma model in combination with quantitative two-dimensional gel electrophoresis (2D-PAGE). Hyperplastic nodules (HN) were generated in male F-344 rats using the resistant hepatocyte model. Six months after initiation animals bearing HN and untreated control rats were treated with the following compounds known to modulate liver enzymes and proteins: lead nitrate (LN), cobaltheme (CoH), 3-methylcholanthrene (3MC), and phenobarbital (PB). In control animals LN, CoH, 3MC, and PB treatment all resulted in a two- to tenfold increase in the expression of the Yc subunit of glutathione-S-transferase (GST). PB also increased the Yb and Ya subunits fivefold each. LN also increased the expression of a polypeptide tentatively identified as the Yp subunit of the placetal form of GST-P. Neither CoH, 3MC, nor PB had any effect on the expression of this polypeptide. Polypeptide 8, composed of 5 isoelectric point variants (6.00-6.60/66,000) was increased two- to threefold in HN from untreated animals and was similarly increased in normal liver following treatment with each of the four modulators. The order of potency was: LN > CoH > PB = 3MC. Polypeptides 6 (6.60/21,000) and 7 (6.40/16,000) which were expressed at relatively high levels in normal liver (0.5-0.6% of the total integrated density on each gel) were reduced three- to fivefold in HN. Following treatment of normal liver with either LN, 3MC, or CoH, polypeptides 6 and 7 were reduced to 0.1-0.2% and 0.2-0.3%, respectively. Polypeptide 3 (5.90/38,000) which is markedly reduced in HN is similarly decreased in normal liver by LN, PB, and CoH. 2D-PAGE of microsomal polypeptides failed to reveal any common polypeptide changes between HN and modulator-treated normal liver, although numerous qualitative and quantitative differences specific to each modulator were observed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05317-04 LEC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 chereclers or less Title must fit on one line between the borders.)*

Opal Suppressor tRNA in Human and other Genomes

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigetor.) (Name, title, laboretory, and institute affiliation)*

PI:	Dolph L. Hatfield	Research Biologist	LEC NCI
Others:	Byeong Jae Lee	Visiting Fellow	LEC NCI
	Malini Rajagopalan	Visiting Fellow	LEC NCI
	O. Wesley McBride	Section Head	LB NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Experimental Carcinogenesis

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The only naturally occurring nonsense suppressor tRNAs described in higher eukaryotes are two opal suppressor serine tRNAs that occur in vertebrate tissues. These tRNAs have several unique features: (1) they are 90 nucleotides in length and thus are the longest tRNAs sequenced to date; (2) they are phosphorylated on their serine moiety to form phosphoseryl-tRNA; (3) they have few modified bases compared to other tRNAs; (4) they are encoded by a single gene even though several pyrimidine transitions occur post-transcriptionally and one of the transitions occurs in the anticodon; and (5) the primary transcript arises, unlike any other known tRNA, without processing on the 5' side of the gene product. Among animals, the gene occurs in members of the Phyla Chlordata (tunicate, amphioxus, lamprey, hag fish, horned shark, winter flounder, Xenopus, chicken and bovine), Arthropoda, Mollusca, Aschelminthes and Porifera. The gene was also detected in the genomes of representatives from the Monera, Plant and Protist Kingdoms. The genes encoding the opal suppressor tRNAs which have been isolated and sequenced from human, rabbit, chicken and Xenopus genomes are transcribed in vivo in Xenopus oocytes and are transcribed in vitro in HeLa cell extracts. Fingerprints of the processed transcript from the Xenopus gene show that the gene is faithfully transcribed and that initiation of transcription occurs at the first nucleotide within the gene. The 3' trailer sequence is removed by purified 3' processing enzyme. The triphosphate on the 5' nucleotide is preserved in transport of the gene product from the nucleus to the cytoplasm and remains intact in the cytoplasm, suggesting that it may have a function on the mature tRNA. The gene was mapped to human chromosome 19 and the corresponding pseudogene to human chromosome 21.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP5373-04 LEC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Purification and Characterization of a Rat Liver-Derived Growth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Anthony C. Huggett	Visiting Associate	LEC	NCI
Others:	Henry C. Krutzsch	Expert	LEC	NCI
	Mrunal S. Chapekar	Senior Staff Fellow	LEC	NCI
	Peter J. Wirth	Expert	LEC	NCI
	James B. McMahon	Expert	DDRG	NCI
	Anita B. Roberts	Senior Investigator	LCP	NCI
	Snorri S. Thorgeirsson	Chief	LEC	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Experimental Carcinogenesis

SECTION

Biopolymer Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project is to isolate and characterize a protein from adult rat liver that produces a reversible inhibition of the proliferation of liver-derived epithelial cells. An improved analytical-scale purification procedure has been developed that produces a preparation with a specific activity about 1000-fold greater than previously reported. The inhibitory activity was labile at low pH, at temperatures over 50 degrees C, in the presence of sulphydryl reducing agents, and it could be completely abolished by trypsin under mild denaturing conditions. Its isoelectric point was determined to be 5.5 by chromatofocusing. The growth inhibitory activity, which could be eluted from SDS-PAGE at 17-19 kD, was compared to that of TGF-beta. The ID-50 of the liver-derived inhibitor was similar to that of TGF-beta in rat liver epithelial cells and also in primary hepatocyte cultures. In contrast to TGF-beta the activity of the liver-derived growth inhibitor was unaltered in the presence of a neutralizing antibody raised against TGF-beta. In addition the liver-derived inhibitor did not stimulate the growth of NRK cells in soft agar. Current efforts are focused on the large-scale purification of the liver-derived inhibitor such that antibody production and amino acid sequence analysis can be performed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05374-04 LEC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural and Physicochemical Studies of Proteins Relevant to Tumorigenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter P. Roller	Head, Biopolymer Chemistry Section	LEC NCI
Others:	Snorri S. Thorgeirsson	Chief	LEC NCI
	Chien-Hua Niu	Expert	LEC NCI
	Anthony C. Huggett	Visiting Associate	LEC NCI
	Preston H. Grantham	Chemist	LEC NCI

COOPERATING UNITS *(if any)*

Kossuth University, Debrecen, Hungary (Dr. Z. Dinya)

LAB/BRANCH

Laboratory of Experimental Carcinogenesis

SECTION

Biopolymer Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.0	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project involves studies on the chemical structure and physicochemical characteristics of certain natural biopolymeric materials and their synthetic analogs with the aim of relating the resulting structural information to their biological mode of action, such as cell growth regulation, cell transformation or differentiation. The modern methods of mass spectrometry, nuclear magnetic resonance spectroscopy, chromatographies, various chemical methods and sequencing are being applied. Projects include: (1) Development of fast atom bombardment mass spectrometric methods for molecular weight and sequence analysis of peptides. Methods are developed for the analysis of disulfide linked dimeric peptides and of cysteine-containing cyclic disulfides. These peptides were best analyzed by reductive alkylation with 4-vinylpyridine, whereby the peptides are linearized and the basic pyridino group imparts better charge-carrying characteristics to the molecule. A number of synthetic peptides were analyzed for ascertaining the correctness of the synthesis and the oxidation state. These included several epidermal growth factor and transforming growth factor-alpha cyclic peptides and several synthetic analogs of a peptide segment of fibronectin that binds to cell surface receptors. (2) Most carcinogens exert their toxic effects by covalently interacting with critical cellular macromolecules. We have succeeded in synthesizing, for the first time one of the previously postulated metabolically activated forms of aromatic amines, specifically the O-acetyl-N-hydroxy-2,4-dinitrophenylamine derivative of 2,4-dinitrophenylamine. This species was stable enough to be isolated and it was characterized by a full battery of spectral techniques.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05379-04 LEC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 chererters or less. Title must fit on one line between the borders.)*

Analysis of Polypeptide Changes During Cellular Differentiation and Transformation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter J. Wirth	Expert	LEC NCI
Others:	Lori L. Hampton	Biologist	LEC NCI
	Snorri S. Thorgeirsson	Chief	LEC NCI

COOPERATING UNITS *(if any)*

Tom Maciag, Microbiologist, American Red Cross; Gene Liau, Biologist, American Red Cross; John P. Kupferschmid, Clinical Associate, IR SU, NHLBI

LAB/BRANCH

Laboratory of Experimental Carcinogenesis

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.7	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

This project was initiated to analyze, both qualitatively and quantitatively, changes in total cellular protein patterns during cellular senescence using the technique of quantitative two-dimensional polyacrylamide gel electrophoresis. A human endothelial cell culture system has been developed which allows one to study changes in cellular polypeptide expression in presenescent and senescent human umbilical vein endothelial cells (HUVEC) and to follow changes in cellular phenotype and polypeptide expression following treatment with a variety of biological growth factors. Two-dimensional polyacrylamide gel electrophoresis of [35-S]-methionine-labelled polypeptides of young presenescent (16 population doublings) and old senescent (55 population doublings) human endothelial cells treated with 12-O-tetradecanoylphorbol-13-acetate (TPA), recombinant gamma-interferon (gamma-IFN), and tumor necrosis factor (TNF) revealed the expression of polypeptides unique to each biological response modifier and also unique to senescence. Presenescent HUVEC express 13 polypeptides that are not expressed in senescent cells and senescent human endothelial cells express 9 polypeptides that young cells do not express. Furthermore, TPA and gamma-IFN revert the expression of a number of polypeptides which are unique to presenescent and old senescent cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05447-03 LEC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Isolation and Characterization of Proteins from Two-Dimensional Polyacrylamide Gels

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Anthony C. Huggett	Visiting Associate	LEC NCI
Others:	Peter J. Wirth	Expert	LEC NCI
	Preston Grantham	Chemist	LEC NCI
	Snorri S. Thorgeirsson	Chief	LEC NCI
	Peter P. Roller	Head, Biopolymer Chemistry Section	LEC NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Experimental Carcinogenesis

SECTION

Biopolymer Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	0.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to develop the analytical technology required for the elution and subsequent microsequencing of proteins from two-dimensional polyacrylamide gels. A number of "interesting" protein spots have been defined whose regulation is markedly altered during the multistep process of neoplastic transformation. Initially, microscale procedures aimed at the recovery and sequence analysis of these proteins from one-dimensional SDS-PAGE have been investigated. Electroelution and passive extraction techniques were found to be suitable only when large amounts (>500 pmoles) of protein were available. With lower protein amounts, the contamination produced by gel components and N-terminal blocking of the proteins which occurred during their isolation from the gels, prevented direct amino-terminal sequence analysis. Standard electroblotting techniques were only successful when more than 200 pmoles of protein was applied to the gels. A number of modifications to this procedure have been made such that 50 pmoles of soybean trypsin inhibitor applied to an SDS-polyacrylamide gel could subsequently be correctly sequenced to 17 cycles. This procedure was successfully applied to the sequence analysis of standard proteins. Work is currently underway to apply this technique to the analysis of unknown "interesting" proteins and also to extend the technique to encompass the isolation of proteins from two-dimensional polyacrylamide gels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05448-03 LEC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Guanosine Triphosphate Binding Site of ras Proteins by NMR and CD Spectroscopy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Chien-Hua Niu	Expert	LEC NCI
Others:	Kyouhoon Han	Visiting Fellow	LEC NCI
	Peter P. Roller	Head, Biopolymer Chemistry Section	LEC NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Experimental Carcinogenesis

SECTION

Biopolymer Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A number of studies revealed that a point mutation at either position 12, 13, 59, or 61 of ras p21 proteins is associated with a fundamental change in their biochemical properties including their ability to transform cells. The main objective of this project is to study the conformational differences between non-transforming and transforming ras p21 proteins as well as their conformational changes upon binding to GTP. A few important observations concerning the conformational changes upon addition of GTP to synthetic N-terminal segments of ras p21 proteins appeared in the last report. Additional significant results are as follows: (1) Upon addition of either the glycine-containing (Gly-peptide) and valine-containing (Val-peptide) 34 amino acid residue peptides of the N-terminal segments of ras p21 proteins to the solution containing GTP or ATP, the line width of all three phosphorus-31 NMR resonance, alpha-, beta-, and gamma-phosphate, were broadened. Simultaneously, all three phosphate resonances shifted downfield upon binding with peptides. However, the degree of their shifts was somewhat different. Beta- and gamma-phosphate resonances shifted downfield noticeably, but the alpha-phosphate resonance was not shifted to any significant degree upon addition of either the Gly-peptide or Val-peptide. (2) It is known that magnesium ion plays an important role in binding guanine nucleotide to ras p21 proteins. Upon addition of magnesium ion to the mixture of the Gly-peptide with GTP, all of three phosphate resonances shifted further downfield without broadening their line widths. (3) The Gly-peptide, in contrast to the Val-peptide, catalyzes the hydrolysis of GTP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05449-03 LEC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 chereters or less. Title must fit on one line between the borders.)*
Conformational Studies of Growth Factors and Transforming Related Peptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor) (Neme, title, leboretory, and institute affiliation)*

PI:	Chien-Hua Niu	Expert
Others:	Kyouhoon Han	Visiting Fellow
	Peter P. Roller	Head, Biopolymer Chemistry Section
	Snorri S. Thorgeirsson	Chief

COOPERATING UNITS *(if eny)*

None

LAB/BRANCH
Laboratory of Experimental Carcinogenesis

SECTION
Biopolymer Chemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are (1) to study the mechanism of mitogenic activities of growth factors based on their molecular conformation and (2) to develop and design specific and competitive peptide inhibitor of cell adhesion and migration during invasion. Results obtained so far include: (1) Four cyclic peptides, analogues of human epidermal growth factor (EGB) and transforming growth factor (TGF)-alpha, have been synthesized by high dilution method and purified by high performance liquid chromatography (HPLC). (2) Using a radioreceptor assay, all four of synthetic cyclic peptides competed binding of (125)I-EGF to the EGF receptor at a concentration of 100 uM. Cyclic [Ala(20)]EGF(14-31) and cyclic EGF(20-31) were able to block 30% and 20% of the binding of (125)I-EGF to the receptor, respectively. In the case of TGF-alpha, clycic [Ala(21)]TGF(16-32) and cyclic TGF(21-32) would displace 20% and 11% of the (125)I-EGF to the receptor, respectively. (3) Using various two-dimensional nuclear magnetic resonance (NMR) techniques, the proton resonances of the individual amino acids for both TGF-17mer and TGF-12mer were assigned, and the internuclear proton-proton distances through space were obtained. The latter information was used to generate energy-minimized peptide structures using a computer program. (4) Four cell recognition peptide analogues have been synthesized and purified. (5) Circular dichroism (CD) studies of GRGDS and GRGES in methanol reveal that GRGDS has a more highly ordered secondary structure. (6) The pK(a) value of Asp and Glu in the GRGXS series, determined by pH titration using CD spectroscopy, were 2.50 and 3.10, respectively, which were both lower than that of the individual amino acids (3.86 for Asp, 4.01 for Glu).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05450-03 LEC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chromatin Structure and Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Gordon L. Hager	Head, Hormone Action & Oncogenesis Section	LEC NCI
Others:	Trevor Archer	Visiting Fellow	LEC NCI
	Diana S. Berard	Microbiologist	LEC NCI

COOPERATING UNITS *(if any)*

Universite de Paris XI, Dept de Chimie Biologique Lab Hormones,
94270 Bicetre, France (Dr. Helene Richard-Foy)

LAB/BRANCH

Laboratory of Experimental Carcinogenesis

SECTION

Hormone Action and Oncogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.4	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The genetic information in mammalian cells exits is organized into a highly condensed nucleoprotein structure whose basic repeating subunit is the nucleosome. The major function of this structure is usually thought to be packaging the very large amounts of DNA into a minimal volume. Recent evidence indicates that nucleosomes can be specifically positioned, or phased, in some regions of the eukaryotic genome. This finding introduces the possibility that the interaction of transacting gene regulatory factors with their DNA-binding sites may be affected by the organization of these sites in chromatin. We have shown that nucleosomes are phased across the steroid-regulated MMTV promoter. The sites to which steroid-receptors bind are displayed on the surface of nucleosome B in this phased array. Hormone activation of the promoter is accompanied by loss, or modification, of this nucleosome. These findings indicate that steroid receptors interact with a highly structured nucleoprotein complex at the MMTV LTR and suggest that chromatin organization may be involved in the transcriptional response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05452-03 LEC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Expression and Development in Transgenic Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Su-yun Chung	Senior Staff Fellow	LEC	NCI
Others:	Snorri S. Thorgeirsson	Chief	LEC	NCI
	Miriam Falzon	Visiting Fellow	LEC	NCI
	Shu-hua Yu	Visiting Fellow	LEC	NCI
	Nancy Sanderson	Chemist	LEC	NCI
	Dennis R. Roop	Microbiologist	LCCTP	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Experimental Carcinogenesis

SECTION

Chemical Carcinogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	2.1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The overall objective of this project is to employ the transgenic mouse system by introducing natural or manipulated gene sequences into the germ line of an animal and to alter its phenotype and genetic background. This system provides a new way of investigating tissue-specific and developmental stage-specific regulation of gene expression. In the past year, we have succeeded in generating transgenic mice following microinjection of recombinant DNA in 1-cell stage embryos. We used two recombinant DNA constructs: (1) an SV40 large T antigen under the control of metallothioneine promoter and (2) a genomic human keratin gene. We are now in the process of establishing transgenic lines by genetic breeding. The genomic localization and expression of the introduced sequences are being characterized. We have also isolated and characterized six homeobox-containing genes from a rat genomic library. DNA sequence analysis and RNA expression studies indicate that these clones contain sequences exhibiting greater than 90% homology to the consensus homeobox sequence and are developmentally regulated in a tissue-specific manner.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05453-03 LEC

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Genetic Determinants in Chemical Hepatocarcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Snorri S. Thorgeirsson	Chief	LEC NCI
Others:	Peter Nagy	Visiting Fellow	LEC NCI
	Ritva P. Evarts	Veterinary Medical Officer	LEC NCI
	Susan H. Garfield	Chemist	LEC NCI
	Michael G. Cordingley	Visiting Associate	LEC NCI
	Michael M. Gottesman	Section Head	LMB NCI

COOPERATING UNITS *(if any)*: Laboratory of Immunopathology, NIAID (Dr. H. C. Morse, III)

LAB/BRANCH: Laboratory of Experimental Carcinogenesis

SECTION: Chemical Carcinogenesis Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	0.7	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main objective of this project is to define the genetic determinants for the initiation stage in hepatocarcinogenesis and subsequent evolution of liver tumors that are brought about by chemical carcinogens and other cancer-causing agents. The principal lesions that develop in the rat liver as a result of initiation-promotion protocols are foci of altered hepatocytes. Initiation of these foci by a variety of hepatocarcinogens has been shown to follow an apparent first order dose response, suggesting that the foci are a clonal expansion of the initiated cell. Consequently, the phenotype of initiation should be completely represented by the foci of altered hepatocytes. We have consistently observed significant upregulation of the expression of myc and raf oncogenes during early and late stages of hepatocarcinogenesis. Reconstruction experiments with retroviral vectors containing these and other oncogenes associated with the tumor development in the liver gave the following results: v-raf and H-v-ras were capable of transforming rat liver epithelial (RLE) cells, whereas neither v-myc nor c-myc could transform these cells. The combination of v-raf and v-myc was the most efficient tranforming agent. The transformed RLE cells gave rise to different tumors depending upon the combination of oncogenes used for transformation. Also a strong association was found between transformation of RLE cells and the expression of a multidrug-resistance (mdr) gene. Levels of messenger RNA for the mdr gene, which encodes P-glycoprotein, were elevated in both preneoplastic and neoplastic lesions. Expression of the mdr gene also reached high levels in regenerating rat liver 24 to 72 hours after partial hepatectomy. These results show that the expression of the mdr gene can be regulated in liver and is likely to be responsible for part of the mdr phenotype of carcinogen-initiated hepatocytes and regenerating liver cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05495-02 LEC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Amino Acid at the Suppression Site in Rabbit Beta-Globin Readthrough Protein

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dolph L. Hatfield	Research Biologist	LEC NCI
Others:	Snorri S. Thorgeirsson	Chief	LEC NCI
	Michael Bustin	Research Chemist	LMC NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Experimental Carcinogenesis

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rabbit beta-globin readthrough protein is the only naturally occurring read-through protein in higher eukaryotes which does not involve a viral system. Since suppressor tRNAs have been used in gene therapy experiments and have been implicated in inhibiting viral expression, the readthrough protein has been isolated from rabbit reticulocytes in order to identify the amino acid at the suppression site and, therefore, to characterize the nonsense suppressor tRNA involved in the expression of this unique protein. Specific antibodies against this protein were prepared by synthesizing a 22 amino acid peptide which corresponds to the readthrough portion of the beta-globin readthrough protein, coupling the peptide to KLH protein and injecting the conjugated protein into a sheep. Specific antibodies were produced which were purified and used in combination with HPLC chromatography to isolate the readthrough protein for characterizing the amino acid at the suppression site.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05500-01 LEC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Polypeptide Modulation in MCF-7 Cells by Estrogen and Growth Factors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Snorri S. Thorgeirsson	Chief	LEC NCI
Others:	Peter J. Worland	Visiting Fellow	LEC NCI
	Peter J. Wirth	Expert	LEC NCI
	Lori L. Hampton	Biologist	LEC NCI
	Diane A. Bronzert	Biologist	MB NCI
	Robert B. Dickson	Senior Investigator	MB NCI
	Marc E. Lippman	Section Chief	MB NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Experimental Carcinogenesis

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.5	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project was to utilize the established human mammary tumor cell lines (MCF-7, MCF-7(gpt) [produced by transfection with the Eco-gpt selectable gene marker], MCF-7(ras) [produced by transfection with Eco-gpt and the v-Hras oncogene] and LY2) to investigate the effect of antiestrogens, estrogen and other growth factors on the polypeptide expression of these cells. The growth factors IGF-1 (insulin-like growth factor-1) and TGF-alpha (transforming growth factor alpha) are able to elicit many of the growth stimulating responses of estrogen when applied to the MCF-7 human mammary tumor cell line. An initial baseline study between the MCF-7, MCF-7(gpt) and MCF-7(ras) has found that there are several polypeptides expressed only in MCF-7 (14), MCF-7(gpt) (5) and MCF-7(ras) (3). A number of quantitative differences between the cell lines were apparent, with the major differences occurring between the MCF-7 - MCF-7(gpt) and MCF-7 - MCF-7(ras). Studies to assess the effect of estrogen on the cellular polypeptide expression and the effect of estrogen, IGF-1 and TGF-alpha on cellular and secreted proteins of the MCF-7, MCF-7(gpt) and MCF-7(ras) are in the final analysis stage. Additional to this are experiments using the antiestrogen LY117018 to assess the effect on polypeptide expression and secretion from the antiestrogen-resistant cell line LY2 and the MCF-7 cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05501-01 LEC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular Polypeptides Associated with Metastasis of Rat Mammary Tumor Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Snorri S. Thorgeirsson	Chief	LEC NCI
Others:	Peter J. Worland	Visiting Fellow	LEC NCI
	Peter J. Wirth	Expert	LEC NCI
	Lori L. Hampton	Biologist	LEC NCI

COOPERATING UNITS *(if any)*

Department of Pathology, Roswell Memorial Park Institute, Buffalo, New York (Dr. Untae Kim)

LAB/BRANCH

Laboratory of Experimental Carcinogenesis

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.7	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was initiated to identify those polypeptides that specifically relate to the metastatic process as the first step toward their purification and identification. Utilization of metastasizing and non-metastasizing cells derived from the same parent population of tumor cells is fundamental to this project, and we have confirmed that the TMT-081-ms cells do metastasize in syngeneic rats and that the TMT-081-nm do not metastasize in syngeneic rats. Radiolabelling of these cells with 14C amino acids has revealed several qualitative and quantitative differences in their polypeptide patterns. The most intensely labelled spots that occurred only in the metastatic cells were (MW/pI) 67/5.5 and 50/4.5 and the most intense spots occurring only in the non-metastatic cells were 46/6.7 and 38/6.1. When 32P was used to radiolabel these cells, the resultant polypeptide patterns were markedly different from those obtained with 14C amino acid labelling. A number of the 32P-labeled polypeptides could not be observed on the 14C autoradiograms. There were again several qualitative and quantitative differences that could be observed from visual inspection of the autoradiograms between the metastatic and non-metastatic cell lines. The most intensely labelled spots occurring only in the metastatic cells were 98/4.7 and 24/4.5. Those polypeptides that were approximately threefold greater in intensity in the metastatic cells compared to the non-metastatic were 14/5.2, 15/5.4 and 12/6.3. The polypeptides of 40/5.9 and 40/5.8 were at least threefold greater in the intensity of 32P label compared to the corresponding polypeptides in the metastatic cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05502-01 LEC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In Vivo Protein-DNA Interactions Probed by UV-crosslinking

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Gordon L. Hager	Head, Hormone Action & Oncogenesis Section	LEC NCI
Others:	Anna Tate Riegel	Visiting Fellow	LEC NCI
	Diana S. Berard	Microbiologist	LEC NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Experimental Carcinogenesis

SECTION

Hormone Action and Oncogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The interaction of specific DNA-binding proteins with their recognition sites in the eukaryotic chromosome is central to the mechanisms of gene regulation that occur in these cells. The techniques used to study these interactions rely primarily on methodology designed to detect preferential binding between proteins at various levels of purity from broken cell preparations and purified DNA sequences. It is often assumed that if a high affinity binding protein is present in a given cell type, that protein will interact with its recognition sequence. We recently observed (see Project Z01CP04986-10 LEC) that two tight-binding transcription factors are excluded from MMTV chromatin in vivo. These findings indicate the importance of techniques that permit an unambiguous determination as to when a protein occupies (or is excluded from) its recognition site in vivo. We have undertaken to apply the technique of UV DNA-protein cross-linking to this problem. Several advantages would accrue from the successful development of this application. Utilizing amplified minichromosomes, based on the bovine papilloma virus (BPV) vector (see Project Z01CP05450-03 LEC), we have detected specific cross-links between minichromosome DNA two types of proteins, RNA polymerase II and histones. Using the MMTV hormone-inducible promoter on BPV minichromosomes, the interactions of both proteins are modified by steroid induction. Attempts are underway to extend this technology to the use of very short (40 nanosecond) irradiations, which would offer a new and powerful approach to the study of transcriptional regulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05503-01 LEC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biological Effects of a Rat Liver-Derived Growth Inhibitor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mrunal S. Chapekar	Senior Staff Fellow	LEC	NCI
Others:	Snorri S. Thorgeirsson	Chief	LEC	NCI
	Peter P. Roller	Head, Biopolymer Chemistry Section	LEC	NCI
	Peter J. Wirth	Expert	LEC	NCI
	Anthony C. Huggett	Visiting Associate	LEC	NCI
	James B. McMahon	Expert	DDRG	NCI
	Robert I. Glazer	Pharmacologist	LBC	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Experimental Carcinogenesis

SECTION

Biopolymer Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The principle goal of this project is to assess the growth modulatory effects of a protein isolated from rat liver that causes reversible inhibition of the proliferation of rat liver epithelial (RLE) cells in culture. A highly potent preparation of this inhibitor protein has been obtained using a new purification procedure involving DEAE-cellulose and gel filtration chromatography followed by high resolution chromatofocusing and hydrophobic interaction FPLC. Normal RLE cells were markedly sensitive to the antiproliferative effects of this inhibitor (ID-50 200 pg/ml), whereas aflatoxin-transformed RLE cells exhibited low sensitivity (ID-50 1.5 ng/ml). Rat hepatoma cells UVM 7777 and human hepatoma cells Hep-G2 were resistant to the cytostatic effects of the inhibitor; however, human breast carcinoma cells (MCF-7) and rat hepatoma cells (Reuber) were affected at relatively higher concentrations (ID-50 1.0 ng/ml). On the contrary, proliferation of normal rat kidney fibroblasts (NRK) and human foreskin fibroblasts was stimulated in response to this inhibitor. Measurement of tyrosine kinase activity in RLE cells treated with this liver-derived growth inhibitor using a novel non-denaturing gel electrophoretic assay, indicated a reduction in cytoplasmic tyrosine kinase which was accompanied by an increase in membrane-associated kinase activity. The identity of these kinases and their role in the growth regulation is currently under investigation. Experiments examining the cell cycle specificity of this inhibitor using microcinematography technique are also underway.

PHS 6040 (Rev 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05504-01 LEC

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Isolation of Hepatocyte Plasma Membrane Proteins from Normal & Neoplastic Rat Liver

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Chien-Hua Niu	Expert	LEC NCI
Others:	Timothy Benjamin	Chemist	LEC NCI
	Peter P. Roller	Head, Biopolymer Chemistry Section	LEC NCI
	Snorri S. Thorgeirsson	Chief	LEC NCI
	Anthony C. Huggett	Visiting Associate	LEC NCI

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Laboratory of Experimental Carcinogenesis

SECTION: Biopolymer Chemistry Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	0.7	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our previous investigations of glycoproteins isolated from the plasma membranes of normal and neoplastic rat livers using two-dimensional gel electrophoresis (2D-PAGE) have revealed many differences, both qualitative and quantitative. The main goal of this project is to isolate, purify, and structurally characterize the specific glycoproteins whose expression is markedly altered during chemically induced hepatocarcinogenesis in order to understand their role either as markers or causal agents in the process of cell transformation. Results obtained so far are as follows: Attempts have been made to isolate a specific glycoprotein (molecular weight, 200 KD; pI 5.8), which is downregulated during cell transformation, from normal rat liver by perfusion, homogenization, ultracentrifugation, and brief sonication. The glycoprotein was further purified by Concanavalin-A (ConA) affinity chromatography and then gel filtration using a Superose-12 column eluted with Tris buffer containing 6 M guanidine. Following desalting by dialysis of the solution against 1.0 M acetic acid, subsequent purification has been achieved by ion-exchange chromatography using a Mono S column eluted with a linear gradient of 1.0 M sodium chloride (NaCl) in ammonium acetate buffer (pH 5.0). Two-dimensional gel electrophoresis has been used to monitor the progress of purification of the glycoprotein at each step.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04491-11 LEP

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Quantitative Studies on Concurrent Factors in Neoplastic Transformation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: U. Saffiotti Chief LEP NCI

COOPERATING UNITS *(if any)*
Mario Negri Pharmacol. Res. Insititute, Milan, Italy (F. Bertolero); Commission of European Commun. Research Center, Ispra, Italy, (E. Sabbioni); Lab. of Applied Toxicology, Istituto Superiore di Sanita', Rome, Italy (M. Bignami, E. Dogliotti); Inst. of Histology and Embryology, Univ. of Padua, Italy (S. Garbisa).

LAB/BRANCH
Laboratory of Experimental Pathology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.2	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on mechanisms of transformation in the mouse embryo BALB/3T3 clone A-31-1-1 cell line were continued. (1) Transformation was obtained with several metals. The mechanisms of toxicity and transformation by both trivalent and pentavalent arsenic (As) were investigated. Trivalent As was found to be 4 times more active than pentavalent As. Cellular uptake was higher for trivalent than pentavalent As, and the latter was reduced in the cells to the trivalent form by a metabolic reduction process partly dependent on glutathione. When correlated with the calculated As cell burden, toxicity and transformation frequency were found to be the same for both forms of As. Cells exposed to pentavalent As released the trivalent form into the medium. No methylated metabolites were found, indicating lack of this detoxification mechanism in this cell line. The results indicate that trivalent As is the form responsible for the toxic and transforming effects, independent of the valence state of the As exposure.

(2) Previous studies on the induction kinetics of ouabain-resistant mutations induced by N-ethyl-N-nitrosourea in BALB/3T3 cells were repeated and fully confirmed using larger numbers of cells.

(3) When inoculated in nude mice, BALB/3T3 cells transformed by As, by other carcinogens, or spontaneously, were found to be tumorigenic but not metastasizing, in keeping with the lack of type IV collagenolytic activity observed in these cell lines. Induction of metastatic activity by further treatment with the ras oncogene or with carcinogens is under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04493-09 LEP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Bioenergetic Pathways in Chemically-Transformed Epithelial Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A. E. Kaplan Research Chemist LEP NCI

COOPERATING UNITS *(if any)*

Laboratory of Applied Studies, Division of Computer Research and Technology, NIH, Bethesda, MD (B. Bunow); Department of Microbiology, Harvard Medical School, Boston, MA (H. Amos); Program Resources, Inc., Frederick, MD (R. L. Brown)

LAB/BRANCH

Laboratory of Experimental Pathology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of routes of energy losses from neoplastic cells continued using a model consisting of a control rat hepatocyte line and its N-nitroso-N-methlyurea-transformed counterpart. Studies of energy loss focused on the enzyme, lactic dehydrogenase (LDH, E.C. 1.1.1.27), because it is known to produce lactic acid in larger quantities in neoplastic cells and to export it into the medium, representing an energy loss. Five subtypes of LDH were identified with isoelectric points between pH 5.98 and 9.44 in control cells but only two in tumorigenic cells. The latter correlate with markedly increased rate of synthesis and excretion of lactic acid.

The availability of new monoclonal antibodies against tubulin, cytokeratin and actin resulted in more clearly defined differences between the control and tumorigenic rat hepatocyte lines. In the latter, cytokeratin is markedly diminished in fluorescence, but the aggregation pattern is not altered; tubulin and actin are altered in the patterns of their individual aggregates and also show a slight overall decrease in fluorescence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05265-06 LEP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 chereclers or less. Title must fit on one line between the borders.)*

Effects of Chemical Carcinogens on Transforming DNA Sequences and Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Pnncipel Investigetor.) (Name, title, laboratory, and institute affiliation)*

PI:	U. Saffiotti	Chief	LEP	NCI
Others:	M. I. Lerman	Expert	LIB	NCI
	S. F. Stinson	Biologist	LEP	NCI

COOPERATING UNITS *(if any)*

Laboratory of Central Nervous System Studies, National Institute of Neurological and Communicative Disorders and Stroke, NIH (D. Y. Goldgaber, D. C. Gajdusek).

LAB/BRANCH

Laboratory of Experimental Pathology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.4	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project is to identify, characterize, and clone those genes that drive the development of neoplasia whose malignant potential results from changes caused by chemical carcinogens. (A). DNAase I-hypersensitive (HS) sites were identified as targets for rapid binding and repair following in vivo exposure to benzo[a]pyrene (BP), both in total liver cell DNA and in the c-Ha-ras-1 proto-oncogene. The kinetics of BP adduct formation and repair were first determined in total liver DNA from hamsters given tritiated BP intraportally. Isolation of nuclei at selected times from BP treatment showed that 80% of the adducts were DNAase I-HS at early times after BP exposure (30 min), whereas the adducts remaining when repair was 90% complete (60 min) were no longer DNAase I-HS. The Ha-ras gene was analyzed under the same conditions of BP exposure in hamster liver DNA and showed a marked DNAase I-HS response at the early time points (15-30 min), but not after repair completion (120 min), indicating that BP binding and repair occur preferentially at DNAase I-HS sites. DNAase I-HS sites were also found in the Ha-ras locus in human liver DNA, where two such sites were recognized, probably in the promoter and enhancer regions. (B). The gene encoding the polypeptide that forms the brain amyloid in Alzheimer's disease and in adult Down's syndrome was isolated, characterized and sequenced. The gene was identified by synthesis of a 59-oligonucleotide probe with deoxyinosine in every third position, hybridization to a clone from a human brain cDNA library, and sequencing. This gene was found to encode a single mRNA species, to be transcribed in normal tissues, to be conserved in distant species and to be localized in chromosome 21.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05274-06 LEP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Respiratory Carcinogenesis by Chemical and Physical Factors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	U. Saffiotti	Chief	LEP NCI
Others:	S. F. Stinson	Biologist	LEP NCI

COOPERATING UNITS *(if any)*

Department of Pathology, University of Maryland, School of Medicine, Baltimore, MD (E. M. McDowell, K. P. Keenan)

LAB/BRANCH

Laboratory of Experimental Pathology

SECTION

Respiratory Carcinogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.2	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Multifactorial induction of epithelial cancers from different respiratory tract segments in animal models is studied by combined treatments with chemical, physical and biological factors. Results of a complex multifactorial study were analyzed. Fourteen groups of hamsters were treated with the following variables: single intralaryngeal instillation of N-methyl-N-nitrosourea (MNU) at 5 weeks of age; 15 weekly instillations of benzo[a]pyrene (BP) adsorbed on ferric oxide (Fe-O) in saline (or of Fe-O or saline alone); instillation was either only at the larynx or through the length of the trachea (abrasion of the tracheal epithelium induced reparative hyperplasia and inflammation). The three major determinants of the carcinogenic response were found to be MNU, BP and tracheal wounding, which was a key factor in the induction of carcinomas not only in the trachea but also in the intrapulmonary bronchi. In another hamster study, concurrent intraperitoneal injection of dimethylsulfoxide increased the incidence and severity and decreased the latency of respiratory tumors induced by intratracheal administration of BP/Fe-O suspensions, more so when DMSO was given with BP, than 5 days after. Binding of BP in the respiratory tract after intratracheal administration of BP/Fe-O was determined by quantitative autoradiography. In the hamster, it was high in the larynx, trachea and bronchi, and low in the terminal bronchioles. In the rat, binding was high in the trachea, intrapulmonary bronchi and terminal bronchioles, and low in the larynx and extrapulmonary bronchi. Maximum binding was reached within 48-72 hours in hamsters, but only within 3 hours in rats. Silica-induced pulmonary epithelial proliferative lesions were further studied for their pathogenetic relationship to granulomatous cell reaction and to cellular mediators of inflammation, in conjunction with long-term carcinogenesis studies of different forms of silica (quartz, cristobalite, tridymite).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05276-06 LEP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Growth Control in Epithelial Cells and its Alteration in Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. E. Kaighn	Expert	LEP	NCI
Others:	U. Saffiotti	Chief	LEP	NCI

COOPERATING UNITS *(if any)*

Mario Negri Pharmacol. Res. Institute, Milan, Italy (F. Bertolero)

LAB/BRANCH

Laboratory of Experimental Pathology

SECTION

Tissue Culture Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mouse keratinocytes were found to undergo spontaneous "immortalization" without a crisis, in a newly developed serum-free medium, LEP/MK2, consisting of low calcium MEM with non-essential amino acids supplemented with eight factors. Three lines have been isolated to date (MK1, MKDC4, and MK/2057C). The MK1 line has now undergone more than 400 doublings. Giemsa banding has revealed significant karyotypic changes in MK1 as early as the 4th passage, leading to a near-tetraploid karyotype with random loss and gain of individual chromosomes. Minute chromosomes, but no stable markers, have been observed. After these initial changes, the karyotype has remained essentially stable at later passage levels. Line MKDC4 has undergone more than 200 doublings to date and was also found to be subtetraploid at the 7th passage. Line MK/2057C was derived from line MKDC4 at passage 6, found to be resistant to transforming growth factor-beta (TGF-β) and maintained in LEP/MK2 medium with 1.0 ng/ml TGF-β. This line has doubled more than 150 times since isolation and remains subtetraploid. Growth parameters were determined for these cell lines at increasing passage levels. Changes with passage level included increased plating efficiency, a reduced requirement for bovine pituitary extract, increased resistance to the growth-inhibitory activity of serum and serum-derived factors including TGF-β, and decreased response to hormones and growth factors. The established lines, like primary and secondary keratinocytes, remain responsive to calcium-induced terminal differentiation and are non-tumorigenic in athymic nude mice. This serum-free system is currently used for transformation studies with oncogenes and chemical carcinogens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05494-02 LEP

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular and Molecular Studies in Normal and Neoplastic Human Prostatic Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. E. Kaighn	Expert	LEP	NCI
Others:	J. F. Lechner	Microbiologist	LHC	NCI
	R. Reddel	Expert	LHC	NCI

COOPERATING UNITS *(if any)*
Departments of Urology and Surgery, Northwestern University Medical School, Chicago, IL (J. Kozlowski); Laboratory of Oral Medicine, National Institute of Dental Research, NIH (M. I. Lerman); University of Texas Medical School, Houston, TX (D. Sirbasku)

LAB/BRANCH
Laboratory of Experimental Pathology

SECTION
Tissue Culture Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A major goal of this project is to investigate the role of known oncogenes and genomic DNA from prostatic cancer cells in transforming normal human prostatic epithelial cells. The "immortalization" of a normal human prostatic epithelial cell line, NP-2s, has been accomplished. This line (Lechner et al., JNCI, 60: 797, 1978) was recovered from liquid nitrogen and cultured in serum-free medium (P4-8F) consisting of PFMR4 (Lechner et al., Methods in Cell Biology, Vol. 21B, pp. 195-225, 1980), without trace element concentrate, supplemented with selenite, 50 nM; calcium, 0.5 mM; epidermal growth factor, 0.5 ng/ml; insulin, 5.0 µg/ml; bovine pituitary extract, 0.5%; bovine serum albumin, 250 µg/ml; phosphoethanolamine, 0.5 µM; and cholera toxin, 0.1 nM. Overnight cultures of cells near the end of their lifespan were transfected with plasmid p-RSV-T consisting of the RSV-LTR promoter and the gene encoding the SV40 large T-antigen (Brash et al., Mol. Cell. Biol., May 1987). The treated cells formed rapidly-growing, multilayered colonies within 2 weeks at a frequency of 1-2/10,000 cells at risk in 4 repeat experiments, whereas the untreated cells became quiescent and formed no colonies. Individual transfected colonies were isolated, expanded and tested for growth in suspension, tumorigenicity in nude mice, karyotype and response to growth factors. All 13 colonies tested are non-tumorigenic and remain anchorage-dependent. There was significant extension of the life span of NP-2 cells following transfection with constructs contining ras, v-myc or both. These non-tumorigenic lines will be used to investigate further steps toward neoplasia.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05192-07 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Repair of Carcinogen-Induced DNA Damage in Human Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Curtis C. Harris	Chief	LHC	NCI
Others:	Simon Plummer	Visiting Fellow	LHC	NCI

COOPERATING UNITS *(if any)*

Department of Physiology, Hershey Medical Center, Hershey, PA (A.E. Pegg); Department of Pathology, University of Maryland School of Medicine, Baltimore, MD (B.F. Trump); Karolinska Institute, Stockholm, Sweden (R.C. Grafstrom)

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Carcinogen Macromolecular Interaction Section

INSTITUTE AND LOCATION

NCI NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Normal adult human tissues and cultured bronchial epithelial cells and fibroblasts exhibit O6-alkylguanine-DNA alkyltransferase activity in vitro by catalyzing the repair of the promutagenic alkylation lesion O6-methylguanine from DNA. Alkyl-transferase activity varies in the different human tissues tested in the decreasing order of liver, colon, esophagus, peripheral lung and brain. Formaldehyde inhibits repair of O6-methylguanine and potentiates the mutagenicity of an alkylating agent, N-methyl-N-nitrosourea, in normal human fibroblasts. In some experimental studies, repeated exposure to alkylating agents has led to an increase in O6-methylguanine-DNA alkyltransferase activity, i.e., an adaptive response. We have shown that human bronchial epithelial cells do not adapt and increase their DNA repair capability. This finding has important implications in carcinogenesis caused by low doses of N-nitrosamines. The effects of cigarette smoke condensate, catechol and smoke "conditioned" media on the activity of O6-methylguanine-DNA alkyltransferase (O6MT) and uracil-DNA glycosylase (UDG) on cultured human bronchial epithelial cells, HUT 292 cells and Beas-12 cells is currently under investigation. The activity of these two DNA repair enzymes is also being measured in the alveolar macrophages and peripheral blood lymphocytes of smokers and nonsmokers. Interindividual and intraindividual variation in these activities is up to 100-fold and 6-fold, respectively. Preliminary results indicate a significant rise in UDG activity in the macrophages of smokers compared to nonsmokers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05293-06 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

ras Oncogene Transfection of Human Lung Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	George H. Yoakum	Senior Staff Fellow	LHC	NCI
Others:	John F. Lechner	Section Chief	LHC	NCI
	Ainsley Weston	Visiting Fellow	LHC	NCI
	James C. Willey	Biotech Training Fellow	LHC	NCI
	Paul Amstad	Visiting Fellow	LHC	NCI
	Curtis C. Harris	Chief	LHC	NCI
	Lance Liotta	Chief	LP	NCI
	D. C. Rao	Visiting Fellow	LP	NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Carcinogen Macromolecular Interaction Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanism of v-Ha-ras transformation of NHBE cells was investigated by testing the effect of v-Ha-ras expression on chromosomal stability. To determine the effect of v-Ha-ras on chromosome structure plasmid H1 containing v-Ha-ras was transfected by protoplast fusion, and mitotic NHBE cells were examined 24 hours later to observe effects on chromosomal structure. Increased numbers of chromosome breaks and gaps were observed in v-Ha-ras-transfected NHBE cells. Multistage progression of v-Ha-ras-transfected NHBE cells was studied by characterization of the tumorigenic growth in nude mice, cell surface antigens, and biochemical properties of TBE-1, TBE-1SA, and tumor cell lines derived from TBE-series cells. An in vitro model was developed to study the multistage progression in malignancy of human bronchial epithelial cells that were transformed to immortal cell lines with measurable malignant potential following transfection with Harvey ras oncogene (v-Ha-ras). Progressively malignant cell lines derived from this transformation were selected by continued growth in tissue culture (TBE-1), anchorage-independent growth in soft agar (TBE-1SA), and xenogeneic transfer of TBE-series tumor tissues between mice. The TBE-1SA cell line has a shorter average latency period for subcutaneous primary tumors in athymic nude mice, higher frequency of successful transplantation, and more frequent metastasis to the liver, spleen, and lungs from primary tumors than tumorigenic cell lines selected for progression by continued growth in cell culture. The secondary growth of tumors that were passaged between mice also led to increased malignancy for each type of TBE-cell line tested.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05324-05 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Studies of Tumor Suppression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Curtis C. Harris Chief LHC NCI

COOPERATING UNITS *(if any)*

University of California at Irvine, Irvine, CA (E. Stanbridge)
University of Maryland, Baltimore, MD (E. Gabrielson)

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Carcinogen Macromolecular Interaction Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.1	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genetic changes related to carcinogenesis are being studied using hybrids of human lung carcinoma cells with normal human bronchial epithelial cells. Initial studies suggest that a limited population doubling potential (mortality) is the dominant genetic trait in hybrid cells. Other hybrid cell lines have been isolated and are being characterized for doubling potential, karyotype and tumorigenicity in athymic nude mice. The effects of individual chromosomes are being assessed by fusion with mini-cells containing single marked chromosomes.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05325-05 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

DNA Cytosine Methylation, Cellular Physiology, and Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Vincent L. Wilson	Sr. Staff Fellow	LHC	NCI
Others:	Curtis C. Harris	Chief	LHC	NCI
	Tohru Masui	Visiting Associate	LHC	NCI

COOPERATING UNITS *(if any)*

Gerontology Research Center, NIA, Baltimore, MD (R.G. Cutler); Lab. Environmental Carcinogenesis, Copenhagen, Denmark (H. Autrup).

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Biochemical Epidemiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	0.7	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role DNA methylation plays in the promoter regions of two separate gene systems has been clarified. Demethylation of at least one Hpa II restriction site upstream from the structural coding sequences of human gamma globin or the hepatitis B core antigen gene is necessary but not sufficient for the initiation of transcription. The final conversion of a quiescent, demethylated gene (gamma globin or hepatitis B core antigen) to an active state requires some endogenous or exogenous inducing agent, which may be highly specific for any given gene or gene complex. Clonal selection is not responsible for observed changes in gene expression, since we have clearly shown that cells remethylate DNA that has been demethylated by 5-azacytidine treatment.

New micro techniques have been developed which enable the analytical quantitation of 5-methylcytosine in less than one microgram of DNA isolated from any source. Thus, the genomic 5-methylcytosine content of normal human bronchial epithelial and pulmonary mesothelial cells has been measured for the first time. These techniques have enabled the determination of changes in the genomic content of 5-methylcytosine during normal physiological processes. The genomic content of 5-methylcytosine in normal human bronchial epithelial cells and in rodent tissues decreases with increasing in vivo age. Significant decreases in DNA 5-methylcytosine occur concomitantly with the induction of squamous differentiation in normal human bronchial epithelial cell cultures. These techniques have also provided for the demonstration that chemical carcinogens can induce decreases in DNA 5-methylcytosine levels in dividing normal human bronchial epithelial cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05326-05 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

HLA Antigens: Structure, Function and Disease Association

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Dean L. Mann	Section Chief	LHC	NCI
Others:	William Blattner	Chief, Family Studies Section	EEB	NCI
	James Geodert	Expert	EEB	NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Biochemical Epidemiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

HLA typing was performed on lymphocytes from patients with a common disease or from families where more than one individual had a common disease type. HLA typing was performed in a cohort of individuals with AIDS, either Kaposi's sarcoma, or opportunistic infections, or individuals at risk for this disease. A total of 250 individuals have been HLA typed. One hundred of these patients have been followed over a 4-5 year period. The objectives of these studies are to examine possible genetic susceptibility to the development of AIDS or AIDS-related complex that is related to expression of histocompatibility antigens. The HLA-DR1 phenotype is increased in frequency in all AIDS patients compared to HIV sero-positive controls. HLA-DR3 is significantly decreased in the patients with Kaposi's sarcoma. In the HIV seropositive individuals followed for 54 months, 22 have developed AIDS (opportunistic infection). These individuals have a significant increase in the HLA-DR1 and/or DR3 phenotype. HLA-DR antigen frequencies were compared with antibody production to DNA and RNA antigens in patients with systemic lupus erythematosus. Individuals with the HLA-DR3 and DR4 phenotypes were found to have antibodies to different nucleic acids.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05328-05 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunologic Studies of Human T-Cell Lymphoma Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Dean L. Mann	Section Chief	LHC	NCI
Others:	Mikulas Popovic	Medical Officer	LTCB	NCI
	Robert Gallo	Chief	LTCB	NCI
	William Blattner	Chief, Family Studies Section	EEB	NCI
	Jeffrey Clark	Senior Staff Fellow	EEB	NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Biochemical Epidemiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The human T-cell lymphoma virus, HTLV-I, has been found to be associated with patients with adult T-cell leukemia. Studies are underway to understand the mechanism of malignant transformation of cells infected with this virus and the immunologic response of individuals who are infected with this virus and who demonstrate malignancies, or those who are carriers of the virus but have not developed malignancies. Patients with sestemic lupus erythematosis and other autoimmune diseases were examined for evidence of infection with HTLV-I or-II or HIV by testing serum for antibody to these viruses and probing DNA from their lymphocytes for retroviral sequences. None were found. Chronic lymphocytic leukemia (CLL) cells were obtained from patients who were HTLV seropositive; however, their malignant B-cells did not contain the HTLV-I retrovirus. Using hybridoma technology, CLL cells were fused with a B-lymphoblastoid cell line and the immunoglobin captured. In one instance, the captured immunoglobin reacted with the HTLV-I p24 gag proteins and, in the other instance, the large envelope protein from HTLV-I. Immunoglobin gene rearrangement present in the B CLL cells was demonstrated in the hybridoma cell line. The results indicate that the CLL cells were antigen-committed cells prior to malignant transformation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05341-05 LHC

PERIOD COVERED

October 1, 1986 to September 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Model Systems for Studying Physical Carcinogens at the Cellular Level

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	John F. Lechner	Section Chief	LHC	NCI
Others:	Angela Somers	Visiting Fellow	LHC	NCI
	Brenda Gerwin	Research Chemist	LHC	NCI
	Helen Reddel	Guest Researcher	LHC	NCI
	Curtis C. Harris	Chief	LHC	NCI

COOPERATING UNITS *(if any)*

Duke University, Department of Pharmacology, Durham, NC (G. Rosen); Baltimore V.A. Hospital, Baltimore, MD (E. Gabrielson); Institute of Occupational Health, Helsinki, Finland (K. Linnainmaa)

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

In Vitro Carcinogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Methods to culture human pleural mesothelial (NHM) cells have been improved. Pure cultures are initiated by pelleting the mesothelial cells from pleural effusion fluid and inoculating the resuspended cells into dishes containing LHC basal nutrient medium supplemented with serum (3%), hydrocortisone (0.5 micromoles), insulin (5 micrograms/ml) epidermal growth factor (EGF) (5 ng/ml), transferrin (10 micrograms/ml), trace elements, and 2% chemically-reduced (factor-free) serum (FFS). Using this pseudo-defined protocol, mesothelial cell cultures have been established from more than 200 donors. The cells can be subcultured at clonal density with a colony-forming efficiency of more than 10% and high density cultures can be subcultured four to six times before senescence. We have now established that transforming growth factor beta (TGF-beta) and platelet-derived growth factor (PDGF) will induce serum-starved cells to undergo one round of DNA synthesis in the absence of serum. However, for sustained growth to ensue, the medium must also be supplemented with insulin and high density lipids (HDL). We have further found FFS to be both a good source of HDL and essentially free of other growth factor activities. Surprisingly, we have found that NHM cultures, on average, respond equally well in mitogen assays to numerous purified peptide growth factors including: interleukin 1, interleukin 2, EGF, fibroblast growth factor, PDGF, TGF-beta, beta-interferon, gamma-interferon and cholera toxin. Further, insulin is required for sustained growth and transferrin potentiates the activities of the other factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05403-04 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Analysis of Gene Regulation and Proliferative Control in Human Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Brenda I. Gerwin	Research Chemist	LHC	NCI
Others:	Roger Reddel	Expert	LHC	NCI
	John Lechner	Res. Microbiologist	LHC	NCI
	Tohru Masui	Visiting Associate	LHC	NCI
	Peter Wirth	Expert	LHC	NCI
	Snorri Thorgeirsson	Chief	LEC	NCI
	Anita Roberts	Research Chemist	LC	NCI
	Michael Sporn	Chief	LC	NCI

COOPERATING UNITS *(if any)*

Hazelton Labs, Rockville, MD (M. Moore); Institute of Occupational Health, Helsinki, Finland (K. Linnainmaa)

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Carcinogen Macromolecular Interaction Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These experiments have shown that human mesothelial cells, as compared to fibroblasts, are more sensitive to induction of structural chromosomal aberrations by exposure to asbestos fibers. In addition, it has been shown that malignant mesothelioma cell lines produce PDGF A-chain and B-chain mRNA at much higher levels than do normal cells. PDGF-like activity is detected in medium conditioned by tumor cells, but not by normal cells. TGF-beta mRNA is expressed at similar levels in normal cells and tumor cells, but TGF-beta protein is secreted in greater amounts by normal cells. Normal cells respond to mitogenic stimuli from PDGF and possess PDGF receptors. These findings suggest the possibility of an autocrine mechanism for the generation of mesothelioma.

Two-dimensional gel analysis of normal human bronchial epithelial cells after TPA or TGF-beta treatment has indicated several protein alterations which might correlate with squamous differentiation. The magnitude of the alterations is not great, implying that this technique may not display the most critical changes. It is of interest that Northern blot analysis indicates that a 2-hour treatment of human bronchial epithelial cells with TPA, but not TGF-beta, can induce an increase in IL-1 beta.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05409-04 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Control of Growth and Differentiation of Human Bronchial Epithelial Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	John F. Lechner	Section Chief	LHC	NCI
Others:	Tohru Masui	Visiting Associate	LHC	NCI
	Yang Ke	Visiting Fellow	LHC	NCI
	Curtis C. Harris	Chief	LHC	NCI

COOPERATING UNITS *(if any)*

Univ. of MD School of Medicine, Balt., MD (B.F. Trump); Georgetown Univ. School of Medicine, Washington, DC (H. Yeager); VA Hospital, Washington, DC (P. Schafer)

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

In Vitro Carcinogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.2	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Defined methods to grow replicative cultures of normal human bronchial epithelial (NHBE) cells without serum have been developed. These cells can be subcultured several times; will undergo 35 population doublings; and have expected epithelial cell characteristics of keratin, desmosomes and cell surface antigens. NHBE cells inoculated at clonal density will multiply with an average generation time of 28 hr; the majority of the cells are small and migratory and have few tonofilaments. Adding human whole blood-derived serum (BDS) depresses the clonal growth rate of NHBE cells in a dose-dependent fashion. In contrast, human lung carcinomas either replicate poorly or fail to grow at all when inoculated at clonal density in serum-free medium. Their rates of multiplication increase in direct proportion to the amount of BDS added to the optimized medium. Type beta transforming growth factor (TGF-beta) was found to be the primary differentiation-inducing factor in serum for NHBE cells, while TGF-beta was not growth inhibitory for malignant cells. These differential effects of TGF-beta on normal versus malignant cells are not because of lack of TGF-beta-specific receptors on malignant cells. Epinephrine antagonized the effect of TGF-beta without altering characteristics of TGF-beta-specific receptors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05426-03 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization and Mode of Action of the raf Subfamily of Oncogenes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	George E. Mark, III	Expert	LHC	NCI
Others:	Andrea Pfeifer	Visiting Fellow	LHC	NCI
	Paul Amstad	Visiting Fellow	LHC	NCI
	Dean L. Mann	Section Chief	LHC	NCI
	Curtis C. Harris	Chief	LHC	NCI
	Snorri S. Thorgeirsson	Chief	LEC	NCI

COOPERATING UNITS *(if any)*

Dept. of Genetics, Harvard Medical School (N. Perrimon); Dept. of Radiation Medicine, Georgetown Univ. School of Medicine (U. Kasid).

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Carcinogen Macromolecular Interaction Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The raf proto-oncogene shows significant homologies to protein kinase-C in regions involved with ligand binding and kinase regulation (activation). More specifically, the cysteine finger which exists in a duplicated form in pk-C are present in an identical context within the raf protein and show approximately 50% sequence relatedness. Raf, when non-activated, may be seen as a diffuse cytoplasmic protein. In neuroepitheliomas, where we believe raf to be activated, the protein is found concentrated in the golgi apparatus (i.e., in the particulate fraction of the cell).

To test the transforming and tumorigenic potential of the c-raf-1 proto-oncogene, retroviral recombinants were constructed using Mulligan's pLJ and pZip vectors. Injection into newborn mice of infected cells, or the G418 selected psi-am clones containing the c-raf-1 sense construct, caused tumors demonstrable within 1-2 weeks. Similar short latency periods were seen with pLJ-PDGF A chain transfected psi-am cells. It is concluded that the normal c-raf-1 gene product may act in a transforming capacity in the absence of structural modifications.

Raf has been found to be related to the transformed phenotype of a chemically induced (AAF) human B-cell malignancy and a laryngeal carcinoma (SQ20B). Two additional head and neck carcinoma DNAs have also been found to transform 3T3 cells, in which we subsequently identified altered human raf loci. SQ20B cells transfected with DNA capable of anti-sense raf RNA transcription are non-tumorigenic.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05429-03 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Retroviral Shuttle Vectors for Infection of Oncogenes into Human Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Curtis C. Harris	Chief	LHC	NCI
Others:	Paul Amstad	Visiting Fellow	LHC	NCI
	Andrea Pfeifer	Visiting Fellow	LHC	NCI
	George E. Mark, III	Expert	LHC	NCI
	Roger Reddel	Expert	LHC	NCI

COOPERATING UNITS *(if any)*

Dept. of Pathology, University of Uppsala Hospital (C. Betsholtz)
Fox Chase Cancer Center (A. Klein-Szanto)

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Carcinogen Macromolecular Interaction Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Viruses carrying either sense or anti-sense orientations of the following genes have been produced: v-raf, c-raf, v-Ha-ras, c-myc(mouse), and PDGF A chain (normal and carboxyl-deleted cistrons). The sense constructs of ras, v-raf, and PDGF A chain were found to produce transformed foci on mouse 3T3 cells. Most produced tumors in nude mice with relatively short latencies.

Zip-Ha-ras virus was used to infect immortalized human bronchial epithelial cells (Beas 12). Within 5 weeks from subcutaneous injection of 5 million cells into nude mice, tumors appeared in 80% of the mice. The karyotype of these cultured cells shows them to be human in origin.

Zip-Ha-sar virus (ras anti-sense) was used to infect TBE-1 cells (primary human bronchial epithelial cells which were transformed after they were essential with a v-Ha-ras oncogene). We conclude from this experiment that the Ha-ras gene function is necessary to maintain TBE-1 cell proliferation since infected cells, which should grow in the presence of G418, do not as a consequence of anti-sense abrogation of function. The Zip-Ha-sar infected cells lack the previously introduced v-Ha-ras mRNA and have instead the expected 5.4-kb mRNA representing the sar transcript.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05431-03 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transfection of myc Oncogenes into Human Bronchial Epithelial Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Brenda Gerwin	Research Chemist	LHC	NCI
Others:	Curtis C. Harris	Chief	LHC	NCI
	George Yoakum	Sr. Staff Fellow	LHC	NCI
	Paul Amstad	Visiting Fellow	LHC	NCI
	George Mark	Expert	LHC	NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Carcinogen Macromolecular Interaction Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Normal human bronchial epithelial (NHBE) cells were transfected with a variety of different oncogenes: raf, v-Ha-ras, a combination of raf and v-myc on the same plasmid and the translocated c-myc frame of the CA46 Burkitt's lymphoma (BL) cell line. The transfected cells were then selected for resistance to inducers of differentiation by treating them with blood-derived serum (BDS) or TPA. The CA46 translocated c-myc gene was the most effective oncogene in inducing resistance to differentiation in normal human bronchial epithelial (NHBE) cells. T-Antigen immortalized cells which can be induced to differentiate were transfected with the CA46 myc construct. A series of differentiation resistant clones have been generated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05432-03 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Biological Activity of Fecapentaene-12 in Human Tissues and Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Curtis C. Harris	Chief	LHC	NCI
Others:	Simon M. Plummer	Visiting Fellow	LHC	NCI
	Jin-Su Choi	Visiting Fellow	LHC	NCI
	Dean L. Mann	Section Chief	LHC	NCI
	Vincent Wilson	Sr. Staff Fellow	LHC	NCI

COOPERATING UNITS *(if any)*

Dept. Toxicology, Karolinska Institute, Sweden (R. Grafstrom)
Microbiological Associates, Inc., Bethesda, MD (R. Curren, L.L. Yang)

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Biochemical Epidemiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	2.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Fecapentaene-12 (fec-12), a candidate carcinogen in the pathogenesis of colon cancer, is cytotoxic, mutagenic and induces DNA single strand breaks (SSB), sister chromatid exchanges (SEC) and unscheduled DNA synthesis (UDS) in normal human fibroblasts. DNA repair-deficient fibroblasts are more sensitive than normal fibroblasts to the cytotoxic and mutagenic effects, which are dose dependent. Accumulation of SSB as a result of inhibition of the polymerase component of the excision repair mechanism suggests that SSB may be mediated, in part, by DNA repair mechanisms. These results indicate that fec-12 is genotoxic, mutagenic and causes direct DNA damage in human cells. Further support for the hypothesis that fec-12 is an initiating agent in colon cancer comes from the finding that this compound induces transformation in murine Balb 3T3 cells.

Plasmid assays investigating the mechanism of fec-12-DNA damage have shown evidence of interstrand DNA cross-links and direct SSB. Fec-12 induces plasmid mutations in excision repair-deficient (uvra-) E. coli. Restriction digest analysis and DNA sequencing of plasmids isolated from mutants indicated that approximately 10% had marked DNA rearrangements.

Possible covalent binding of 3H fec-12 to calf thymus DNA is indicated by cesium chloride density gradient centrifugation. Separation of fec-12-DNA adducts by enzymic digestion of DNA and HPLC is currently in progress. Preliminary results with the 32P-postlabelling technique indicate that fec-12-DNA adducts may be present in DNA extracted from human fibroblasts exposed to 3H fec-12 in vitro.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05434-03 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunology of AIDS and AIDS-Related Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Dean L. Mann	Section Chief	LHC	NCI
Others:	William Blattner	Chief, Family Studies Section	EEB	NCI
	J. J. Goedert	Expert	EEB	NCI
	R. J. Bigger	Medical Officer	EEB	NCI
	Stanley H. Weiss	Medical Staff Fellow	EEB	NCI
	R. C. Gallo	Chief	LTCB	NCI
	Mikulas Popovic	Medical Officer	LTCB	NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Biochemical Epidemiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Acquired immunodeficiency syndrome (AIDS) is characterized by the profound loss of ability to respond to environmental antigens as well as the development of Kaposi's sarcoma. We have studied the percentage of T-cell subsets in patients with the disease and patients at risk for the disease. In a prospective study, we evaluated the total numbers of T4 positive lymphocytes in 86 HIV antibody-positive AIDS-free homosexual men, 19 of whom developed AIDS between June 1982 and 1985. In evaluation of these T-cell subsets, it was found that the highest degree of correlation with development of the disease was with low numbers of T4 positive cells at the time that the studies were initiated. In skin biopsies from 7 of 40 patients with Kaposi's sarcoma, AIDS, or individuals who were HIV sero-positive, we have identified the HIV retrovirus in Langerhans' cells. HIV has been demonstrated to bud from Langerhans' cells and HIV has been rescued from skin biopsies. The result indicates that these macrophage-like cells harbor HIV. In studies of the early events of binding of the HIV retrovirus to T-cells, we have determined that the epitope defined by a monoclonal antibody detecting the T4A antigen on the T4 molecule is the specific receptor for HIV binding. Also involved in the binding is the HLA-DR molecule, while other products of the HLA-D region appear not to be involved in HIV binding. Peripheral blood monocytes, as well as cell lines with monocyte function and characteristics, have been infected with HIV. HLA-DR expression increases on infected monocytes. Infected monocytes have slightly diminished function in mixed lymphocyte reaction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05435-03 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of Hydrocarbon-Macromolecular Adducts in Humans and Cancer Risk

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Ainsley Weston	Visiting Associate	LHC	NCI
Others:	Curtis C. Harris	Chief	LHC	NCI
	Glennwood E. Trivers	Res. Biologist	LHC	NCI
	Simon M. Plummer	Visiting Fellow	LHC	NCI
	Vincent Wilson	Sr. Staff Fellow	LHC	NCI
	Dean L. Mann	Section Chief	LHC	NCI
	David Manchester	Expert	LHC	NCI

COOPERATING UNITS *(if any)*

University of Oulu, Finland (K. Vahakangas), Louisiana State University, Baton Rouge, LA (M. J. Newman), M.R.C., Carshalton, England (P. Farmer), University of MD Sch. of Med., Baltimore, MD (B. F. Trump)Cooperating Units

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Biochemical Epidemiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Classical epidemiology and xenobiochemical studies have led to a better understanding of the genotoxic effects of environmental contaminants, for example, polycyclic aromatic hydrocarbons (PAHs) in humans. Development of assays for carcinogen exposure at the molecular level in humans has therefore been a major focus of research and from the battery of assays that are currently available, both immunological and physico-chemical approaches are being developed. Since each type of assay system clearly has its own advantages and disadvantages, the use of more than one corroborative assay has been of importance. The problems that appear to confront the development of assay systems for human biomonitoring are essentially twofold: the levels of material present in macromolecular complexes challenge the detection limits of conventional assay systems, and complex mixtures of adducted materials confound simple assay systems. A model system using six synthetically modified DNAs was recently proposed to assist in the development of these assays. Two types of immunoassays have been used in these studies: 1) an enzyme-linked immunosorbant assay (ELISA) has been used to detect for PAH-DNA adducts in human DNA and to measure anti-PAH-DNA antibodies in human serum; and 2) ultra-sensitive enzyme-linked radioimmunoassay (USERIA) has been used to determine the presence of PAH-DNA adducts in human lymphocytes. In addition, High Pressured Liquid Chromatography and synchronous fluorescence spectroscopy (SFS) have been used to obtain evidence for the formation of benzo(a)pyrene-diol-epoxide-DNA and benzo(a)pyrene-diol-epoxide-hemoglobin adducts in human peripheral blood.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05477-02 LHC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Activation of Proto-oncogenes by Ultraviolet Light

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Douglas E. Brash Sr. Staff Fellow LHC NCI

COOPERATING UNITS *(if any)*
Department of Dermatology, Massachusetts General Hospital, Boston, MA (H. Baden)

LAB/BRANCH
Laboratory of Human Carcinogenesis

SECTION
Carcinogen Macromolecular Interaction Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

DNA has been isolated from 3 human basal cell carcinomas, as well as several tumor cell lines carrying known oncogenes. These genomic DNAs were restriction-digested, electrophoresed, and probed with oligonucleotide probes to detect single-base mismatches in the Harvey, Kirsten, and N-*ras* proto-oncogenes at the codon 12 region and codon 61 region. Thus far, one of the skin carcinomas has been found to carry a mutant Ki-12-*ras* allele.

We transfected the UV-irradiated cloned c-Ha-*ras* gene and the c-Ki-*ras* minigene into NIH 3T3 cells to locate sites of activation. Transformed foci were recovered. A number of foci were also obtained with unirradiated plasmids; we believe this to be due to methylation of cytosines at active sites during propagation of the plasmid in *E. coli*, followed by deamination. Therefore, we are repeating the experiment after subcloning all proto-oncogenes into the same vector and transforming into a non-methylating (dcm-) host.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05479-02 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Detection of Carcinogen DNA Adducts by 32P Postlabeling

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Vincent L. Wilson	Sr. Staff Fellow	LHC	NCI
Others:	Simon M. Plummer	Visiting Fellow	LHC	NCI
	Philip Smith	Hall-Shields Fellow	LHC	NCI
	Dean Mann	Section Chief	LHC	NCI
	Curtis C. Harris	Chief	LHC	NCI

COOPERATING UNITS *(if any)*

Department of Nutrition and Food Science, Massachusetts Institute of Technology, Boston, MA (J.M. Essigman); Department of Toxicology, Karolinska Institute, Stockholm, Sweden (R.C. Grafstrom).

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Biochemical Epidemiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The ability to detect low levels of carcinogen DNA adducts in the tissues of people environmentally exposed to chemical carcinogens is invaluable to epidemiological studies of the incidence of cancer in selective populations. A number of selective methodologies have been developed to quantitate carcinogen DNA adducts. The Randerath 32P-postlabelling technique provides a fingerprint analysis of only polycyclic aromatic hydrocarbon type DNA adducts. However, the 32P-postlabelling method has been adapted in the present study to enable the detection of small alkylation type carcinogen DNA adducts. The detection and quantitation of O6-MeGua adducts in DNA has been shown, by the use of standards, to be accurate as low as one adduct in at least 1,000,000 guanine residues. The presence of unidentified 32P labeled spots has also been observed from the analysis of DNA from cells treated with acrolein and DNA treated with fecapentaene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05480-02 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Polymorphisms and Human Lung Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Ainsley Weston	Visiting Associate	LHC	NCI
Others:	Curtis C. Harris	Chief	LHC	NCI
	James C. Willey	Biotech Training Fellow	LHC	NCI
	Brenda I. Gerwin	Research Chemist	LHC	NCI
	Dean L. Mann	Section Chief	LHC	NCI

COOPERATING UNITS *(if any)*

New England Medical Center, Boston, MA (T. Krontiris); Children's Hospital of LA, Los Angeles, CA (W. Benedict); National Institute of Occupational Health, Oslo, Norway (A. Haugen)

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Biochemical Epidemiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There is a clear association between smoking and lung cancer, but it is still not known why some individuals, who are heavily exposed to large concentrations of chemical carcinogens, do not develop tumors, whereas others do. These observations provide circumstantial evidence for the involvement of a genetic factor that predisposes for tumor formation. Recent restriction fragment length polymorphism (RFLP) studies have shown that the loss of normal cellular sequences from chromosome 13 (in the case of retinoblastoma), chromosome 11 (in the case of Wilm's tumor and bladder cancer and breast cancer), chromosome 1 (in the cases of melanoma), chromosome 22 (in the case of acoustic neuroma) and chromosome 3 (in the case of small cell carcinoma of the lung) have been associated with malignancy. It appears that this method might be generally applied to the analysis of inherited susceptibility to cancer and therefore be informative in risk assessment for lung cancer. Tumor and normal tissue from high molecular weight DNA samples have been collected from more than 60 cancer patients for restriction enzyme digestion and Southern analysis. Initial experiments have centered on examination of genes located on the short-arm of chromosome 11; loss of allelic fragments during tumorigenesis have been detected at the cellular Harvey ras locus, the insulin locus, the calcitonin locus, the beta-globin locus, the catalase locus and the Int-2 locus (homologous to the MMTV locus). Experiments that examine additional loci throughout the human genome for these DNA samples are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP5505-03 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

SV40 Large T-Antigen Transformation of Normal Human Lung Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	R. R. Reddel	Expert	LHC	NCI
Others:	Y. Ke	Visiting Fellow	LHC	NCI
	M. McMenamin	Biologist	LHC	NCI
	J. Quintero	Biologist	LHC	NCI
	B. I. Gerwin	Research Chemist	LHC	NCI
	C. C. Harris	Chief	LHC	NCI
	J. Rhim	Res. Microbiologist	LCMB	NCI
	T. McLemore	Sr. Staff Fellow	LETM	NCI

COOPERATING UNITS *(if any)*

Fox Chase Cancer Center, Philadelphia, PA (A. Klein-Szanto)

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Carcinogen Macromolecular Interaction Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is a project to obtain immortalized nontumorigenic cell lines of human mesothelial and bronchial epithelial origin. Five lines have been established from normal human bronchial epithelial (NHBE) cells, one by infection with adenovirusI2-SV40 hybrid virus, two by infection with wild type SV40 virus, and two by transfection via strontium phosphate coprecipitation with a plasmid, pRSV-T, containing origin-minus SV40 early region sequences. One line has been established from normal mesothelial cells by transfection with the plasmid, pRSV-T. These cell lines are being used to study aspects of multistage carcinogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOICP05506-03 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Oxidant-Induced DNA Damage

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Curtis C. Harris	Chief	LHC	NCI
Others:	Philip C. Smith	Hall Shields Fellow	LHC	NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Biochemical Epidemiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Oxidative damage to DNA in cell cultures of bronchial epithelial cells is used as a model to study the mechanism for such damage in vitro. Oxidant-induced damage may be significant in vivo due to challenges presented from free radicals generated from cigarette smoke. Primary human bronchial epithelial cells, an immortalized cell line derived from these cells (Beas-12) and the HUT 292 tumor cell line are exposed to the model oxidants, hydrogen peroxide and menadione, or to cigarette smoke-conditioned media then assayed for biochemical alterations and oxidative DNA damage. Biochemical markers include glutathione, glutathione peroxidase, glutathione reductase, intracellular Ca++ mobilization, peroxide levels and cell viability. Representative measures of DNA damage are 8-OH-deoxyguanosine (8-OH-dG) and thymine glycol content. These measures allow the examination of the hypothesis that substantial oxidative DNA damage does not occur until the cellular protective mechanisms for scavenging active oxygen species are compromised or depleted. The response of human bronchial epithelial cells to oxidant-induced stress caused by components in cigarette smoke may have importance in the etiology of lung cancer caused by smoking.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05507-03 LHC

PERIOD COVERED

October 1, 1986 - September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cell Surface Antigens on Human Lung Carcinomas

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Dean L. Mann	Section Chief	LHC	NCI
Others:	George Mark	Expert	LHC	NCI
	Roger Reddel	Expert	LHC	NCI
	John Lechner	Section Chief	LHC	NCI
	Curtis Harris	Chief	LHC	NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Biochemical Epidemiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The expression of a variety of cell surface antigens were studied on normal bronchial epithelial cells, small cell lung carcinomas, mesothelial cells, mesotheliomas, as well as bronchial epithelial cells, fibroblasts and mesothelial cells transfected with T antigen. The monoclonal antibodies used to study these cell surface antigens defined the determinants normally expressed on a variety of different cell types, mainly those cells of hematopoietic and lymphoid origin. Small cell lung carcinoma cell lines and freshly explanted tumor expressed antigens defined by monoclonal antibodies MY4 and MY9, as well as certain monoclonal antibodies that detect antigens usually associated with the B cells or, in some cases, epithelial cells. In contrast, normal bronchial epithelial cells did not express the MY4 and MY9 antigens. Normal bronchial epithelial cells had been transfected with the H-ras oncogene also expressed in the MY9 antigen. The expression of MHC class I antigens was variable, while MHC class II antigen expression was also variable, but low in these cell lines. Normal mesothelial cells expressed low levels of all of the myeloid-associated antigens. Mesotheliomas varied in their expression of these antigens and there was no consistent pattern which appeared to identify mesotheliomas. In mesothelial cells transfected with T antigen, MY7 and MY4 increased, while MY9 was expressed at variable levels. These findings indicate that bronchial epithelial cells as well as mesothelial cells, both normal and malignant, may express cell surface antigens that are commonly found on other cell types and that expression of certain surface antigens do not necessarily indicate the origin of a malignant cell.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05508-03 LHC

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Isolation of Growth and Differentiation Genes by Subtraction Libraries

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	George E. Mark, III	Expert	LHC	NCI
Others:	Andrea Pfeifer	Visiting Fellow	LHC	NCI
	Curtis C. Harris	Chief	LHC	NCI
	John Lechner	Section Chief	LHC	NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Human Carcinogenesis

SECTION: Carcinogen Macromolecular Interaction Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To perform subtraction identification of clones relevant to epithelial cell differentiation, a novel subtraction cloning procedure has been developed. Radioactive sense and cold anti-sense RNAs from the two cell types being subtracted are synthesized in vitro from libraries representing the transcripts of these two cell types, hybridized overnight in solution, and the unique single-stranded RNA sequences are easily separated from the common double-stranded RNA sequences by chromatography. Libraries of 200,000 members per microgram of polyadenylated RNA have been constructed.

As a spin-off of this subtraction procedure it has been realized that genomic subtraction can also be accomplished using a slight modification of the basic protocols. This would enable the direct identification of "recessive" genes such as the ones involved in retinoblastoma, Wilm's tumor, and muscular dystrophy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05509-03 LHC

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Detection of Mutations in Proto-Oncogenes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Douglas E. Brash	Sr. Staff Fellow	LHC	NCI
Others:	Curtis C. Harris	Chief	LHC	NCI

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Laboratory of Human Carcinogenesis

SECTION: Carcinogen Macromolecular Interaction Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have isolated DNA from 6 human lung tumor cell lines as well as several tumor cell lines carrying known oncogenes. These genomic DNAs were restriction-digested, electrophoresed, and probed with oligonucleotide probes to detect single-base mismatches in the Harvey, Kirsten, and N-ras proto-oncogenes at the codon 12 region and codon 61 region.

Thus far, two previously uncharacterized lung tumor lines appear to carry mutations in the codon 12 region of Ki-ras, with the second allele being normal. A line previously shown to have a Ki-12-ras mutation has been found to have lost the normal allele.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05510-03 LHC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Activities of Promoters/Enhancers in Human Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Roger R. Reddel	Expert	LHC	NCI
Others:	Douglas E. Brash	Sr. Staff Fellow	LHC	NCI
	Brenda Gerwin	Research Chemist	LHC	NCI
	Curtis C. Harris	Chief	LHC	NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Human Carcinogenesis

SECTION

Carcinogen Macromolecular Interaction Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is to investigate the relative promoter strengths of various cloned mammalian promoter regions in human cell types of particular interest to the research program of LHC: normal bronchial fibroblasts, mesothelial cells and bronchial epithelial cells, as well as the recently constructed immortalized mesothelial and bronchial epithelial cell lines. The assayed promoter/enhancer regions included those from the Rous sarcoma virus (RSV) long terminal repeat (LTR), SV40 virus, Moloney sarcoma virus (MSV) LTR, and adenovirus major late promoter (MLP) with or without SV40 enhancer sequences, HTLV-I LTR or HIV LTR with or without their respective trans-activating proteins, metallothionien with or without cadmium, and mouse mammary tumor virus (MMTV) LTR with and without dexamethasone. The sequences were assayed for their promoter/enhancer activity using the chloramphenicol acetyl transferase (CAT) assay system.

In the immortalized lines, SV40-enhanced adenovirus MLP, transactivated HTLV-I LTR and transactivated HIV LTR were highly active. Under the conditions of the assay, the metallothionien promoter was measurably active, but not inducible by cadmium, and MMTV was inducible but showed weak activity. These promoter/enhancer regions are now being tested in normal cells. The very high level of expression by the enhanced adeno-5 MLP and the HTLV-LTR promoters will facilitate construction of vectors for efficient expression of genes in these human cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04496-10 LMC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Chromosomal Proteins and Chromatin Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael Bustin	Acting Section Chief	LMC	NCI
Others:	David Landsman	Visiting Associate	LMC	NCI
	Thyagarajan Srikantha	Visiting Fellow	LMC	NCI
	Nirmolini Soares	Lab. Tech. (Microbiol.)	LMC	NCI

COOPERATING UNITS *(if any)*
Chester Beatty Laboratories, England (Dr. Graham Goodwin)
Laboratory of Biochemistry, Georgetown University (Dr. M. Smulson)

LAB/BRANCH
Laboratory of Molecular Carcinogenesis

SECTION
Protein Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of chromosomal proteins in maintaining the structure and regulating the function of chromatin and chromosomes is investigated. Present efforts are concentrated on learning the cellular function of two non-histone chromosomal proteins, HMG-14 and HMG-17. These two proteins are the only known nucleoproteins whose main binding site in the nucleus is on the nucleosome. Various experiments suggest that they may be involved in modulating the structure of transcriptionally active chromatin. We have isolated and sequenced the human cDNAs for both HMG-14 and HMG-17. We found that the transcripts have unusual features including extremely long 3' untranslated regions (65% of the sequence) and highly GC-rich 5' untranslated regions (73% GC). Each of the proteins is encoded by a distinct multigene family. The HMG-17 multigene family is the largest known human retro-pseudogene family with 50 copies per genome. Each family transcribes a single-size mRNA whose synthesis is regulated in a cell-cycle specific manner. The sequences of the two cDNAs are distinct except in the region coding for the DNA binding domains of the proteins. The DNA binding domains of the proteins are similar in many ways, suggesting that they recognize distinct regions on the nucleosome. Transfection of various HMG-14- and HMG-17-containing vectors into COS and yeast cells allows modulation of the cellular level of the proteins. These studies will further the understanding of gene structure and function in normal and neoplastic cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04517-11 LMC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

DNA Repair in Human Cancer-Prone Genetic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: K. H. Kraemer Research Sci. LMC NCI

Others:	S. Seetharam	Visiting Fel.	LMC	NCI	J. Scotto	Biometrician	BB	NCI
	H. Waters	Tech. (Biol.)	LMC	NCI	G. Peck	Sr. Invest.	DB	NCI
	M. Seidman	Guest Res.	LMC	NCI	J. Robbins	Sr. Invest.	DB	NCI
	F. Kanai	Guest Res.	LMC	NCI	M. Tucker	Sr. Invest.	EEB	NCI
	T. Runger	Guest Res.	LMC	NCI	R. Tarone	Biometrician	BB	NCI
	D. Brash	Staff Fel.	LHC	NCI				

COOPERATING UNITS *(if any)*

Dept. of Pathology, New Jersey School of Medicine (W. C. Lambert); New York Blood Center (J. German); Dept. Dermatol., Hosp. of Univ. of PA (W.H. Clark); Lab of Radiobiology, Harvard School of Public Health (J. Little)

LAB/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	5	4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Molecular, cellular and clinical abnormalities in patients with xeroderma pigmentosum (XP) and with the dysplastic nevus syndrome (DNS) of hereditary cutaneous melanoma are being studied. We have developed new assays utilizing plasmids as tools to measure DNA repair and mutagenesis at the molecular level. In DNA repair-deficient XP cells, we demonstrated that both dimer and non-dimer photoproducts block expression of a transfected gene to a greater extent than with normal cells, while apurinic sites yield normal expression. We established the first permanent simian virus 40 (SV40) transformed cell line from XP complementation group D. We used the shuttle vector plasmid pZ189 to determine that there is a restricted spectrum of mutations induced in UV-treated DNA replicating in XP cells of complementation groups A and D. There are fewer plasmids found with multiple mutations and with transversion-type base substitution mutations than with normal cells. The major UV-photoproduct, the T-T cyclobutane dimer, was found to be only weakly mutagenic with XP and normal lines. Cytosine containing photoproducts produced 90-95% of the mutations and both cyclobutane dimer and non-dimer photoproducts were mutagenic. We determined that photoproduct frequency was not the major determinant of UV mutation frequency in DNA replicated in human cells. We found evidence for activity of an error-prone polymerase in human cells that may be relevant to generation of immunoglobulin diversity. We compiled the largest retrospective study of XP patients to date (830 patients) and found the median age of onset of skin cancer to be 8 years, a 50-year reduction in comparison to the US general population. There was a greater than 1000-fold increase in basal cell or squamous cell carcinomas or melanomas of the skin and in tumors of the anterior (sun exposed) portion of the eye and tongue. A prospective Registry of XP patients has been established. A clinical trial of skin cancer prevention in XP patients is in progress studying oral oral 13-cis retinoic acid as a chemopreventive agent. We formulated the most widely used classification for DNS and estimated that there is a sevenfold increased melanoma risk for people with dysplastic nevi without a personal or family history of melanoma.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01OP05086-09 LMC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study on Cross-reactivity of Monoclonal Antibodies to Rat Cytochrome P-450

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	S. S. Park	Expert	LMC NCI
Others:	H. V. Gelboin	Chief	LMC NCI
	G. M. Sundaresan	Chemist	LMC NCI
	Y. C. Lee	Guest Researcher	LMC NCI

COOPERATING UNITS *(if any)*

Univ. of Oulu, Finland (O. Pelkonen); Vanderbilt Univ., School of Medicine, Nashville, TN (F. P. Guengerich)

LAB/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Metabolic Control Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20982

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Metabolism and sensitivity to drugs and chemical carcinogens differ among tissues, organs, individuals and species. Cytochrome P-450 (P-450) is a key component of the mixed function oxidase system which metabolize many drugs and endobiotics. The type and quantity of specific forms of cytochrome P-450 determine the extent of activation and/or detoxification of particular substrates. MAbs to cytochrome P-450 which identify human cytochromes P-450 are very useful tools for identification of particular human isozymes. Hybridomas were made by fusion of myeloma cells with spleen cells of mice immunized with cytochrome P-450 derived from rats treated with 3-methylcholanthrene, pregnenolone 16 alpha-carbonitrile and ethanol. The MAbs were tested for cross-reactivity with P-450 isozymes which were purified from different animals and also used for identification of human cytochromes P-450 The respective forms of cytochrome P-450 were studied by radioimmunoassay (RIA) and reaction phenotyping with microsomal preparations of human placenta, blood cells and livers. In Western blotting, pregnenolone-16 alpha-carbonitrile and ethanol-inducible cytochome P-450 were also found in human liver microsomes with MAbs specific for the two P-450s. These MAbs have proven useful in phenotyping and immunopurification of human cytochrome P-450 in different tissues and organs of individuals exposed to different environments.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05125-07 LMC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Preparation and Characterization of Monoclonal Antibodies to Cytochrome P-450

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. S. Park	Expert	LMC	NCI
Others:	H. V. Gelboin	Chief	LMC	NCI
	G. M. Sundaresan	Chemist	LMC	NCI
	Y. S. Hong	Guest Researcher	LMC	NCI

COOPERATING UNITS *(if any)*

Dana-Farber Cancer Institute, Boston, MA (D. J. Waxman)

LAB/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Metabolic Control Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cytochrome P-450 (P-450) is a key component of the mixed-function oxidases which metabolize numerous xenobiotics and endobiotics such as prostaglandins, fatty acids, and steroid hormones. P-450s occur in multiple forms, several of which are induced by the administration of a variety of inducers. P-450s are expressed constitutively, and some of these have been shown to be developmentally regulated and age-dependent. The isolation and characterization of individual forms of cytochrome P-450 have been important steps for understanding the function of the different P-450 forms and their role in determining individual differences in the metabolism of xenobiotics and endobiotics. Our approach is to prepare and use monoclonal antibodies (MAbs) as specific probes for individual and classes of cytochrome P-450. Five MAbs have been prepared to a constitutive form of P-450 (P-450RLM5) which metabolizes steroid hormones. All MAbs belonged to mouse immunoglobulin subtype IgM, bind to P-450RLM5, and distinguish this P-450 from another constitutive form, cytochrome P-450RLM3, in double immunoprecipitation reactions. One of the MAbs to P-450RLM5 regiospecifically inhibits 16 alpha-hydroxylation of androstenedione and testosterone in microsomal and reconstituted systems of P-450RLM5. RIA studies indicated that the expression of P-450RLM5 is developmentally regulated in male rats. MAbs to P-450RLM5 will be very useful for reaction phenotyping and quantitative RIA of tissues, immunopurification of P-450RLM5 and for the study on P-450 isozyme interaction in hormone metabolism and with respect to chemical carcinogenesis in animals and humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05208-07 LMC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Phenotyping of Human Cytochrome P-450

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Fred K. Friedman	Research Chemist	LMC	NCI
Others:	Haruko Miller	Bio. Lab. Tech.	LMC	NCI
	Sang S. Park	Expert	LMC	NCI
	Harry V. Gelboin	Chief	LMC	NCI

COOPERATING UNITS *(if any)*

Hebrew University, Jerusalem, Israel (H. Kapitulnik)
University of Oulu, Finland (O. Peikonen)

LAB/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Metabolic Control Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.4	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The individual forms of cytochrome P-450 display unique substrate specificity and reactivity profiles toward a variety of drugs and carcinogens. Differences in cytochrome P-450 phenotype may relate to individual differences in sensitivity to certain drugs and susceptibility to carcinogenesis. Monoclonal antibodies (MAbs) to cytochromes P-450 have been used as specific probes for the cytochromes P-450 in human liver. Western blot analysis with antisera to rat ethanol-induced P-450, a form with high nitrosamine metabolizing activity, detected a P-450 in human liver homogenates. Individual variation in the level of this P-450 was observed. MAb 1-98-1 to this rat P-450 also detected a related human P-450 in liver microsomes by Western blot analysis. A radioimmunoassay for P-450 in human liver microsomes was developed using this MAb. Further refinement will provide a rapid, efficient method for screening large numbers of samples from human tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05318-05 LMC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization and Regulation of Cytochrome P-450

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Fred K. Friedman	Research Chemist	LMC	NCI
Others:	Richard C. Robinson	Biologist	LMC	NCI
	Sang S. Park	Expert	LMC	NCI
	Harry V. Gelboin	Chief	LMC	NCI

COOPERATING UNITS *(if any)*

Uniformed Services University of the Health Sciences (A. Alvares).
National Institute of Aging, NIH (J. Rifkind).

LAB/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Metabolic Control Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	0.7	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cytochromes P-450 metabolize a wide array of compounds, including xenobiotics such as drugs and carcinogens, and endogenous compounds such as steroids. The focus of this project is the characterization of structure-function relationships and regulation of the multiple forms of this enzyme. Monoclonal antibodies (MAbs) to rat P-450s are a tool in these studies. A 3-methylcholanthrene (MC)-inducible P-450 was immunopurified from the livers and lungs of rats. On the basis of amino acid sequence analysis, peptide mapping, and molecular weight, the liver and lung P-450s were indistinguishable. Using a MAb to ethanol-inducible rat liver P-450, a P-450 has been purified from both rat and human liver. These differed in primary structure as evidenced by different amino terminal sequences and peptide maps. Developmental regulation of P-450 was examined by studying P-450-dependent testosterone metabolism in 3- and 24-month old rats. Ring hydroxylation patterns, as well as content of liver constitutive P-450s, varied with age. While P-450 activities generally declined with age, the 7 alpha-hydroxylase and corresponding P-450 form responsible for this activity increased with age.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05436-03 LMC

PERIOD COVERED
October 31, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of Cytochrome P-450 and Their Role in Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	N. Battula	Expert	LMC	NCI
Others:	G. K. Townsend	Biologist	LMC	NCI
	F. J. Gonzalez	Senior Staff Fellow	LMC	NCI
	H. V. Gelboin	Chief	LMC	NCI

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH
Laboratory of Molecular Carcinogenesis

SECTION
Metabolic Control

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cytochrome P-450s are a superfamily of enzymes, some of which are capable of metabolizing xenobiotics such as drugs and carcinogens as well as endobiotics such as steroids and prostaglandins. In animals, multiple forms of these enzymes are expressed simultaneously either constitutively or after administration of specific inducers. These enzymes display overlapping substrate specificities. Thus, a single cytochrome P-450 may metabolize multiple substrates and a single substrate may be acted upon by several cytchrome P-450s. Some of the cytochrome P-450 catalytic products bind to cellular macromolecules and thus are presumed to initiate mutagenesis and carcinogenesis. In order to define the contribution of a given cytochrome P-450 to the metabolism of specific drugs and carcinogens, it is important to express these enzymes individually. For this purpose, we have begun to develop expression systems in which an individual cytochrome P-450 protein is synthesized from its full length cDNA. Success in this effort will enable us to define the contribution of each of these enzymes to mutagenesis and cell transformation mediated by chemical carcinogens.

For this purpose, we employed two types of expression systems, namely recombinant vaccinia virus and recombinant retrovirus. We have constructed infectious recombinant vaccinia virus and infectious recombinant retrovirus containing the full length coding cDNA sequences for mouse cytochrome P1-450 and P3-450. Human and mouse cells infected with the recombinant viruses expressed high levels of the authentic size proteins as detected by immunoblotting. The expressed proteins are enzymatically active and displayed substrate specificities characteristic of the respective enzymes. Experiments to determine the catalytic specificities and the contribution of the enzymes to mutagenesis are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05519-01 LMC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Developmental Regulation of Cytochrome P450s

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Frank J. Gonzalez	Sr. Staff Fellow	LMC	NCI
Others:	Harry V. Gelboin	Chief	LMC	NCI
	Tamihide Matsunaga	Visiting Fellow	LMC	NCI
	James Gillette	Chief	LCP	NHLBI
	Kiyoshi Nagata	Visiting Fellow	LCP	NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Carcinogenesis

SECTION
Nucleic Acids Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Certain P-450 genes are under complex and poorly understood developmental control. Among the most interesting developmentally regulated P-450s is testosterone 7 alpha-hydroxylase (P-450a). To examine the mechanism by which P-405a is controlled, we purified the enzyme from rat liver microsomes, produced specific polyclonal antibody and isolated its cDNA clone. The antibody and cDNA were used as probes to study levels of P-450a and its mRNA. Three mRNAs of approximately 2.0, 2.6 and 3.0 kilobase (Kb) in length hybridized with the P-450a cDNA probe in young adult male rats. Analysis of mRNA from 3-methylcholanthrene-treated rats revealed that only the 2.0 and 3.0 Kb mRNA were induced to a similar extent as the P-450a protein. In addition, levels of these two mRNAs correlated with levels of P-450a during development. The 2.6 Kb mRNA was only present in adult males and was absent throughout the life of females. These data suggest that the 2.0 and 3.0 Kb mRNAs are derived from the same gene via the use of different polyadenylation sites and that the 2.6 Kb mRNA is derived from a separate related differentially regulated gene. This supposition was confirmed by the isolation of two related genes from a rat gene library. The P450a gene (2.0 and 3.0 Kb mRNAs) and the P-450a2 gene (2.6 nucleotide mRNA) are highly homologous and both contain nine exons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05520-01 LMC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene Structure and Regulation of N-nitrosodimethylamine Demethylase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Frank J. Gonzalez	Sr. Staff Fellow	LMC	NCI
Others:	Harry V. Gelboin	Chief	LMC	NCI
	Morio Umeno	Visiting Fellow	LMC	NCI
	Tamihide Matsunaga	Visiting Fellow	LMC	NCI

COOPERATING UNITS *(if any)*
Department of Biochemistry, New Jersey Medical School, Newark, NJ (Chung S. Yang)
National Institute on Alcohol Abuse and Alcoholism (Byung J. Song)

LAB/BRANCH
Laboratory of Molecular Carcinogenesis

SECTION
Nucleic Acids Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

N-nitrosodimethylamine demethylase (P-450j) is a major nitrosamine metabolizing enzyme in human and rat liver. Under certain dietary and pathophysiologic conditions, this enzyme can also be found in kidney and lung. Metabolism of nitrosamines by P-450j results in the production of electrophilic intermediates that can bind and mutate DNA. Regulation of the levels of this enzyme, therefore, may be important in nitrosamine-mediated carcinogenesis.

P-450j can be elevated five- to sixfold in rat liver, kidney and lung through administration of ethanol. This increase is due to post-transcriptional events since P-450j mRNA levels do not change. In the chemically induced diabetic rat, P-450j is also markedly elevated; however, this increase is accompanied by an elevation in P-450j mRNA. Transcription run on experiments confirmed that P-450j mRNA is specifically stabilized in the liver, lung and kidney of the diabetic rat. In contrast to these instances of post-transcriptional regulation, P-450j is transcriptionally activated during development. To explore the mechanism of this developmental regulation, the rat and human P-450j genes were first isolated and sequenced. Both genes contain nine exons and share considerable nucleotide similarity immediately upstream of their transcription start sites. This region may be an important cis-acting regulatory domain in the P450j gene. Analysis of the rat and human P-450j genes during development revealed that cytosines upstream of both the rat and human genes are specifically demethylated coincident with their developmental activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CP05521-01 LMC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Polymorphic Drug Oxidation: The Human and Rat Debrisoquine 4-Hydroxylase Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Frank J. Gonzalez	Sr. Staff Fellow	LMC	NCI
Others:	Harry V. Gelboin	Chief	LMC	NCI
	Shioko Kimura	Visiting Associate	LMC	NCI
	Morio Umeno	Visiting Fellow	LMC	NCI
	Eiji Matsunaga	Visiting Fellow	LMC	NCI
	Tamihide Matsunaga	Visiting Fellow	LMC	NCI
	Jullia Pastewka	Chemist	LMC	NCI

COOPERATING UNITS *(if any)*

Argonne National Laboratory, Argonne, IL (James P. Hardwick)
Biocenter, University of Basel, Switzerland (Urs A. Meyer)

LAB/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Nucleic Acids Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Human polymorphic drug oxidation has been recognized for over 30 years. The most extensively studied is the debrisoquine 4-hydroxylase polymorphism in which 6% to 8% of the Caucasian population in Europe and North America cannot metabolize this drug. Studies in our lab and others confirmed that this polymorphism is due to a cytochrome P-450. We have isolated and produced antibody against the rat debrisoquine 4-hydroxylase (dbl) and this antibody was used to obtain the rat and human cDNA clones. These were sequenced and, by comparison to the known P-450 sequences and dbl, were found to constitute a separate P-450 gene subfamily. Gene cloning revealed that at least four active genes related to dbl exist and are expressed in rat; only one of these genes may have debrisoquine hydroxylase activity. In contrast, in humans, only one active gene and pseudogene exist. Cloning and sequencing of genes from human livers that do not possess the dbl protein revealed the presence of mutant genes. Three mutant genes were characterized that produce incorrectly spliced mRNA. The dbl probe may be useful for analysis and detection of mutant genes in human populations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05522-01 LMC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Characterization of Human Thyroid Peroxidase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Shioko Kimura	Visiting Associate	LMC	NCI
Others:	O. Wesley McBride	Section Head	LB	NCI

COOPERATING UNITS *(if any)*

Miyazaki Medical College Hospital, Miyazaki, Japan (Sachiya Ohtaki, Tomio Kotani)

LAB/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Nucleic Acid Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Peroxidases represent a group of hemoproteins that are ubiquitous in both the plant and animal kingdoms. They reduce H_2O_2 and other organic peroxides, while oxidizing a great variety of chemicals. During this reaction, free radicals are produced, which can bind irreversibly to DNA. In certain tissues which are low in the level of xenobiotic-biotransforming cytochrome P-450s, it could be possible that peroxidases provide alternate pathways for xenobiotic metabolism. Furthermore, the reaction of cytochrome P-450s with substrates is composed of a series of steps, one of which is similar to the peroxidative reaction. This also suggests the evolutionary relationship between cytochrome P450s and peroxidases. Therefore, the studies on the peroxidases in terms of the regulation of their expression and the structure-function relationships will help in the understanding of those of the cytochrome P-450s. Although peroxidases exist throughout the human body, the levels and the types of peroxidases in different tissues are not clear. The thyroid gland is one of the tissues whose peroxidase has been intensely studied. This peroxidase is involved in thyroid hormone synthesis and recently has been indicated to be one of the major antigens of the thyroid autoimmune diseases such as Graves' disease and Hashimoto's thyroiditis. The level of the peroxidase is high in patients with the former disease and low in patients having the latter disease and also thyroid cancer. We have started characterization of the thyroid peroxidase by means of molecular biology. Two cDNA clones for human thyroid peroxidase were isolated and sequenced. Both cDNAs are identical except that the 171-nucleotide sequence is deleted in one of the clones, without any reading frame shift. Two mRNAs are expressed in all thyroid tissues examined, suggesting that the alternative splicing of the same gene generated two thyroid peroxidases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05523-01 LMC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of P-450 DNAs in Mammalian Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Shioko Kimura	Visiting Associate	LMC	NCI
Others:	Toshifumi Aoyama	Visiting Fellow	LMC	NCI
	Frank J. Gonzalez	Senior Staff Fellow	LMC	NCI
	Harry V. Gelboin	Chief	LMC	NCI

COOPERATING UNITS *(if any)*

Argonne National Laboratory, Argonne, Illinois (James P. Hardwick)

LAB/BRANCH
Laboratory of Molecular Carcinogenesis

SECTION
Nucleic Acid Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The microsomal cytochrome P-450s have broad substrate specificities and metabolize exogenous materials such as carcinogens and drugs as well as endogenous agents such as cholesterol, prostaglandins, leukotriene, steroid hormones and fatty acids. By expressing various rat and human P-450 cDNAs in a variety of cell lines and characterizing the expressed P-450s, their material substrates and products, most of which have not been identified, could be determined. This furthermore provides another approach to study the structure-function relationship of each P-450 and its mode of action in vivo. We have inserted 10 forms of P-450 cDNAs obtained from rat and human tissue into the vaccinia virus-T7 expression vector. One of the P-450 cDNAs inserted into the vector, P-450LA, was expressed with a high efficiency in a variety of mammalian cell lines and possessed the catalytic activity towards 15- and 16-hydroxylation of palmitic acid. The expressed P-450 was also compared with the protein purified from rat liver microsomes. This P-450 was found to be constitutively expressed in mouse and rat hepatoma cell lines and inducible in the latter cell line by the hyperlipodemic drug, clofibrate. Other forms of P450 will be expressed similarly in this vaccinia virus system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04265-22 BB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Consulting in Statistics and Applied Mathematics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J. J. Gart	Chief, MSAMS	BB	NCI
Others:	R. E. Tarone	Mathematical Statistician	BB	NCI
	H. M. Pettigrew	Mathematician	BB	NCI
	D. G. Thomas	Mathematical Statistician	BB	NCI
	J. Nam	Mathematical Statistician	BB	NCI
	A. M. Smith	Statistician (Health)	BB	NCI

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Biostatistics Branch

SECTION

Mathematical Statistics and Applied Mathematics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is the purpose of this study to collaborate with NCI researchers on mathematical problems related to many areas of cancer research. Consulting assistance in statistical methodology and applied mathematics is provided for NCI investigators and to some extent for NCI contractors. In general, the study is devoted to accelerating the use of quantitative methodology in various aspects of the NCI intramural and extramural programs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04267-22 BB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Research in Mathematical Statistics and Applied Mathematics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	J. J. Gart	Chief, MSAMS	BB NCI
Others:	R. E. Tarone	Mathematical Statistician	BB NCI
	H. M. Pettigrew	Mathematician	BB NCI
	D. G. Thomas	Mathematical Statistician	BB NCI
	J. Nam	Mathematical Statistician	BB NCI
	A. M. Smith	Statistician (Health)	BB NCI

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Biostatistics Branch

SECTION

Mathematical Statistics and Applied Mathematics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is the purpose of this project to conduct research in mathematical statistics, probability, and applied mathematics, and especially to develop new statistical methodology which is applicable to the biomedical sciences. Particular subjects of interest are the methodology of analyzing survival curves and proportions, and statistical methods in cancer epidemiology and statistical genetics, such as the analyses of the relative risk and human leukocyte antigen (HLA) data.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04269-16 BB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biomedical Computing - Consultation, Research and Development, Service

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J. Michael Stump	Chief, IRMS	BB	NCI
Others:	D. J. Grauman	Computer Systems Analyst	BB	NCI
	R. I. Ramsbottom	Computer Specialist	BB	NCI
	B. L. Stephenson	Computer Specialist	BB	NCI
	R. S. Wolfson	Computer Programmer/Analyst	BB	NCI

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Biostatistics Branch

SECTION

Information Resources Management Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	5.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Information Resources Management Section's mission includes: 1) planning and conducting research and development work to improve methodology in the application of computers and data processing techniques in support of research conducted and coordinated by NCI investigators and their collaborators; 2) serving as the focal point in the Epidemiology and Biostatistics Program for the procurement, management and monitoring of support services contracts, and for the evaluation and procurement of automatic data processing (ADP) and word processing equipment as well as data resources used by staff investigators; 3) providing liaison, consultation and collaboration to NCI investigators on the design, development and operation of data processing and information systems; and 4) representing the Division of Cancer Etiology in providing consultation, guidance and assistance to the National Cancer Institute and the Division of Computer Research and Technology (DCRT) on ADP and office automation issues, problems and operations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04475-10 BB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Skin Cancer and Solar Radiation Program

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J. Scotto	Health Services Director	BB	NCI
Others:	T. R. Fears	Mathematical Statistician	BB	NCI

COOPERATING UNITS *(if any)* Interfederal Agency Task Force on Health Effects of Solar Ultraviolet, Environmental Protection Agency(J.Hoffman); National Oceanic and Atmospheric Admin.(G.Cotton,L.Machta); National Aeronautic and Space Adm. (J.Frederick); Temple Univ.(F.Urbach); Smithsonian Institute(B.Goldberg)

LAB/BRANCH

Biostatistics Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project provides statistics and analyses of epidemiologic and photobiologic data relevant to the etiology of skin cancer, including malignant melanoma. Through these studies, NCI provides research in response to Public Law 95-95 (Amendment to the Clean Air Act) and the federal stratospheric ozone protection policy program. Recently, worldwide non-aerosol production of chlorofluorocarbons has increased, and significant depletions of ozone and increases of solar ultraviolet radiation, specifically ultraviolet (UVB) radiation (290nm-320nm) exposure on earth, accompanied by increased incidence in skin cancer have been predicted. We detected no significant increases in surface measurements of solar ultraviolet radiation (UVB) over a 12 year period, 1974-85, however, from 20 locations within the United States. We calculated skin cancer incidence rates for groups at high and low risk living in areas with varying UVB exposure. Refined estimates made during the year suggest that a 10% increase in UVB may result in a 16-20% increase in basal cell carcinoma of the skin, a 20-40% increase in squamous cell skin cancers, and a 6-10% increase in melanoma. Other skin cancer risk factors were also identified, with relative and attributable risk estimates derived for several constitutional and environmental variables.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04500-10 BB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Methodologic Studies of Epidemiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	M.H. Gail	Medical Statistical Investigator	BB NCI
Others:	J. Benichou	Guest Researcher	BB NCI
	R. Brookmeyer	Visiting Biostatistician (IPA)	BB NCI
	W. Blot	Chief, Biostatistics Branch	BB NCI
	T. Fears	Mathematical Statistician	BB NCI
	J. Lubin	Health Statistician	BB NCI
	J. McLaughlin	Senior Staff Fellow	BB NCI
	S. Wacholder	Senior Staff Fellow	BB NCI

COOPERATING UNITS *(if any)* Harvard University (J. Robins, Mayo Clinic (S. Wieand), Univ. of Paris (C. Chastang), Committee on Biological Effects of Ionizing Radiation of the National Academy of Sciences, Memphis State University (Y. Tan), Chinese Academy of Medical Sciences (Y. Liu), NIEHS (C. Weinberg)

LAB/BRANCH

Biostatistics Branch

SECTION

Epidemiologic Methods Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.90	3.80	0.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Work continued on appropriate methods for selecting controls and on the reliability of exposure data in case-control studies of cancer. New methods were developed for analyzing case-control studies in which controls were selected by cluster sampling. A paper appeared that adapts logistic regression to the case in which the exposure and stratification factors are confounded. Surrogate respondents were found to be reliable sources of information on cigarette use. Methods for projecting the minimum size of the acquired immunodeficiency syndrome (AIDS) epidemic were published. A manuscript is in press that describes biases in the conventional analysis of prevalent cohort data, such as seropositive persons at risk for AIDS. Methods for cancer risk projecting for individuals and populations were applied to cohorts at high risk for breast cancer and to those exposed to radiation. Variance calculations for such risk projections were derived for cohort data. Indirect corrections for confounding were studied for occupational cohort data in which confounder information is not available for individuals. The case-cohort design was examined, and a paper is in press that describes the calculation of standardized mortality ratios and their variances from such data. Computational methods for binary regression were published, and additional computer software is under development for epidemiologic analyses. Sample size calculations were carried out for new tests designed to detect qualitative interactions in clinical trials and observational studies, and case-control sample size formulas were developed for logistic regression models with continuous covariates and general relative risk functions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04779-11 BB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Field Studies in High Risk Areas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	W. Blot	Chief	BB	NCI
Others:	J. Fraumeni, Jr.	Associate Director	E&B	NCI
	R. Hoover	Chief	EEB	NCI
	T. Mason	Chief, PSS	EEB	NCI
	B. Stone	Mathematician	BB	NCI

COOPERATING UNITS *(if any)* LA St. Univ. (P. Correa); Univ. TX (P. Buffler); Med. Univ. SC (S. Schuman); NJ Dpt. Health (A. Stemhagen); Chinese Acad. Med. Sci. (B. Li); Shanghai Cancer Inst. (Y. Gao); Center Prev. Med. (E. Buiatti); Univ. So. CA. (S. Preston-Martin); Emory Univ. (R. Greenberg); CA. Hlth. Dpt. (D. Austin)

LAB/BRANCH

Biostatistics Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.5	6.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to identify and describe environmental and host determinants of cancer in areas at high risk of cancer through the use of analytical epidemiologic and biometric techniques, particularly case-control studies of specific cancers. Completed during the year were case-control studies of respiratory cancer in New Jersey, Texas, and Louisiana, esophageal cancer in coastal South Carolina, while data collection was completed for a case-control study of oral cancer in Atlanta, New Jersey, Los Angeles, and San Francisco. The lung cancer investigations revealed elevated risks among several occupational groups, including shipyard workers in New Jersey and construction workers in Louisiana and Texas. Smoking of hand-rolled cigarettes was linked to the exceptionally high risk of lung cancer among Cajuns in southern Louisiana. Analyses from South Carolina showed that esophageal cancer risk is strongly increased among heavy users of alcohol, especially moonshine, but that low intake of fruits and vegetables also contributes to elevated mortality from this tumor. Several international studies are underway to take advantage of unique opportunities to evaluate diet and other factors, including air pollution, in the etiology of cancer. Interviewing was completed for case-control studies of cancers of the esophagus, stomach, and lung and choriocarcinoma in areas of China at high risk of these cancers. Smoking was shown to be the dominant cause of lung cancer among men in Shanghai, while exposures to cooking oil volatiles were implicated in the high risk of lung adenocarcinoma among women, most of whom were nonsmokers. A case-control study of gastric cancer continued in areas of Italy that have among the world's highest rates of this malignancy. Also in operation is a randomized intervention trial in Linxian, China, to assess the role of vitamin/mineral supplementation on reducing this extraordinarily high cancer risk.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05498-02 BB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Consulting on Epidemiologic Methods

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	M.H. Gail	Chief, Epidemiologic Methods Section	BB NCI
Others:	R. Brookmeyer	Visiting Biostatistician (IPA)	BB NCI
	T. Fears	Mathematical Statistician	BB NCI
	J. Lubin	Health Statistician	BB NCI
	J. Benichou	Guest Researcher	BB NCI
	S. Wacholder	Senior Staff Fellow	BB NCI

COOPERATING UNITS *(if any)* Lung Cancer Study Group, Committee on Biological Effects of Ionizing Radiation of The Natl. Academy of Sciences; Univ. of California at Los Angeles (R. Elashoff); New York Univ. Med. Center (R. Shore); Univ. of Chicago (A.B. Schneider); Cancer Inst. of the Chinese Academy of Med. Sciences (J.Y. Li)

LAB/BRANCH

Biostatistics Branch

SECTION

Epidemiologic Methods Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	2.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Major efforts included: 1) collaboration with the Committee on Biological Effects of Ionizing Radiation of the National Academy of Sciences to evaluate available data on risk to alpha-emitting radionuclides, 2) analysis of the interactive effects of joint carcinogen exposures in large rodent studies, 3) the planning and implementaion of cohort and case-control studies in China to quantify the joint effects of smoking and exposure to arsenic and radon on lung cancer risk and to investigate risk factors for penile cancer, 4) studies on the effects of ultraviolet radiation on skin cancer, 5) evaluation of data on the risks from smokeless tobacco, 6) evaluation of case-control data on dietary risk factors for esophageal cancer, 7) collaboration and consultation on the design and analysis of cohort studies in groups at risk of acquired immunodeficiency syndrome, 8) joint evaluation of serum markers for lung cancer, 9) analysis of lung cancer clinical trials, and 10) consultation with the Division of Cancer Prevention and Control, NCI, on large-scale prevention and intervention trials.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04377-16 CEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Familial, Congenital, and Genetic Factors in Malignancy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John J. Mulvihill	Chief, Clinical Genetics	CEB	NCI
Others:	D. M. Parry	Geneticist	CEB	NCI
	P. Madigan	Research Technician	CEB	NCI
	C.A. Collins	Research Assistant	CEB	NCI

COOPERATING UNITS *(if any)*

Atomic Energy of Canada, Ltd. (M. Paterson); UCLA (R. Sparkes); Biotech Laboratory (S. Tsai); Yale University (U. Francke); Health Research (A. Sandberg); Brookhaven Laboratory (R. Setlow); Litton Bionetics (J. Ivett)

LAB/BRANCH

Clinical Epidemiology Branch

SECTION

Clinical Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER:
2.8	2.0	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Study of preneoplastic genetic diseases with a high risk of cancer may help detect environmental and genetic influences in carcinogenesis, especially when appropriate laboratory assays are used. Neurofibromatosis, an autosomal dominant disorder with a predisposition to cancer, received emphasis. Results on 12 families show linkage to the gene for the receptor for nerve growth factor, with a lod score of 4.4 at a recombination distance of 14 centimorgans. Forty-year follow-up of 212 neurofibromatosis patients in Denmark permitted life-table analysis: survival was worst for females who were the original probands, slightly better in male probands, and only slightly less than rates expected in the general population in affected relatives. The relative risk for malignant neoplasms was 4.0 in probands, but only marginally elevated in relatives. Similar multidisciplinary approaches to three other preneoplastic syndromes revealed, in the nevoid basal cell carcinoma syndrome, a lod score of 1.2 to amylase 1 on chromosome 1p, and an association with auditory defects; in the dysplastic nevus syndrome, a possible excess of chromosome breaks; on multiple endocrine neoplasia, type 1, no firm linkage to 28 polymorphic protein loci.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04400-22 CEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Epidemiology of Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Frederick P. Li	Chief, Clinical Studies	CEB	NCI
Others:	R. W. Miller	Chief	CEB	NCI
	J. J. Mulvihill	Chief, Clinical Genetics	CEB	NCI
	D. M. Parry	Geneticist	CEB	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Epidemiology Branch

SECTION

Clinical Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

Persons who have exceptionally high risk of developing cancer are studied to find explanations for their susceptibility. These unusual individuals are identified through referral by practitioners or self-referral and through clinical observations at the bedside. With informed consent, epidemiologic inquiries are made to identify predisposing host and environmental factors, and concurrent laboratory studies help to clarify biologic mechanisms of cancer susceptibility. Results show that carriers of cancer genes develop cancer at very high rates in a few tissues. Early cancer detection has been achieved through screening of high-risk persons, and counseling has been provided to appropriate patients. High-risk patients also tend to develop multiple primary cancers in childhood, and nearly 1000 patients are under prospective observation for second cancers through the Registry of Survivors of Childhood Cancer in Boston. An additional series of nearly 2,000 survivors of childhood retinoblastoma in New York and Boston are being registered for long-term follow-up.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05139-08 CEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

NIH Interinstitute Medical Genetics Program: The Genetics Clinic

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dilys M. Parry	Geneticist	CEB	NCI
Others:	J. J. Mulvihill	Chief, Clinical Genetics	CEB	NCI
	C. A. Collins	Research Assistant	CEB	NCI

COOPERATING UNITS *(if any)*

CC (S. Schlesinger); NEI (M. Kaiser-Kupfer); NIADDK (D. Camerini-Otero, B. White); NICHD (W. Gahl, J. Sidbury, M. Zasloff); NIDR (K. Brown, C. Hughes) NINCDS (R. Eldridge, N. Barton)

LAB/BRANCH

Clinical Epidemiology Branch

SECTION

Clinical Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.80	0.70	0.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Genetics Clinic is a collaborative undertaking by researchers from six NIH institutes and the NIH Clinical Center. Consequently, clinic patients constitute a broad spectrum of genetic disease. The patient load during the clinic's fifth year comprised 213 individuals representing some 60 different diagnostic categories. Of these, 53 patients (25%) were seen by members of the Clinical Epidemiology Branch (CEB). For our Branch, the Clinic provides a multidisciplinary setting in which to study unusual patients who either have cancer or an increased risk of developing malignancy. Patients are ascertained through special referrals from outside physicians and from inhouse requests for etiologic consultations. With informed consent, the approach to the patient includes detailed physical examination and, where applicable, epidemiologic studies of the environmental and genetic background and laboratory studies to clarify biologic mechanisms of carcinogenesis. Categories include patients with genetic diseases predisposing to malignancy, patients with birth defects and cancer, families with childhood sarcomas and breast cancer in blood relatives, and any other families with an excessive occurrence of cancer of any type.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05146-08 CEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Morbidity in Childhood Cancer Survivors and Their Offspring

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John J. Mulvihill	Chief, Clinical Genetics	CEB NCI
Others:	J. M. Byrne	Epidemiologist	CEB NCI
	R. R. Connelly	Statistician	SORB, DCPC NCI
	M. H. Gail	Head, Epidemiologic Methods	BB, DCE NCI

COOPERATING UNITS *(if any)*

NICHD (R. Sherins); Queens Hospital, New York, NY (F. Rosner); VA Medical Center, Newport, NY (H. Zarrabi)

LAB/BRANCH

Clinical Epidemiology Branch

SECTION

Clinical Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Fertility and reproductive histories of cancer patients, especially of long-term survivors of childhood and adolescent cancer, and of men and women who reproduced during cancer therapy, are studied for information on the gonadal toxicity and possible mutagenicity and teratogenicity of cancer treatment, and also to uncover hereditary patterns of cancer. Current phases include intensive analysis of data from interviews and medical records of 2498 cancer survivors and their 3604 sibling controls to learn about their subsequent health and fertility and the health of their offspring. In 7117 offspring, 18 cancers occurred -- not a significant excess over expected numbers. Survivors of childhood brain tumors were less likely to complete 8th grade, or to enter college after high school graduation. Both male and female survivors reported 30% fewer pregnancies than controls; treatment with combined radiation and alkylating agents depressed fertility in survivors to only one-third that of controls. In the subset of subjects from Kansas, survivors had more difficulty than controls getting life or health insurance. In the Connecticut subset, survivors had the same frequency of major depressive episodes as controls. A second phase is a voluntary registry of pregnancies in women with cancer. Preliminary results suggest no excess of birth defects, but some excess wastage of pregnancies conceived within 12 months of completing chemotherapy. An International Conference on Reproduction and Human Cancer was held in May 1987, and its Proceedings are in preparation. Additional studies at the NIH Clinical Center, a national cooperative clinical trial group and a multinational study group are in development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05194-06 CEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

National Cancer Mortality Studies by Computer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Robert W. Miller	Chief	CEB	NCI
Others:	F. W. McKay	Computer Systems Analyst	CEB	NCI
	R. E. Tarone	Biostatistician	BB	NCI
	P. Madigan	Research Assistant	CEB	NCI
	J. Byrne	Visiting Associate	CEB	NCI

COOPERATING UNITS *(if any)*

National Center for Health Statistics (R. Israel)

LAB/BRANCH

Clinical Epidemiology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.3	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have used information from the National Center for Health Statistics (NCHS) and Bureau of the Census to create a comprehensive data base concerning mortality and population information at the county level. Data are available, 1950-1981, for cancer mortality, and 1965-78, for deaths from other causes. Population data will be extended and corrected when the 1980 census data become available. Three-dimensional graphs employing these data are one example of the value of the data collection. Under development are systems for mapping counties in black-and-white, for projecting cancer mortality in coming decades, and for grouping counties by economic subregions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05279-05 CER

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Epidemiologic Data Resources

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. W. Beebe	Statistician (Health)	CEB, NCI
Others:	R. Spirtas	Biostatistician	EEB, NCI
	J. D. Boice	Chief	REB, EBP, NCI
	B. F. Hankey	Biostatistician	SORB, DCPC, NCI
	T. J. Mason	Chief, Population Studies	EEB, NCI
	Z. Hrubec	Expert	REB, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Clinical Epidemiology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To facilitate the development of data resources for cancer epidemiology, a working group was established by the Director, NCI, in 1978. The membership includes those named above plus others, with Dr. Beebe as chairman. The present functions of the group include creating a national data base for occupational mortality, reviewing Master Order Agreement-Request for Proposals, oversight of the Veterans Administration hospital discharge file, liaison with National Center for Health Statistics in regard to the National Death Index, improving access to Federal record systems, and pursuing new leads. A number of contracts or interagency agreements have been initiated in support of this program, especially with Social Security Administration, Internal Revenue Service, and the National Academy of Sciences. A legislative initiative has been drafted in the Office of the Assistant Secretary for Health to widen the access of medical investigators to the address file of the IRS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05280-05 CEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Carcinogenic Effects of Ionizing Radiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	G. W. Beebe	Statistician (Health)	CEB NCI
Others:	C. E. Land	Statistician	REB, EBP, NCI
	J. D. Boice	Chief	REB, EBP, NCI
	B. W. Wachholz	Chief	REB, CPCP, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Epidemiology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A-bomb survivors, Atomic Energy Commission--Department of Energy workers, the population exposed to fallout from atmospheric tests at the Nevada Test Site, etc., have been studied for their potential to provide low-dose risk estimates for radiogenic cancer. Only some combination of experimental and theoretical work, with epidemiologic studies at higher doses, will provide a reliable guide to such risks. Sources of variation in risk estimates for radiogenic cancer are explored for their significance to research on carcinogenic mechanisms and to give direction to epidemiologic research. Dr. Beebe serves as Assistant Project Officer for the study of thyroid nodules in the high background area of China. He also represents Department of Health and Human Services on the Science Panel of the Committee on Interagency Radiation Research and Policy Coordination and NIH on the Public Health Service Group for Input and Communication Regarding Radiation Protection Activities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05329-04 CEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hepatitis B Virus and Liver Cancer in Army Veterans of WWII

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Gilbert W. Beebe Statistician (Health) CEB NCI

COOPERATING UNITS *(if any)*

Medical Follow-up Agency, National Research Council, NAS (J. Norman); Veterans Administration, Six Hospitals (L. Seeff); Liver Diseases Section, DIR, NIDDK (J. Hoofnagle)

LAB/BRANCH

Clinical Epidemiology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The study is based on the epidemic of 50,000 cases of viral hepatitis in the United States Army in 1942, traced to yellow fever vaccine prepared by the Rockefeller Foundation and contaminated with a virus of hepatitis, now shown to have been the hepatitis B virus (HBV). A serologic survey to identify the virus with certainty has been completed on 597 men--about 200 who suffered from acute hepatitis during the 1942 epidemic (Group I), 200 who received vaccine from one of the seven contaminated lots but were not clinically ill (Group II), and 200 who did not receive the Rockefeller vaccine (Group III). Two epidemiologic studies are being performed: 1) a mortality study of 55,000 men divided into three cohorts of approximately equal size, each defined as in the serologic survey, with primary liver cancer the chief end-point; and 2) a case-control study of 2,800 WWII Army Veterans discharged from Veterans Administration hospitals for liver cancer and 2,800 matched controls, the comparison to be based primarily on immunization history with attention to the lot number of the yellow fever vaccine.

In the serologic survey, testing for anti-HBs and anti-HBc has identified the B virus as the source of the infection. In addition, anti-HB levels are high, and only one carrier (HBsAg+) was identified in Group I, none in Group II or III. The mortality study reveals no excess mortality from cirrhosis among either of the two groups infected with the B-virus, and at most a small excess of liver cancer, nothing like that expected from the Asian studies of carriers. The case-control study is still in the process but will be finished during the coming year. A report on the serologic survey was published in the New England Journal of Medicine and the paper on the cohort mortality study is in the final stages of preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04378-12 EEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

U.S. Cancer Mortality Survey and Related Analytic Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T. Mason	Chief, PSS	EEB	NCI
Others:	L. Pickle	Health Statistician	EEB	NCI
	N. Dalager	Epidemiologist	EEB	NCI
	R. Falk	Health Statistician	EEB	NCI
	B. Stephenson	Computer Specialist	BB	NCI
	R. Ramsbottom	Computer Specialist	BB	NCI

COOPERATING UNITS (if any) National Center for Health Statistics, Bureau of the Census (Sam Davis); Environmental Protection Agency (Wilson Riggan)

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Population Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.25	3.0	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The overall objective of this project is to examine the cancer mortality experience in the United States relative to cancer etiology. Special emphasis is placed upon the selection of areas in the U.S. for intensive study. Publications from this area of interest have facilitated the design of ongoing analytical investigations to test specific etiologic hypotheses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04410-11 EEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Persons at High Risk of Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M.A. Tucker	Coordinator of Family Studies	EEB	NCI
Others:	W.A. Blattner	Chief, Family Studies Section	EEB	NCI
	D.L. Mann	Chief, Biochemical Epidemiology Section	LHC	NCI
	S.J. Bale	Staff Fellow	EEB	NCI
	N. Caporaso	Medical Staff Fellow	EEB	NCI
	Y. Liu	Fogarty Fellow	EEB	NCI
	R.C. Young	Chief	MB	NCI
	J.J. Mulvihill	Chief, Clinical Genetics Section	CEB	NCI

COOPERATING UNITS (if any)

Biological Research Faculty & Facility (T. Shimada); Braton Biotech (S. VedBrat); Biotech Laboratories (A. Bodner); Westat, Inc. (J. Cahill); CSG/ORI (K. Boyd/D. Switalski)

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Family Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
7.5	6.2	1.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to (a) conduct and coordinate interdisciplinary studies on members of cancer-prone families and other high-risk populations to clarify the role of genetic mechanisms and host-environmental interactions in human carcinogenesis; and (b) assess, quantify, and elucidate the determinants of the cancer risks associated with therapeutic exposure to cytotoxic drugs. Project staff also conduct or collaborate with other EEB investigators in epidemiologic case-control studies of specific cancers or cohort studies of specific exposures that are particularly relevant to this project. A series of project resources has been developed in support of our research, including (1) a computerized registry of cancer-prone families; (2) a biospecimen repository which processes, stores and distributes biological samples from persons at high risk of cancer; (3) a fibroblast repository/tissue culture facility; and (4) a series of contract-supported laboratories which provide immunologic, cytogenetic, and DNA repair assay capabilities. Persons at high risk of cancer are evaluated clinically and donate biological samples. Clinical, epidemiologic, genetic, and laboratory studies are combined to elucidate mechanisms of cancer susceptibility. The familial melanoma project is a prototype of this approach, in which clinical (dysplastic nevi), genetic (autosomal dominant transmission of a gene possibly linked to the Rh locus) and biologic (enhanced sensitivity to the cytotoxic and mutagenic effects of UV radiation) risk factors have been identified. The therapeutic administration of cytotoxic drugs provides an opportunity to explore the carcinogenic effects of these agents in man. Case-control and cohort studies of cancer patients treated with specific cytotoxic drugs are conducted. These studies have documented differences in leukemogenic potential among specific alkylating agents, and increasing risk of leukemia with increasing total drug dose. In addition, increased risk of bone cancer associated with alkylating agents independent of radiation therapy has been demonstrated.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04411-11 EEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cancer and Related Conditions in Domestic Animals: Epidemiologic Comparisons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. M. Hayes	Veterinary Medical Officer	EEB	NCI
Others:	R. N. Hoover	Chief	EEB	NCI
	L. W. Pickle	Statistician	EEB	NCI
	B. Sass	Veterinary Medical Officer	OD, DCE	NCI
	K. P. Cantor	Epidemiologist	EEB	NCI

COOPERATING UNITS *(if any)*

Dept. of Vet. Anatomy, Ohio State Univ. (G.P. Wilson, J. Burt); Dept. of Med., Cornell Univ. (B. Tennant)

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Environmental Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The continuing purpose of this project is to identify domestic animal models applicable to further research into the etiology of cancer and related disease in humans. As cases accumulate, it is likely that some types of spontaneous cancers in pet animals can be identified as representing the effects of low-level environmental exposure to carcinogenic agents. The frequency of cancer in these animals would serve as a warning of general environmental hazard(s) to people in the same locale. The topics of current investigation are: 1) environmentally influenced cancer in dogs (e.g., bladder, nasal, and oral cancers); 2) morbidity among pet dogs living in Michigan, potentially exposed to polybrominated biphenyls; 3) the epidemiologic features of prostatic cancer in pet and military working dogs; 4) a case-control study of malignant lymphoma in dogs using owner questionnaires to assess household and yard chemical use; and 5) equine oncology and teratology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04480-11 EEB

PERIOD COVERED

October 1, 1987 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Occupational Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Blair	Chief, Occupational Studies Section	EEB	NCI
OTHERS:	M. Alavanja	Special Assistant	E&BP	NCI
	K. Cantor	Epidemiologist	EEB	NCI
	M. Dosemeci	Visiting Fellow	EEB	NCI
	R. Hayes	Epidemiologist	EEB	NCI
	B. Miller	Epidemiologist	EEB	NCI
	R. Spirtas	Biostatistician	EEB	NCI
	P. Stewart	Industrial Hygienist	EEB	NCI
	T. Thomas	Epidemiologist	EEB	NCI
	S. Zahm	Epidemiologist	EEB	NCI

COOPERATING UNITS *(if any)*

Univ. of NE (D. Weisenberger); Univ. of KS (F. Holmes); U.S. Coast Guard (T. Haas); USDA (J. Teske); U.S. Air Force (S. Birch); NIOSH (H. Amandus, W. Halperin); MO Cancer Control Program (J. Davis)

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Occupational Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
13.0	9.5	3.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Occupational Studies Section supports and conducts epidemiologic studies of occupational groups to identify and clarify the role factors in the workplace play in the origin of cancer. During the past year several studies were completed on cancer risks among workers exposed to pesticides. A mortality study of farmers from Wisconsin noted excesses of cancers of the lymphatic and hematopoietic system, stomach, prostate and eye. Excess deaths from lymphatic cancer were noted among grain workers, particularly those from grain mills where pesticides are used to control insects. A case-control study in Kansas uncovered a striking dose-response between the risk of non-Hodgkin's lymphoma and number of days of use of herbicides, particularly 2,4-D, that rose to over sixfold among farmers with 20 or more days of exposure. A study of industrial workers exposed to formaldehyde uncovered an excess of cancer of the nasopharynx that rose with increasing level of exposure among workers who were also exposed to formaldehyde-containing particulates and a 30% excess of lung cancer that was not associated with level of exposure. Industrial hygiene monitoring for formaldehyde in industry found eight-hour time-weighted averages below 2 ppm in most plants but higher levels occurred in areas where formaldehyde-containing particulates were present. Excesses of leukemia and brain cancer (predominantly gliomas) were seen among anatomists. A case-control study of nasal cancer in the Netherlands found that the well-known excessive risk of adenocarcinoma from exposure to wood dusts (16-fold) did not decrease for at least 15 years after termination of exposure. Annual increases in incidence of mesothelioma between 1973 and 1980 of approximately 12% remained after a histopathologic review by a panel of expert pathologists. A 10-fold excess of astrocytic brain cancer was associated with long-term employment in the electronics industry in a case-control located in Pennsylvania, New Jersey, and Louisiana. Workers producing ceramic plumbing fixtures exposed to talc had over twice the expected mortality from lung cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05128-08 EEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diet and Nutrition in Cancer Etiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Ziegler	Cancer Expert	EEB	NCI
Others:	K. E. Brock	Visiting Fellow	EEB	NCI
	M. H. Schiffman	Clinical Investigator	EEB	NCI
	L. A. Brinton	Chief, ESS	EEB	NCI
	R. N. Hoover	Chief	EEB	NCI
	A. E. Blair	Chief, OSS	EEB	NCI
	T. J. Mason	Chief, PSS	EEB	NCI
	J. F. Fraumeni, Jr.	Associate Director	E&B	NCI

COOPERATING UNITS *(if any)* NCHS (H Barbano); NIA (J Huntly); CA Tum Reg (D Austin); Univ of HI (A Nomura); USC (B Henderson); Kaiser Hlth Plans (A Glass); Walter Reed Army Med Ctr (G Quisp); Beth Naval Hosp (A Robinson); GWU Hosp (L Smith); MN Med Res Cen (S Schwartz); Lipid Nut Unit, USDA (D Nair); LSU (P Corrrea).

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Enviromental Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dietary exposures being assessed in human populations include consumption of specific food groups and food items, such as meat, fruits and vegetables, ethnic dishes, and coffee; macronutrient and micronutrient intake, such as fat, vitamin A, carotenoids, vitamin C, folacin, and trace minerals; general nutritional status; anthropometry; biochemical indices, such as serum cholesterol and serum beta-carotene; and storage and cooking practices. Cancers being studied include those of the colon, rectum, breast, lung, cervix, and larynx. Case-control studies have been initiated in high risk areas with unusually high site-specific cancer mortality, conceivably related to diet, and among migrants whose changing cancer rates appear related to new life-styles, such as Asian-Americans. Analytic case-control studies of specific cancers have assessed nutrition and diet as possible risk factors, and studies of breast cancer and colorectal cancer that are primarily focused on diet have been developed. Selected cohorts with relevant dietary or biochemical data already collected, such as HANES I participants, are being followed for cancer morbidity and mortality. Data from HANES I are being analyzed to test specific hypotheses, and to provide descriptive information on U.S. dietary patterns, diet variation, and determinants of nutrient intake. Laboratory measures of nutritional status are being incorporated into selected case-control studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05319-04 EEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Epidemiologic Studies on Viruses and Genetics in the Etiology of Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Paul H. Levine	Senior Investigator	EEB	NCI
Others:	D. V. Ablashi	Microbiologist	LCMB	NCI
	R. W. Biggar	Senior Investigator	EEB	NCI
	W. A. Blattner	Chief, FSS	EEB	NCI
	R. Gallo	Chief	LTCB	NCI
	M. Robert-Guroff	Senior Investigator	LTCB	NCI
	Z. Salahuddin	Microbiologist	LTCB	NCI
	W. C. Saxinger	Senior Investigator	LTCB	NCI

COOPERATING UNITS *(if any)*

Univ. of Ghana, Accra, Ghana (J. Neequaye, F. Nkrumah); Gorgas Memorial Laboratory, Panama City, Panama (W. Reeves); University of North Carolina, Chapel Hill, N.C. (N. Raab-Traub and J. Pagano)

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Additional evidence for the role of viruses and/or genetics in the etiology of cancer was provided in several studies. Analysis of data including HTLV-I antibody titers on serum samples from more than 42,000 individuals in various geographic locales suggested that in addition to Japan and the Caribbean Islands, HTLV-I or a closely related virus was endemic in Panama, New Guinea and sub-Saharan Africa. Newly identified populations with antibodies reacting against HTLV-I antigens included Indians living in Florida and Panama. The detection of a native born Panamanian Mestizo with HTLV-I-associated adult T-cell leukemia/lymphoma extended our knowledge of people at risk for this disease. The etiologic role of the Epstein-Barr virus (EBV) in nasopharyngeal carcinoma was strengthened by the detection of EBV in biopsies from all American patients entered into a multicenter collaborative study, including all with the more differentiated form (WHO type I) of NPC which had been thought by many not to be EBV-associated. A study of several populations for antibodies to a newly isolated virus (human B-lymphotropic virus or HBLV) from the Laboratory of Tumor Cell Biology, NCI extended our knowledge about its prevalence in the general population and association with several diseases.

Specific findings involving the role of genetics in the etiology of human cancer included: 1) the first report on familial breast cancer in black Americans, 2) the evaluation of a white family with an increased incidence of cancer which included three siblings with NPC, and 3) the identification of a monocyte deficiency in a family with a high frequency of immunologic and hematologic (including acute myelomonocytic leukemia) abnormalities that may be a previously undescribed entity similar to, but distinguishable from, Fanconi's anemia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05400-04 EEB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Epidemiology of Human HBLV Lymphotrophic Viruses: ATL, AIDS and Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W.A. Blattner	Chief, Family Studies Section	EEB	NCI
Others:	R.J. Biggar	Senior Investigator	EEB	NCI
	J.J. Goedert	Coordinator, AIDS Working Group	EEB	NCI
	S.H. Weiss	Medical Staff Fellow	EEB	NCI
	E. Murphy	Medical Staff Fellow	EEB	NCI
	D.L. Mann	Chief, Metabolic Epidemiology Section	LHC	NCI
	R.C. Gallo	Chief	LTCB	NCI
	A. Manns	Biotechnology Fellow	EEB	NCI
	P.H. Levine	Senior Investigator	EEB	NCI
	G. Agius	Guest Researcher	EEB	NCI

COOPERATING UNITS (if any) U.W. Indies, Kingston (W.N. Gibbs); Gorgas Mem. Inst., Panama (W. Reeves); Biotech Labs (A. Bodner); Westat, Inc. (S. Durako); RTI (R. Waddell); Hershey Med. Ctr. (M.E. Eyster); Downstate Med. Ctr. (S. Landesman); NJSDH (R. Altman); NICHD (A. Willoughby); Inst. of Cancer Res., Aarhus, Denmark (M. Melbye)

LAB/BRANCH: Environmental Epidemiology Branch

SECTION: Family Studies Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
8.0	7.0	1.0

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
- [ ] (b) Human tissues
- [ ] (c) Neither
- [X] (a1) Minors
- [X] (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human retroviruses are emerging as etiologic agents of human malignancies. Human T-Lymphotropic Virus Type I (HTLV-I) is linked to adult T-cell leukemia (ATL). Human immunodeficiency virus, (HIV, formerly HTLV-III/LAV) the etiologic agent of the acquired immunodeficiency syndrome (AIDS), is associated with Kaposi's sarcoma and certain forms of Hodgkin's and non-Hodgkin's lymphoma. Our research is focused on characterizing the relationship of this class of virus to human malignancy. Results of our studies document the spectrum of ATL and modes of spread of HTLV-I by heterosexual and homosexual contact and suggest early life transmission in the household. An indirect etiologic mechanism of carcinogenesis is also suggested for HTLV-I in B-cell chronic lymphocytic leukemia (B-CLL), and for HIV in studies of Hodgkin's and non-Hodgkin's lymphoma and Kaposi's sarcoma. A major focus of HIV research has been on cohorts at high-risk for AIDS followed longitudinally since the very beginning of the AIDS epidemic. Results of studies have documented major modes of transmission of HIV in homosexual men, in hemophiliacs, and in drug users and their heterosexual partners, and from mother to offspring. The natural history of progression, the predictors of risk, and the incidence of various outcomes have been defined. Low T-helper cell counts are predictive of AIDS risk and may contribute to heightened transmission of HIV. Among various cofactors, an immunogenetic marker appears to be associated with heightened AIDS risk. Studies are ongoing to utilize epidemiologic approaches to search for persons infected with related viruses, as well as to support work evaluating the human B-cell lymphotrophic virus (HBLV).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05526-01 EEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analytical Investigations of Selected Issues in Human Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. A. Brinton	Chief, Environmental Studies	EEB	NCI
Others:	J. F. Fraumeni, Jr.	Associate Director	E&B	NCI
	R. N. Hoover	Chief	EEB	NCI
	K. P. Cantor	Epidemiologist	EEB	NCI
	P. Hartge	Epidemiologist	EEB	NCI
	M. H. Schiffman	Clinical Investigator	EEB	NCI

COOPERATING UNITS *(if any)* 28 BCDDPs; Hutzel Hosp (J Wolfe); GW Univ (L McGowan); 5 Comp Can Ctrs; Gorgas Mem Lab (W Reeves); Georgetown Univ (R Kurman); NY Health Dept (P Nasca); IL Cancer Council (K Mallin); 3 Kaiser Med Ctrs (A Glass, G Friedman, W Finkle); Mayo Clinic (J Melton); 10 SEER Ctrs; Chin Acad Med Sci (J-Y Li).

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Environmental Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	6.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to investigate, in analytic studies, the etiologies of selected cancers. Specific cancer sites and hypotheses are selected for which the need for investigation is clear but which have been difficult to study elsewhere. Studies focus either on tumors that have not been studied analytically before (e.g., because of the rarity of the tumor) or on hypotheses that are difficult to assess (e.g., because of the prevalence of the exposure or the need to detect an effect at low levels of exposure). Since these studies are often the first or most through to date, they collect data on a wide range of exposures, usually through interviews and medical records. A major emphasis within this project area has been on defining the etiology of female tumors. In many of these studies, as well as in selected others, attempts have been made to assess, more precisely, exposures through interdisciplinary approaches.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04481-11 REB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Radiation-Induced Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. D. Boice, Jr.	Chief	REB	NCI
Others:	C. E. Land	Health Statistician	REB	NCI
	G. W. Beebe	Health Statistician	CEB	NCI
	Z. Hrubec	Expert Statistician	REB	NCI
	R. A. Kleinerman	Epidemiologist	REB	NCI
	E. Ron	Visiting Associate	REB	NCI
	M. Blettner	Expert Statistician	REB	NCI

COOPERATING UNITS *(if any)*
Radiation Effects Research Foundation, Japan (H. Kato); Department of Energy (R. Goldsmith); Chaim Sheba Medical Center, Israel (B. Modan); Tufts University (M. Kaplan); Harvard University (G. Hutchison)

LAB/BRANCH
Radiation Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
11.0	8.0	3.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project (1) examines cancer incidence and mortality among populations exposed to ionizing radiation, especially at low dose levels; (2) characterizes the risk of radiation-induced cancer in terms of tissues at risk, dose response, radiation quality, fractionation of dose, time since exposure, sex, age at exposure and at observation, and possible modifying influences of other environmental and host factors; and (3) examines, tests, and formulates models of radiation carcinogenesis to help define basic mechanisms. Groups studied include the Japanese A-bomb survivors, and several large populations with documented therapeutic (e.g., cervical cancer patients), diagnostic (e.g., tuberculosis patients), and occupational (e.g., x-ray technologists) exposures to ionizing radiation. Program members serve on committees advising the government as well as international agencies.

Results of studies suggest that (1) susceptibility to radiogenic breast cancer declines with increasing age at exposure, and children exposed under age 10 are at high risk; a risk at 8-16 rads has been detected; (2) high-dose radiation to the pelvis induces fewer leukemias than other types of exposures; cell-killing appears to play an important role in defining dose-response relationships; (3) repeated exposure to relatively low radiation doses poses some future risk of breast and thyroid cancer, but not lung cancer; (4) children irradiated for benign conditions of the head and neck are at risk of developing thyroid and brain neoplasia; (5) 9% of all thyroid cancers may be attributed to prior childhood irradiation; (6) radiotherapy for childhood cancer was associated with subsequent cancers of the bone, connective tissue and thyroid, but not leukemia; (7) actinomycin-D does not appear to protect against radiation-induced thyroid cancer; (8) radiation of the adrenal glands may lower breast cancer risk; (9) chromosome aberrations following partial-body irradiation persist in circulating lymphocytes for over 30 years.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05368-04 REB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Drug-Induced Cancer and Multiple Primary Cancers

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. D. Boice, Jr.	Chief	REB	NCI
Others:	R. E. Curtis	Statistician	REB	NCI
	R. A. Kleinerman	Epidemiologist	REB	NCI
	M. A. Tucker	Clinical Investigator	EEB	NCI

COOPERATING UNITS *(if any)*

Danish Cancer Registry (O. Jensen); Connecticut Tumor Registry (J. Flannery); Harvard Medical School (W. Moloney, H. Lisco); Tufts University (M. Kaplan)

LAB/BRANCH

Radiation Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to study the long-term health effects of drugs, especially therapeutic agents, as they may relate to carcinogenicity. In addition, the patterns of occurrence of multiple primary cancers are evaluated in terms of implications for etiologic research. Because many studies of radiation carcinogenesis involve the evaluation of second cancers following radiotherapy for a primary cancer, it is often convenient to evaluate, simultaneously, the effects of chemotherapeutic agents. Populations studied include patients treated in randomized clinical trials, patients reported to cancer registries in the United States and other countries, and patients treated at several large institutions. Additional details can be found in Project No. Z01CP04412-11 EEB, "Carcinogenic Effects of Therapeutic Drugs" and Project No. Z01CP04410-11 EEB, "Studies of Persons at High Risk of Cancer." In addition to the systematic study of therapeutic drugs, occasionally it is possible to evaluate other drug exposures in populations studied primarily for other reasons.

A study of patients given Semustine (methyl-CCNU) as adjuvant therapy for gastrointestinal cancer provided clear dose-response evidence that nitrosoureas are leukemogenic in man. Alkylating agents to treat childhood cancer were associated with an increased risk of leukemia and bone cancer. Women with breast cancer who received chemotherapy are at an increased risk of leukemia. Among ovarian cancer patients, treatment with melphalan appears three times more leukemogenic than with cyclophosphamide. Commonly used drugs were not found to be related to thyroid cancer. Cancer patients have a 31% increased risk of developing a second primary; 49% among 30-year survivors. Smoking may be causally related to cervical cancer. Alcohol may cause breast cancer.