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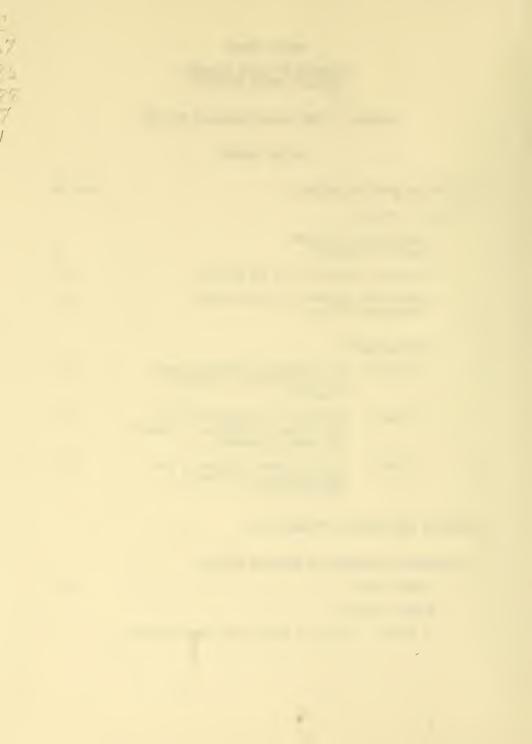
ANNUAL REPORT

DIVISION OF CANCER ETIOLOGY NATIONAL CANCER INSTITUTE

October 1, 1986 through September 30, 1987

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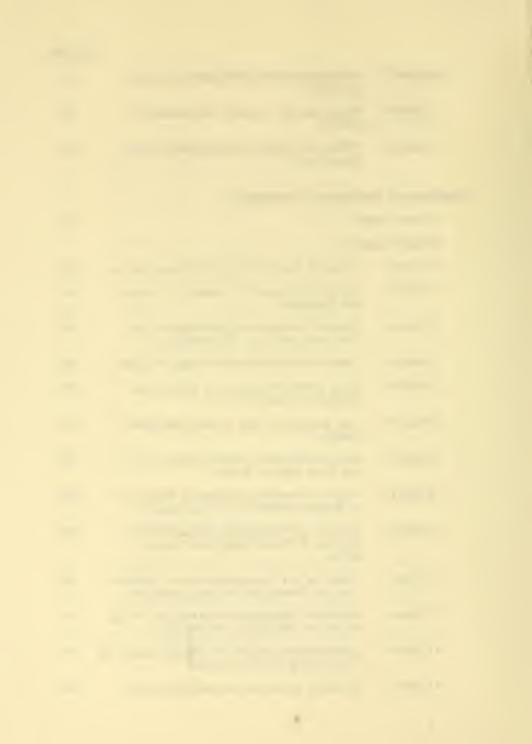
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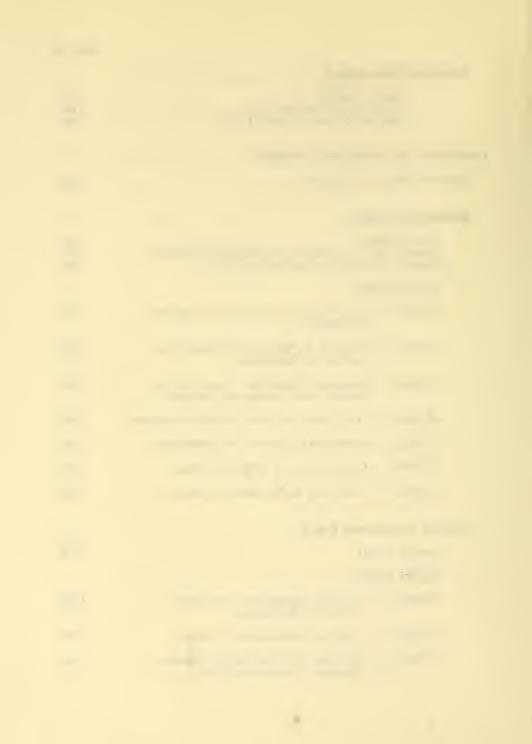
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PROJECT NUMBER

Z01CP03509-24 0D

October 1, 1986 to Sept	tember 30, 1	987				
TITLE OF PROJECT (80 characters or less. Title must lit on one line between the borders.) Carcinogenesis, Chemotherapy and Biological Markers in Nonhuman Primates						
PRINCIPAL INVESTIGATOR (List other pro	ofessional personnel be	low the Principal Inves	tigator.) (Name, title, laboratory	r, and institute affiliation)		
PI: S. M. Sieber	r	Deputy Direc	ctor	OD, DCE	NCI	
Others: R. J. Parker		Expert		OD, DCE	NCI	
COOPERATING UNITS (if any)						
Department of Pathology	/ Louisiana	State Univer	esity Now Orloan	os IA (D. Cons		
Hazleton Laboratories A	merica, Inc.	,, Vienna, VA	(D. Dalgard)	is, th (P. Corr	ea);	
LAB/BRANCH Division of Cancer Etic	vnolo					
SECTION	,109,					
Office of the Director						
NCI, NIH, Bethesda, Mar	yland 20892	2				
TOTAL MAN-YEARS:	PROFESSIONAL:		OTHER:			
CHECK APPROPRIATE BOX(ES)						
(a) Human subjects (a1) Minors	☐ (b) Human	tissues 🖾	(c) Neither			
(a2) Interviews						
SUMMARY OF WORK (Use standard unred						
A wide variety of subst food additives, food co	mnonents and	l onvironment	or and antineopi	astic agents;		
carcinogens; and nitros	n- compounds	: have been o	r are being eval	noder roden	L	
species of nonhuman pri	mates for th	eir potentia	1 carcinogenicit	v and other		
long-term toxic effects	. Of the 29	test compou	nds, 16 have not	as vet		
demonstrated carcinogen	ic activity,	although so	me have been on	test for less		
than 4 years. Nine of	the compound	ls are carcin	ogenic in nonhum	an primates,		
producing tumors in 10- (MNU) induced squamous	cell carcine	treated anim	ais. 1-methyi-1	-nitrosourea		
the esophageal tumors p	ossessina cl	inical and m	orphologic simil	arities to		
human esophageal carcin	oma. Long-t	erm treatmen	t with procarbaz	ine produced		
malignant neoplasms, on	e-half of wh	ich were acu	te nonlymphocyti	c leukemia. T	he	
effects of seven of the	compounds (diethylnitro	samine [DENA], d	ipropyl-		
nitrosamine [DPNA], 1-nitrosopiperidine, aflatoxin B-1, MAM-acetate, urethane						

40.0000

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).



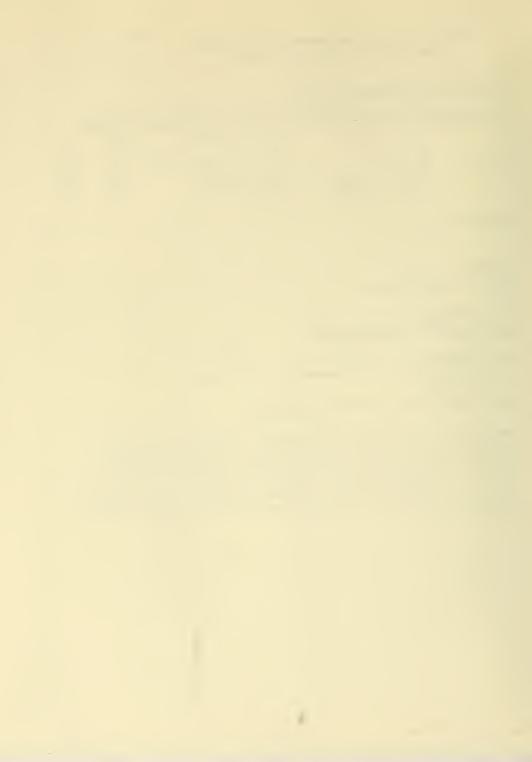
PROJECT NUMBER

701CP04548-15 OD

PERIOD COVERED							
October 1, 19	October 1, 1986 to September 30, 1987						
		Title must fit on one line bet					
Registry of Ex	Registry of Experimental Cancers/WHO Collab. Ctr. for Tumours of Lab Animals						
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Neme, title, laboratory, and institute affiliation)							
P.I.:	Harold L.	Stewart	Scientis	st Emeritus		DCE	NCI
Others:	Carel F.		Guest Re		Officer	DCE DCE DCE DCE	NCI NCI NCI
COOPERATING UNITS (if any)							
LAB/BRANCH							
Office of the	Director						
SECTION SECTION							
INSTITUTE AND LOCATE							
NIH, NCI, Beth	nesda, Mar						
TOTAL MAN-YEARS:		PROFESSIONAL:		OTHER:			
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(a) Human su		(b) Human tissu	es 🗓	(c) Neither			
(a1) Minor							
(a2) Interv							
SUMMARY OF WORK (II)	IMMARY OF WORK (Use standard unreduced type Do not exceed the space proyided)						

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.

OF A PRIME

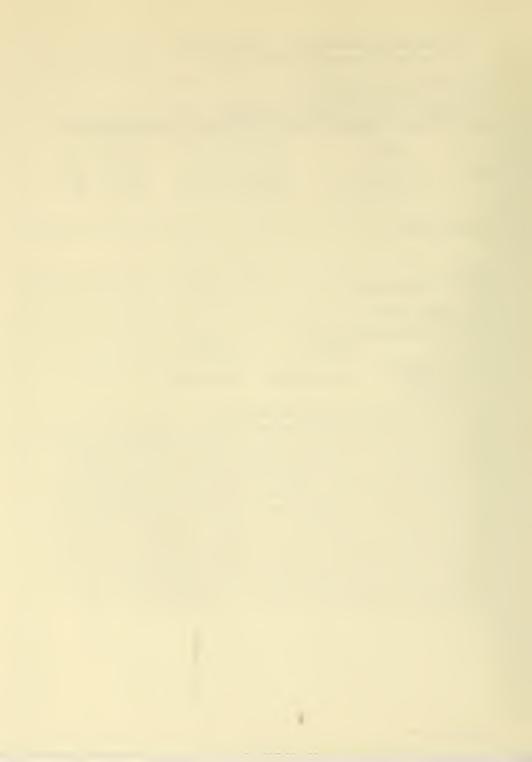


PROJECT NUMBER

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	NOTICE	OF INT	RAMURAL RES	EARCH PE	ROJECT	Z01CP06	5134-12 OD
PERIOD COVER				_			
			mber 30, 198				
			Title must fit on one lin				
					nd Distribution		
PRINCIPAL INV	ESTIGATOR (List other prof	essional personnal belo	w the Principal	Investigator.) (Name, title, la	boratory, and institu	ute affiliation)
PI:	S. M.	Sieber		Deputy	Director	OD, DCE	NC I
Others:	R. J. J. N.	Parker Weinste	in	Expert Senior	Investigator	OD, DCE LMB	NCI NCI
COOPERATING	UNITS (if any,	,					
LAB/BRANCH							
Division o	of Cance	r Etiol	ogy				
SECTION							
Office of		ector					
INSTITUTE AND							
NCI, NIH,	Bethesd	a, Mary	land 20892				
TOTAL MAN-YE	ARS:		PROFESSIONAL:		OTHER:		
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	nan subjec Minors	cts	(b) Human t	issues	□ (c) Neither		
, ,	Interview						
			uced type. Do not exce				
The role	of the 1	ymphati	c system in	the abso	ption and biodi	stribution	of antitumo
agents and	d monocl	onal an	tibodies is i	under in	vestigation. Ar	ititumor ag	gents are
delivered	at high	concen	tration to 1	ymphatics	and regional	lymph nodes	when
are deliv	ered wit	h high	efficiency t	o regiona	al antibodies gi al lymph nodes v	where they	bind
specifica	11y to 1	ymphoid	cells. Ext	ensive me	etabolic and pha	rmacokinet	ic 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.

HE ASSET



PROJECT NUMBER

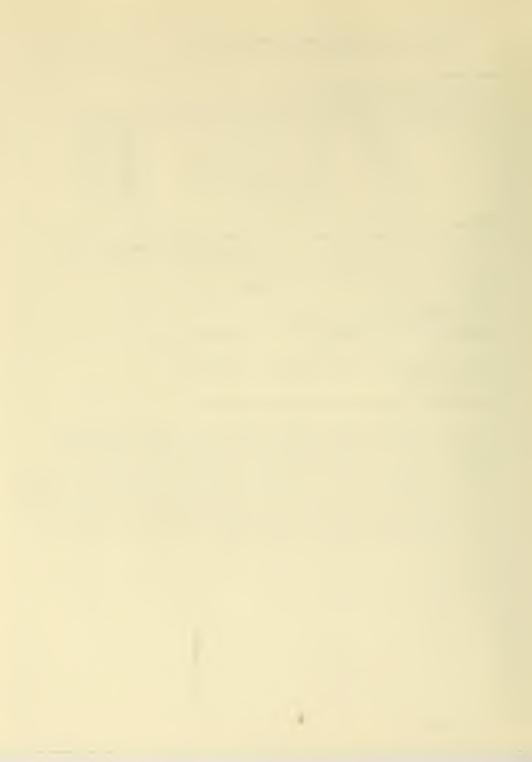
Z01CP04930-16 LCMB

PERIOD COVERED							
October 1, 1986 to September 30, 1987							
TITLE OF PROJECT (80 characters or less		*					
Biology of Natural ar	Biology of Natural and Induced Neoplasia						
PRINCIPAL INVESTIGATOR (List other pro	PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigetor.) (Name, title, laboratory, and institute effiliation)						
PI: P. Arnsteir		rector	LCMB	NCI			
Others: S. A. Aaror	ison Chief		LCMB	NCI			
J. S. Rhim	Research Micr	obiologist	LCMB	NCI			
K. C. Robbi	ins Chief, Mol. 6	enetics Section	LCMB	NCI			
J. Pierce				NCI			
A. Eva		entist	LCMB	NCI			
W. Taylor	Research Biol	ogist	LCMB	NCI			
		J					
COOPERATING UNITS (if any)							
J. Riggs and R. Emmor	is, CA Dept. Health	Services, Berkel	ey, CA; A.	Hackett,			
Peralta Cancer Inst.;							
San Francisco; and K.	. Walen, Children's	Hospital Medical	Ctr., San	Francisco.			
LAB/BRANCH							
Laboratory of Cellula	ar and Molecular Bio	ology					
SECTION							
Office of the Chief							
INSTITUTE AND LOCATION							
NCI, NIH, Bethesda, N	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 \underline{sis} , \underline{erbB} , and \overline{IGF} α are continuing. Two newly integrated oncogenes, \underline{mac} and \underline{dbl} , are also under intensive experimental analysis. Viral constructs containing the above genes inoculated into newborn mice result in distinct patterns of tumorigenicity. The \underline{sis} -containing viruses are uniformly sarcomagenic; \underline{erbB} -containing viruses tend to be more pleiomorphic in their carcinogenesis and induce hepatocellular carcinomas as well as sarcomas; \underline{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.

pit welpter



PROJECT NUMBER

Z01CP04940-20 LCMB

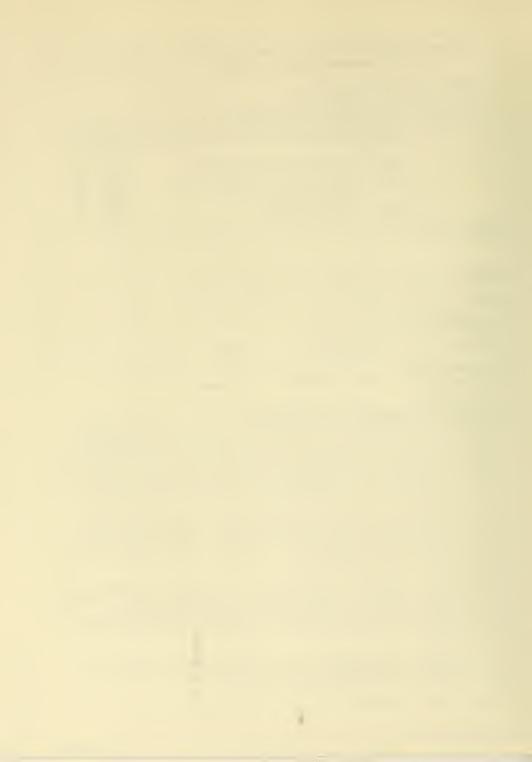
NOTICE OF INTE	MONAL RESEARCH PHOJE	EC1	
October 1, 1986 to Sep	tember 30, 1987		
TITLE OF PROJECT (80 characters or less. Viruses and Iransformi	Title must fit on one line between the border ng Genes in Experimenta	1 Oncogenesis	and Human Cancer
PRINCIPAL INVESTIGATOR (List other profes	ssional personnel below the Principal Invest IN Chief	igator.) (Name, title, labora	lory, and institute affiliation I
Others: S. R. Tronick K. C. Robbins J. H Pierce A. Eva J. C. Lacal M. H. Kraus		etics Section	LCMB NCI
COOPERATING UNITS (if any)			
LABBRANCH Laboratory of Cellular	and Molecular Biology		
SECTION Molecular Biology Sect	ion		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Ma	ryland 20892		
TOTAL MAN-YEARS: 4.0	PROFESSIONAL:	OTHER:	3.0
CHECK APPROPRIATE BOX(ES) (a) Human subjects (a1) Minors (a2) Interviews	(b) Human tissues	(c) Neither	
occurring malignancies genes of retroviruses viruses; (3) the molec and (4) the applicatio for the causes and med During the past year, new human oncogenes, a structure and function	ne the cellular alterat . Topics of present in and cancer cells; (2) t ular biology of retrovi n of knowledge gained f hanisms involved in hum we have isolated and pand have gained importan of the sis and ras once xploitation of two animes.	ions responsib terest include: he biology of e rus replication rom these studi an neoplastic t rtially charact t new insights ogenes. We hav	le for naturally (1) transforming endogenous retro- n and transformation; ies to the search transformation. terized a number of regarding the we also made impor-
carcinoma; <u>dbl</u> , isolat of the tyrosine kinase	nes include the erbB-2 ed in a human diffuse B family closely related ming growth factor alph	cell lymphoma; to but distinc	and <u>arg</u> , a member t from c- <u>abl</u> . Anothe

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.

was characterized as having growth promoting potential but not to be a direct-

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

acting oncogene.



PROJECT NUMBER

DEFANIMENT OF REALITY A	IND HUMAN SERVICES . PUBLIC H	ALTH SERVICE		
NOTICE OF INT	JECT	Z01CP04941-15 LCMB		
PERIOD COVERED				
October 1, 1986 to Se	eptember 30, 1987			
TITLE OF PROJECT (80 characters or less	. Title must fit on one line between the bon	ters.)		
Biochemical Character	ization of Retroviruses	and onc Genes		
PRINCIPAL INVESTIGATOR (List other pro	fessional personnel below the Principal Inve	stigetor.) (Name, title, labora	tory, and institute affiliation)	
PI: S. R. Troni Others: S. A. Aaron		ture Section	LCMB NCI	
K. C. Robbi	ns Chief, Molecular	Genetics Section	n LCMB NCI	
J. E. Dahlb	erg Research Microbio	logist	LCMB NCI	
A. Eva	Visiting Scientis	t	LCMB NCI	
T. Kawakami	Visiting Associat	e	LCMB NCI	
S. Katamine	S. Katamine Guest Researcher			
D. Ron	Visiting Fellow		LCMB NCI	
COOPERATING UNITS (# any) Sackler School of Med	licine, Tel Aviv, Israel	(A. Yaniv)		
LAB/BRANCH				
	ır and Molecular Biology			
SECTION Gene Structure Section	n			
INSTITUTE AND LOCATION				
NCI, NIH, Bethesda, M				
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:		
4.0	1.0	3.0		
CHECK APPROPRIATE BOX(ES)	[V] (b) Human tinguas	7 (a) Maither		
	(b) Human tissues	(c) Neither		
(a1) Minors (a2) Interviews				
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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 protooncogene.

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.



NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04951-11 LCMB

October 1, 1986 to September 30, 1987								
TITLE OF PROJECT (80 characters or lass. Title must fit on one lina between the borders.)								
Molecular Characteriza	Molecular Characterization of Retroviruses							
PRINCIPAL INVESTIGATOR (List other professional personnal below the Principal Investigator.) (Name, title, leboratory, and institute effiliation)								
PI: J. E. Dahlber	`g	Research	Microbiologist	LCMB	NCI			
Others: S. A. Aaronso	on	Chief		LCMB	NCI			
S. R. Tronick	(Chief, Ge	ne Structure Section	LCMB	NCI			
M. Wang		Visiting	Fellow	LCMB	NCI			
T. Kawakami		Visiting	Fellow	LCMB	NCI			
D. Archambaul	lt	Guest Res	earcher	LCMB	NCI			
J. Hallum		Guest Res	earcher	LCMB	NCI			
S. Broder		Chief		COP	NCI			
COOPERATING UNITS (if any)								
Tel Aviv University (A	N. Yaniv); Hebre	w Univers	ity (K. Perk); Dept. F	atholo	gy,			
Colorado State Univers	sity, Fort Colli	ns (J. De	Martini); Dept. Microb	iology	,			
Pathology and Parasito	ology, North Car	olina Sta	te University (L. Cogo	jins).				
LAB/BRANCH								
Laboratory of Cellular	and Molecular	Biology						
SECTION .								
Molecular Genetics Sec	ction							
INSTITUTE AND LOCATION								
NCI, NIH, Bethesda, Ma								
TOTAL MAN-YEARS:	PROFESSIONAL:		OTHER:					
3.0	2.0		1.0					
CHECK APPROPRIATE BOX(ES)		_						
(a) Human subjects	(b) Human tissu	ies 🗀	(c) Neither					
(a1) Minors								
(a2) Interviews								

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

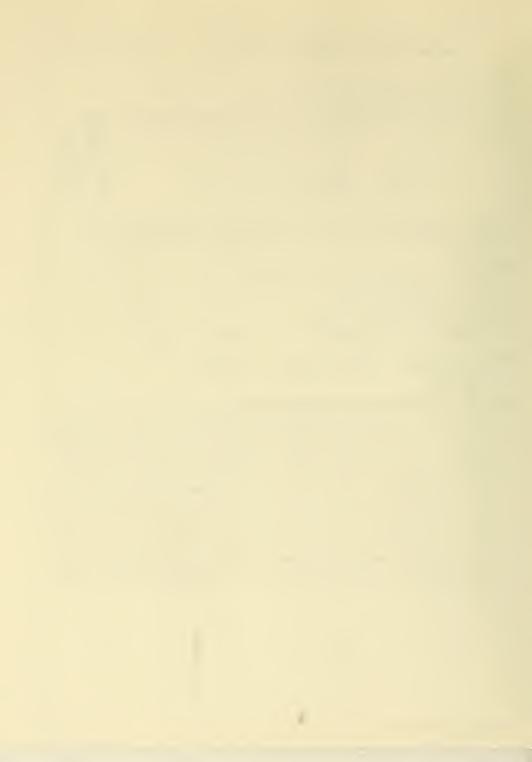
Lentiviruses of sheep, goats, and primates are a genetically distinct group of retroviruses that replicate in immune cells and usually cause disease with a long latent period and slowly progressive course, often leading to death. In order to understand how these viruses interact with their hosts and 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 <u>tat</u>, <u>art</u>, and <u>sor</u>, 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.

PERIOD COVERED



PROJECT NUMBER

NOTICE OF INT	RAMURAL RESEARCH PROJ	ECT	Z01CP04976-10	LCMB
PERIOD COVERED October 1, 1986 to Sep	otember 30, 1987			
TITLE OF PROJECT (80 characters or less Carcinogenesis of Mamm	Title must fit on one line between the bordenalian Cells in Culture	ors.)		
Others: S. Takai J. S. Rhim M. Potter K. H. Kraemer R. E. Tarone M. A. Tucker	Mathematical Statis Oncologist	ogist stician	LCMB LCMB LG LMC BB EEB	NCI NCI NCI NCI NCI NCI NCI
HOWARD UNITS (# any) Howard U. College Med. Benedict); Tel Aviv U. (R. Knight).	(R. Parshad); Childrens (Y. Shiloh); U. NC (M.	Hosp. of Los Swift); Walte	Angeles (W. E. r Reed Dept. Me	d.
	and Molecular Biology			
SECTION In Vitro Carcinogenesi				
NCI, NIH, Bethesda, Ma				
TOTAL MAN-YEARS: 4.0	PROFESSIONAL:	OTHER:	2.0	
CHECK APPROPRIATE BOX(ES) (a) Human subjects (a1) Minors (a2) Interviews		(c) Neither		
prone individuals, as are utilized in evalua DNA damage, deficient transformation. An in during the G-2 phase of to cancer and malignan cancer susceptibility, tion of genes to specificel hybrids, inbred some radiosensitivity appears aspect of this project human epidermal keratiof biologic and bioche associated problem is	blasts and peripheral in well as neoplastic cells well as neoplastic cells ting the relationship be DNA repair, cancer suscence as a circased incidence of chart the cell cycle is assist transformation and can a genetic basis for the fic chromosomes is indicated in the cell cycle is assisted to the cell cycle is assisted transformation and can a genetic basis for the fic chromosomes is indicated in the cell cycle and concern to result from deficit is to develop a reproduct on the cell cycle as an in vitro of the cell cycle as an invitro of the cell cycle as a cell cycl	s transformed atween radiatic ptibility and romatid damage ociated with be no rowide the nis radiosensicated from stugenic mouse stient DNA repaiucible transfomodel for foll o neoplastic te cytomorpholo	in culture or i on-induced chroid malignant neop after x-irradiated a predispose basis of a test tivity with locations. The chror during G-2. The chroid matter ains a programs of the programs of the changes of the changes of the control of the control of the changes of the ch	n vivo, mosomal lastic ation ition for aliza- ic omosoma Another with ession An gnos-
	: gen-q-elegiph-			



PROJECT NUMBER

Z01CP05060-09 LCMB

NOTICE OF INTINAN	MONAL NESEANON PHOSE				
PERIOD COVERED 1, 1986 to September 30, 1987					
Studies on Uncogenic Trans	must fit on one line between the border Sformation in Culture	rs.)			
Others: S. A. Aaronson, J. B. Park		transport (Name, title, laboratory, and institute Advilletion). LCMB NCI			
COOPERATING UNITS (# any) Georgetown University (A. Dritschilo), Washington, D.C.					
Labbratory of Cellular and	d Molecular Biology				
SECTION Office of the Chief					
NCI, NIH, Bethesda, Maryla					
TOTAL MAN-YEARS: 1.5	FESSIONAL: 1.5	OTHER: 0.0			
CHECK APPROPRIATE BOX(ES) (a) Human subjects (a1) Minors (a2) Interviews	(b) Human tissues	(c) Neither			
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					

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.

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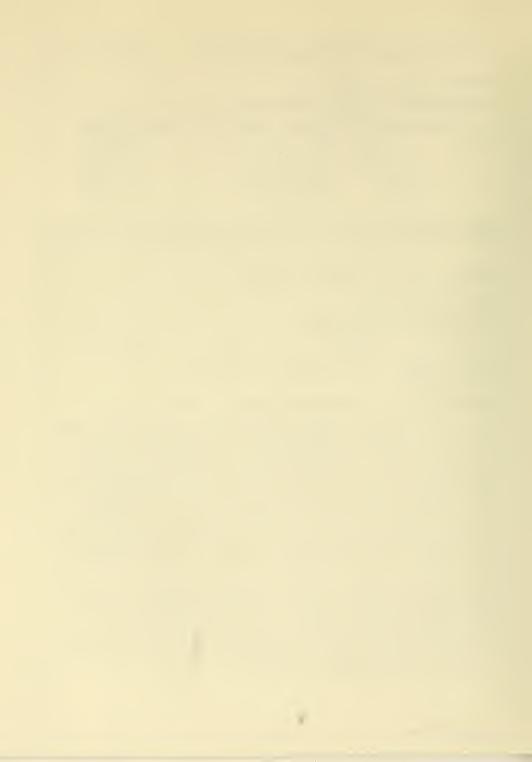
PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT			Z01CP05062-09 LCMB			
PERIOD COVERED, 1986 to September 30, 1987						
TITLE OF PROJECT.(80 characters or less file must lik on one line between the borders themically-Induced Tumors						
Others:	S. A. Aar S. R. Tro S. K. Sri D. Ron L. Varesi J. Ward	onson Chief nick Chief vastava Visit Visit O Visit Chief	Gene Struing Fellow ing Fellow ing Fellow ing Scienti		LCMB LCMB LCMB LCMB LMI LCC	NCI NCI NCI NCI NCI NCI
COOREGATING UNITS (If early Triangle Park, NC (M. Anderson); Dana-Farber Cancer Institute, NIEHS, Research Triangle Park, NC (M. Anderson); Dana-Farber Cancer Institute, Boston, MA (G. M. Cooper); Baylor College of Medicine, Houston, TX (P. Overbeek)						
Laboratory of Cellular and Molecular Biology						
SECTION Molecular Biology Section						
NCI, NIH, Bethesda, Maryland 20892						
TOTAL MAN-YEARS:		PROFESSIONAL:	1.0	OTHER:	0.5	
(a) Human si	ubjects ers	☑ (b) Human t	issu es \square	(c) Neither		

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.



PROJECT NUMBER

нот	CE OF INTRAMURAL RES	EARCH PROJECT	Z01CP05063-09	LCMB	
PERIOD COVERED					
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	characters or less. Title must fit on one lin	The state of the s			
	Epstein-Barr Virus and				
		ow the Principal Investigator.) (Neme, title, labora			
PI:	D. V. Ablashi	Research Microbiologist	LCMB	NCI	
0+60-00	S 7 Salahuddin	Event	LTCD	NCT	
Others:	S. Z. Salahuddin R. C. Gallo	Expert Chief	LTCB	NCI	
		Chemist.	LTCB	NCI	
	S. Joseph F. Wong-Staal		LTCB LTCB	NCI NCI	
	C. Saxinger		LTCB	NCI	
	C. Saxinger	Research Microbiologist	LIUD	NC1	
COOPERATING UNITS (#	any)				
M. Kaplan.	North Shore University	Hospital, Long Island, N	Y: P. D. Markhan	n.	
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					
	of Cellular and Molecu	ılar Biology			
SECTION					
Office of t	he Chief				
INSTITUTE AND LOCATIO					
NCI, NIH, Bethesda, Maryland 20982					
TOTAL MAN-YEARS:	PRÖFESSIONAL:	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.)					
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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.

क्षा कश्चारत



PROJECT NUMBER

1 CMR

NCI

NOTICE OF INTRAMURAL RESEARCH PROJECT Z01CP05164-07 LCMB

Visiting Associate

PERIOD COVERED

PI:

October 1, 1986 to September 30, 1987

J. H. Pierce

M. Kraus

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)

Research Microbiologist LCMB NCI Others: S. A. Aaronson Chief I CMR NCT P. Di Fiore Visiting Associate LCMB NCI J. Falco Medical Staff Fellow LCMB NCT

COOPERATING UNITS III ARVI

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

Laboratory of Cellular and Molecular Biology SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Betheda, Maryland 20892

TOTAL MAN-YEARS PROFESSIONAL:

2.0 1.0 1.0 CHECK APPROPRIATE BOX(ES)

(a) Human subjects

(a1) Minors

(b) Human tissues (c) Neither

OTHER:

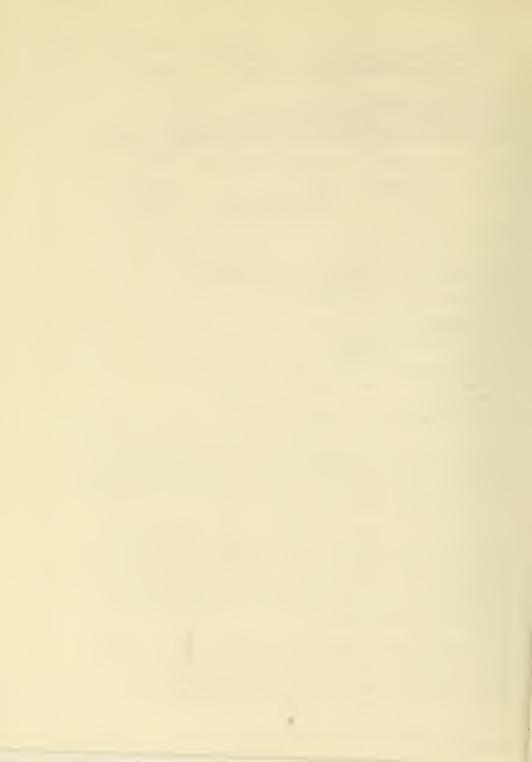
(a2) Interviews

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. erb8-2 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.



PROJECT NUMBER

NOTICE OF IN	Z01CP05167-07 LCMB				
October 1, 1986 to Se	ptember 30, 1987				
TITLE OF PROJECT (80 characters or les Mechanisms of Transfo	s. Title must lit on one line between the border rmation Induced by the <u>si</u>	s.) S Gene			
PRINCIPAL INVESTIGATOR (List other pri	ofessional personnel below the Principal Invest	igator.) (Name, title, leborat	ory, and institute effillation	on)	
PI: K. C. Robbin Others: S. A. Aarons S. R. Tronic T. Miki N. Giese T. Fleming COOPERATING UNITS (H emy)	on Chief		LCMB LCMB LCMB LCMB LCMB LCMB	NCI NCI NCI NCI NCI	
LABUBRANCH Laboratory of Cellular and Molecular Biology					
SECTION Molecular Genetics Se	ction				
NSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892					
TOTAL MAN-YEARS: 2.0	PROFESSIONAL:	OTHER:	. 5		

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

(b) Human tissues

Our previous studies have demonstrated the importance of human $\underline{sis}/\text{PDGF-2}$ 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 $\underline{sis}/\text{PDGF-2}$ mRNA and mitogenically active $\underline{sis}/\text{PDGF-2}$ homodimers. Utilizing $\overline{\text{cDNA}}$ cloning, S1 nuclease mapping, and primer extension techniques, the normal human $\underline{sis}/\text{PDGF-2}$ transcriptional unit has been defined. These studies also suggested the presence of transcriptional and translational regulatory signals within the $\underline{sis}/\text{PDGF-2}$ locus, and have provided an approach for elucidating mechanisms by which this gene is controlled.

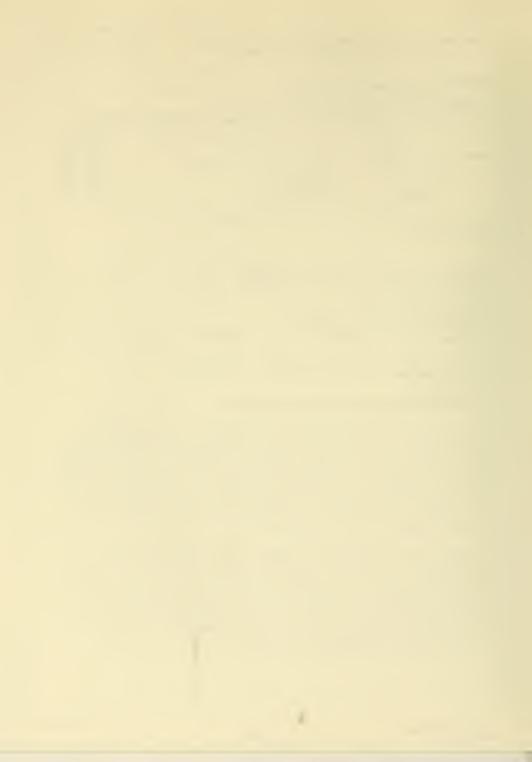
(c) Neither

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.

CHECK APPROPRIATE BOX(ES)

(a) Human subjects

(a1) Minors
(a2) Interviews



PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05362-04 LCMR

PERIOD COVERED

PI:

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) Medical Staff Fellow

J. P. Falco

Chief Others: S. A. Aaronson

Research Biologist

I CMB NCT LCMB NCT

LCMB

W. G. Taylor P. P. Di Fiore

Visiting Associate

LCMB NCI

NCI

COOPERATING UNITS (if env)

Childrens Hospital of Los Angeles (Dr. Bernard Weissman).

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

PROFESSIONAL: TOTAL MAN-YEARS:

1.0

0.0

CHECK APPROPRIATE BOX(ES)

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

OTHER:

(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 (y-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 a. 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.



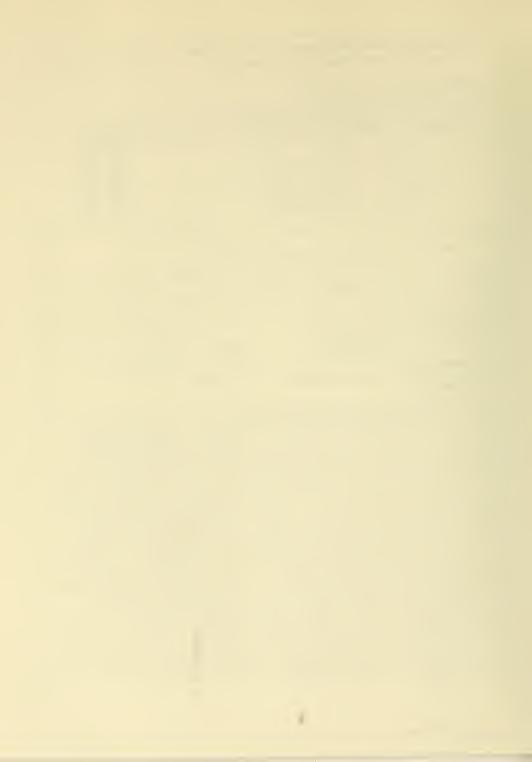
PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05366-04 LCMB

PERIOD COVER						
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		cogenes Encoding				
		fessional personnel below the		gator.) (Name, title		
	M. H. Kraus	Visiting As	sociate		LCMB	NCI
Others:	S. A. Aaronson				LCMB	NCI
	P. P. Di Fiore	3			LCMB	NCI
	J. H. Pierce	Research Mi		ist	LCMB	NCI
	O. Segatto	Visiting Fe			LCMB	NCI
	H. Lacroix	Guest Resea			LCMB	NCI
	N. C. Popescu	Microbiolog	jist.		LB	NCI
COOPERATING	UNITS (if any)					
Meloy, Lal	boratories, Roo	kville, Marylan	id (C. R.	King)		
LAB/BRANCH						
Laborato	ry of Cellular	and Molecular B	Siology			
SECTION						<u> </u>
Molecula	r Biology Sect	on				
INSTITUTE AND						
NCI, NIH	, Bethesda, Mar	'yland 20892				
TOTAL MAN-YE		PROFESSIONAL:		OTHER:		
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stringency hybridization conditions with v-erbB as probe. 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 erb8-2 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, 4to 8-fold <u>erbB-2</u> 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 <u>erbB-2</u> 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.



PROJECT NUMBER

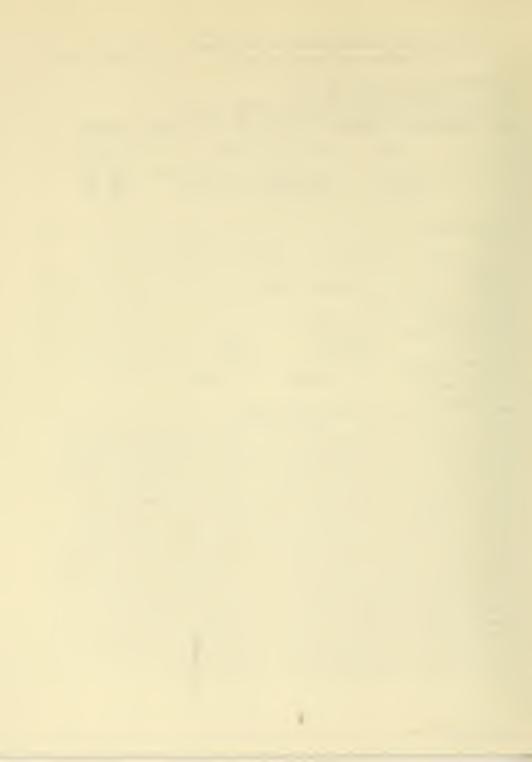
Z01CP05456-03 LCMB

	NOTICE OF INT	RAMURAL RESEARCH	PROJEC	T	2016205450	-U3 LCMB
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		by Viral and Cell				
PRINCIPAL INVES	STIGATOR (List other pro	ofessional personnel below the Prin	cipal Investige	ator.) (Neme, title, laborate	ory, and institute ef	filletion)
PI:	M. S. C. Che	ah Medical St	aff Fell	OW	LCMB	NCI
Others:	K. C. Robbin S. R. Tronic S. Katamine		e Struct	enetics Secti cure Section	on LCMB LCMB LCMB	NCI NCI NCI
(T. J. Le	of Hematology	/Oncology, Univive	rsity of	Washington,	St. Louis,	MO
Laborator Laborator	y of Cellular	and Molecular Bio	logy			
section Molecular	Biology Sect	ion				
NCI, NIH,	Bethesda, Ma	ryland 20892				
TOTAL MAN-YEAR	ns: 1.0	PROFESSIONAL: 1.0	C	O.0		
CHECK APPROPE (a) Huma	an subjects	☑ (b) Human tissues	(c) Neither		

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

(a2) Interviews

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 protooncogene in some facet of granulocytic maturation or function.

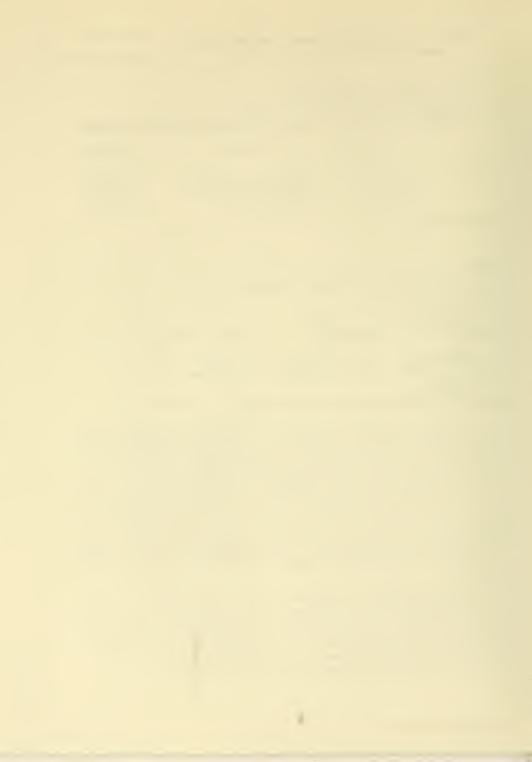


PROJECT NUMBER

DEPARTMENT	OF HEALTH A	NO HUMAN SE	HVICES - PUB	LIC HEA	ALTH SERVICE				
NO	tober 1, 1986 to September 30, 1987 OF PROJECT (80 characters or less. Title must lit on one lina between the borders.) age of Human Tissues CIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute in the content of the professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute in the content of the professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute in the content of the principal Investigator.) (Name, title, laboratory, and institute in the principal Investigator.) (Name, title, laboratory, and institute in the laboratory, and institute in the laboratory, and institute in the principal Investigator.) (Name, title, laboratory, and institute in the laborato	457-03	LCMB						
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PRINCIPAL INVESTIGAT	OR (List other pro	fessional personne	l below the Princi	pal Inves	tigetor.) (Name, tit	tia, laboreto	ry, and institut	e affillation)	
PI:	P. P. Di	Fiore	Visiting	Asso	ciate		LCMB	NCI	
Others:	S. A. Aai	ronson	Chief				1 CMB	NCI	
				Micr	obiologist	t.		NCI	
						•		NCI	
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COOPERATING UNITS (t eny)								
None									
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LAB/BRANCH									
Laboratory of	Cellular	and Molec	ular Biol	ypo					
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SUMMARY OF WORK (U	se standard unred	uced type. Do not	exceed the space	provide	d.)				

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.

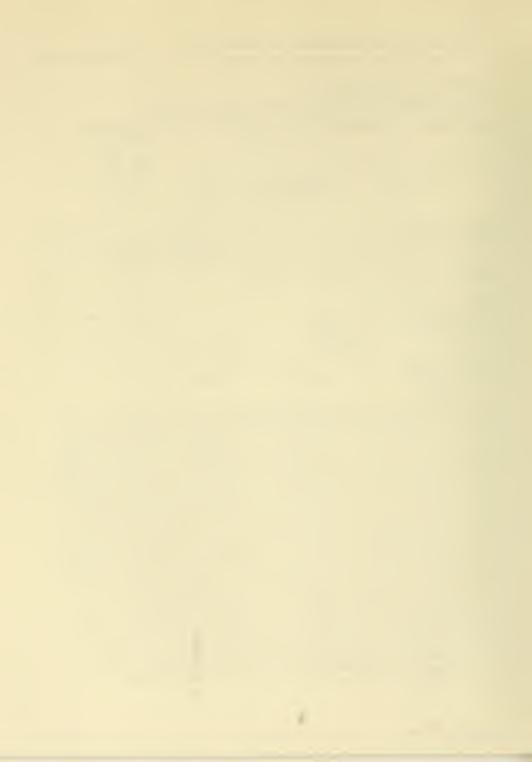
A murine pseudotype of the v-erbB gene has been employed to alter the growth properties and the differentiation program of an in vitro cell line of mouse keratinocytes (MKB). As already demonstrated for many other oncogenes, v-erbB is capable of relieving MKB cells from their dependence on EGF for growth. 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).



PROJECT NUMBER

701CP05459-03 LCMB

NOTICE OF INT	NOTICE OF INTRAMURAL RESEARCH PROJECT					
October I, 1986 to Sep						
TITLE OF PROJECT (80 cheracters or less Structural and Functio	Title must fit on one line between the born nal Characterization of	ras p21 Protei	ns			
PRINCIPAL INVESTIGATOR (List other pro	dessionel personnel below the Principal Inve Visiting Associate	stigator) (Name, title, labore	atory, and institute effiliation) LCMB NCT			
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 (M any) Centro de Biologia Mol Department of Medicine cina y Cirugia Experim	ecular, Universidad Auto , SUNY, Stony Brook, NY ental, Hospital Provinc	(N. Hagag); De	partmento de Medi-			
Laboratory of Cellular	and Molecular Biology					
Molecular Biology Sect	ion					
NCI, NIH, Bethesda, Ma	ryland 20892					
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER: 1.5				
☐ (a1) Minors ☐ (a2) Interviews		(c) Neither				
proteins in E. coli. activities. We have for mechanisms of activation in sms implies an increation that mutations the off-rate for GDP and p21 at positions 12 or specifically blocks the change prebound guaning by which Y13-259 intermutated ras p21 protein ester down-regulated SCC restores the phorbol have been able to demonfor the mitogenic activegulated (protein king Coinjection of ras p21 also demonstrated that pH after microinjection These data, together wp21 function is mediate protein kinase C can pt	e generated to produce Purified proteins were a cound that <u>ras</u> p21 proteins on of their transforming ase of the off-rate of phat substitute Thr 59 for the substitute Thr 50	darge amounts of analyzed for in a shave at lead properties. It is a share at lead properties. It is a share at lead at monoclonal and at monoclonal and activity of large at leading the share that this might all activity. Using the share at leading the share at leading to a share a sha	vitro and in vivo st two different One of these mecha-activity. We have al p21) increased to normal or mutated antibody Y13-259 oteins to interbe the mechanism both normal and d that in phorbol fied protein kinase ing this system, we protein kinase C of p21 into down-DNA synthesis. tivity. We have rise in intracellular corter system. C, implies that ras ave observed that			

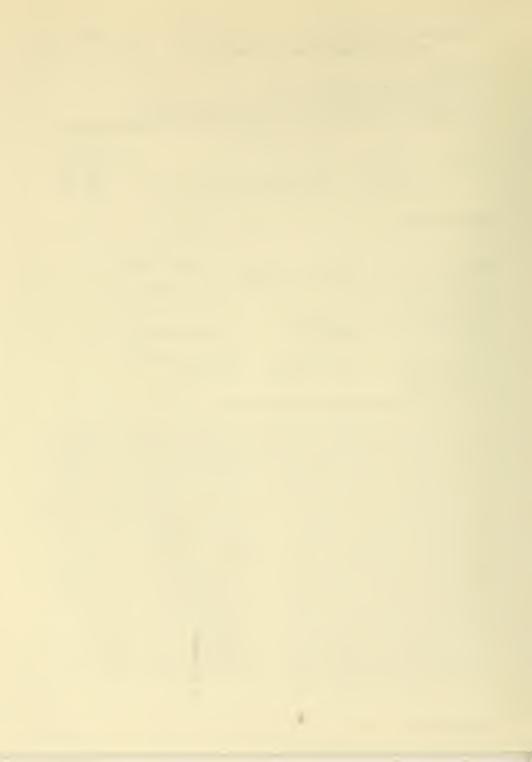


PROJECT NUMBER

Z01CP05460-03 LCMB

October		to Septer	nber 30, 19	37					
Regulati	CT (80 char On Of	acters or lass. Title 1 SSUE-SPEC	must fit on one line	between the SSION O	borders.) f C-Sis/	PDGF-2 Ger	ne		
PRINCIPAL INVES	PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)								
PI:	C. D.	Rao	Visiting	Associa	ate			LCMB	NCI
Others:	M. W.	Aaronson Pech Robbins	Chief Visiting Chief, M			cs Section	1	LCMB LCMB LCMB	NCI NCI NCI
None	INITS (if an)	"							
Laborato	ry of (Cellular an	nd Molecula	r Biolog	ЭУ				
SECTION Molecula	r Biolo	ogy Section	1						
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SUMMARY OF W	ORK (Use s	tandard unreduced	type. Do not exceed	the space p	rovided.)				

(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.



PROJECT NUMBER

DEPART	MENT OF HEALTH AND	HUMAN SERVICES - PUBL	IC HEALTH SERV	ICE	
	NOTICE OF INTRA	AMURAL RESEARCH F	ROJECT	Z01	CP05461-03 LCMB
		36 to September 30, 1987 Paracters or lass. Title must fit on one line between the borders.) Join of Normal Counterpart of dbl Oncogene R (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation, Eva Visiting Scientist LCMB NCI Ron Visiting Fellow LCMB NCI A. Aaronson Chief LCMB NCI A. Aaronson Chief LCMB NCI Jany) Cellular and Molecular Biology Logy Section Note the professional: Nother:			
October		ember 30, 1987			
				е	
PRINCIPAL INVE	STIGATOR (List other profes	sional personnel below the Princip	al Investigator.) (Nam	e, title, laboratory, and	institute affiliation)
PI:	A. Eva	Visiting Scient	ist	LCMB	NCI
Others:	D. Ron	Visiting Fellow	٧	LCMB	NCI
	S. A. Aaronson			LCMB	NCI
COOPERATING (UNITS (if any)				
Laborato	ry of Cellular	and Molecular Biolo	ogy		
Molecula	r Biology Section	on			
NCI, NIH		land 20892			
TOTAL MAN-YEA		ROFESSIONAL:	OTHER:	0.0	
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(a) Hum	ian subjects	(D) Hulliall (ISSUES	(c) Mair	1101	

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

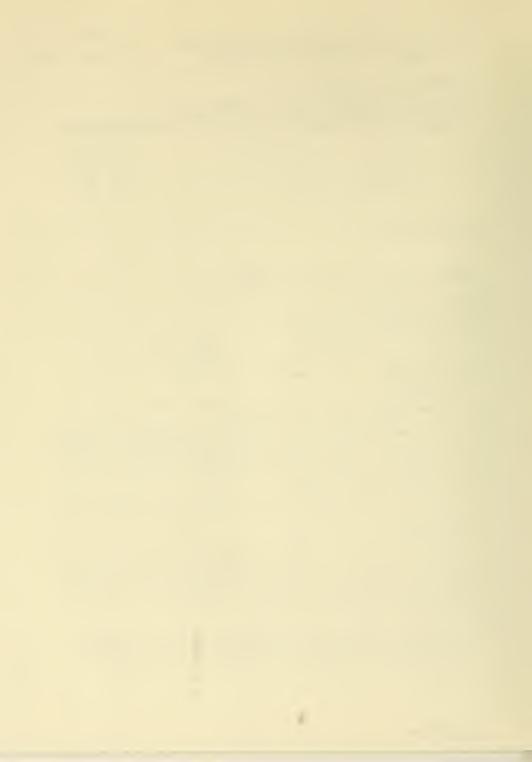
The entire coding sequence of \underline{dbl} proto-oncogene was determined. The sequence analysis revealed that \underline{dbl} proto-oncogene codes for a 925-amino acid protein. It is a hydrophilic protein with a characteristic $\underline{\alpha}$ -helical coiled-coil structure similar to that of intermediate filaments. The \underline{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 $\underline{\mathsf{dbl}}$ revealed that the transforming gene was rearranged with respect to its aminoterminal domain.

The oncogenic potential of the \underline{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 \underline{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 $\frac{dbl}{cell}$ proto-oncogene product was detected for the first time utilizing the COS $\frac{dbl}{cell}$ system and an expression vector driven by SV40 early promoter. The size of the dbl proto-oncogene protein was determined to be $\sim 10~\text{kd}$.

(a1) Minors
(a2) Interviews



PROJECT NUMBER

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Cellular a	and Oncogene Produ	st fit on one line between the borders.) cts which Participate in Gro		
PHINCIPAL INVEST	IGATOR (List other professional	personnel below the Principal Investigator.) (Neme, tit	ile, laboratory, and institute i	effillation)
PI:	W. G. Taylor	Research Biologist	LCMB	NCI
Others:	S. A. Aaronson J. P. Falco	Chief Medical Staff Fellow	L CMB L CMB	NCI NCI
			COND	NCI
COOPERATING UN	ITC (if only)			
COOPERATING ON	113 (ir eny)			
None				
AB/BRANCH				
	of Cellular and	Molecular Biology		
SECTION		- 33		
Office of	the Chief			

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892 TOTAL MAN-YEARS: PROFESSIONAL:

1.0

OTHER: 1.0

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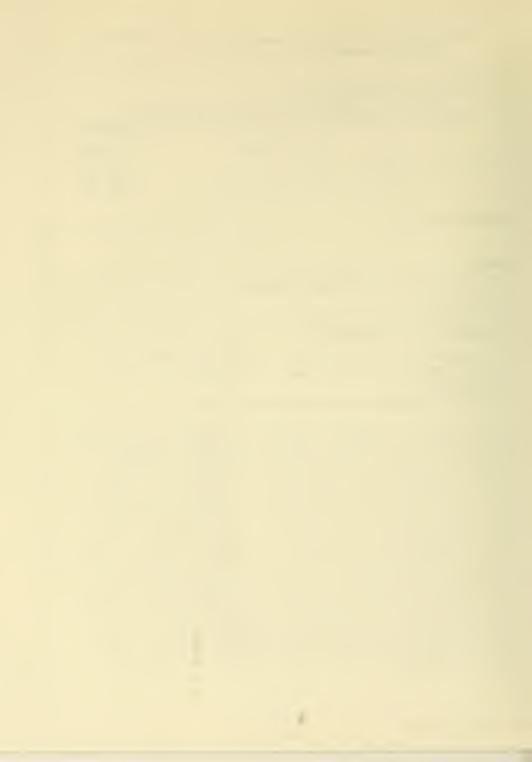
(a) Human subjects (b) Human tissues (c) Neither

(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 thead to strategies for counteracting their tumorigenic potential.



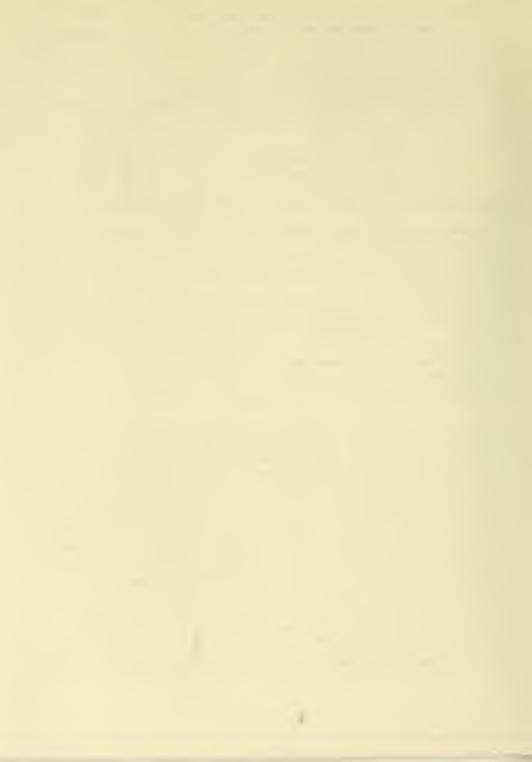
701CP05466-02 LCMB

PROJECT NUMBER

PERIOD COVERED .								
October 1, 1986 to September 30, 1987								
TITLE OF PROJECT (80 cheracters or less. Title must lit on one line between the borders.)								
· ·	Role of Human Transforming Growth Factor $lpha$ in Neoplasia							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)								
PI:	E. Finzi	E. Finzi Medical Staff Fellow LCMB NCI						
Others:	S. H. Yuspa	Chief		LCCTP	NCI			
	A. E. Kilker	ny Expert		LCCTP	NCI			
		ig Guest Research		LCMB	NCI			
	O. Segatto	Visiting Fel	low	LCMB	NCI			
	S. A. Aarons	on Chief		LCMB	NCI			
	J. H. Pierce	Research Micr	robiologist	LCMB	NCI			
COOPERATING UN	ITS (if eny)							
Departmen	t of Molecula	r Biology, Genente	ech, Inc., South	San Francis	sco, CA (T.S.			
Bringman	and R.K. Dery	nck).						
LAB/BRANCH								
Laboratory	y of Cellular	and Molecular Bio	ology					
SECTION								
Office of	the Chief							
INSTITUTE AND LO	CATION							
NCI, NIH,	Bethesda, Ma	ryland 20892						
TOTAL MAN-YEARS	S:	PROFESSIONAL:	OTHER:					
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(a) Human	subjects	(b) Human tissues	(c) Neither					
☐ (a1) M	inors							
☐ (a2) In	terviews							

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

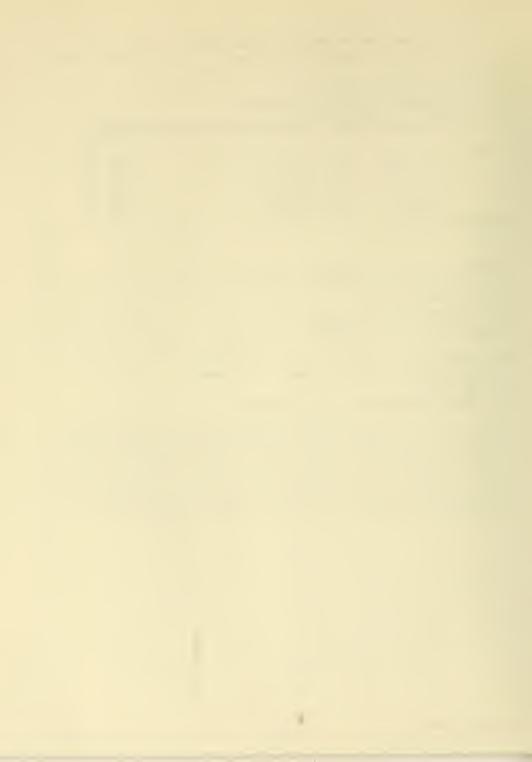
Previously, we have shown that TGF ∞-expression vectors failed to induce morphological transformation upon transfection of NIH/3T3 cells. Transfected cells were shown to secrete large amounts of $TGF\alpha$ into the medium, to grow to high saturation density, to have down-regulated EGF receptors, and to be growth-inhibited by TGF α monoclonal antibody. However, TGF α monoclonal sublines were not tumorigenic in nude mice. These and other results suggest that the normal coding sequence for $TGF\alpha$ 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 $TGF\alpha$ retrovirus were shown to synthesize and secrete $\mathsf{TGF}\,\alpha_{ullet}$ We are investigating the role of $\mathsf{TGF}\,\alpha$ in epithelioid cancer using a nude mouse skin graft model. Recent work indicates that although infection of primary epithelial cells with the TGF a 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 $TGF\alpha$ in the clonal expression of the premalignant lesion.



PROJECT NUMBER DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE 701CP05467-02 LCMB NOTICE OF INTRAMURAL RESEARCH PROJECT PERIOD COVERED 0ctober 1, 1986 to September 30, 1987 TITLE OF PROJECT (80 cheracters or less. Title must fit on one line between the borders.) Cloning of Human c-fqr Proto-oncogene cDNA PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute efficiency PI: K. C. RODDINS Chief, Molecular Genetics Section LCMB NC.) Others: S. Katamine Visiting Fellow LCMB NCI S. R. Tronick Chief, Gene Structure Section LCMB NC.I 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 (# any) None Laboratory of Cellular and Molecular Biology Molecular Genetics Section NSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892 PROFESSIONAL: TOTAL MAN-YEARS: OTHER: 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-fqr 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.

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PROJECT NUMBER

01CP05468-02 LCMB

NOTICE OF INT	RAMURAL RESEARCH PRO	DJECT	ZU1CPU5466-UZ LCMB
October 1, 1986 to Sep	tember 30, 1987		
TITLE OF PROJECT (80 characters or less Implications of Human	. Title must fit on one line between the bo Tyrosine Kinase Gene,		genesis
PRINCIPAL INVESTIGATOR (List other pro	fessional personnel below the Principal In	vestigetor.) (Name, title, lebore	tory, end institute effiliation)
PI: T. Kawakami	Visiting Associat	e	LCMB NCI
Others: K. C. Robbins Y. Kawakami	Guest Researcher	Genetics Section	LCMB NCI
T. Matsui	Visiting Fellow		LCMB NCI
S. A. Aaronso			LCMB NCI LB NCI
N. C. Popescu	Microbiologist		LD NCI
None			
LAB/BRANCH Laboratory of Cellular	and Molecular Biology		
SECTION			
Molecular Genetics Sec	tion		
NCI, NIH, Bethesda, Ma	ryland 20892		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER:	0.0
CHECK APPROPRIATE BOX(ES) (a) Human subjects (a1) Minors (a2) Interviews	🗵 (b) Human tissues	☐ (c) Neither	
SUMMARY OF WORK (Use standard unred	fuced type. Do not exceed the space pro-	vided.)	

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

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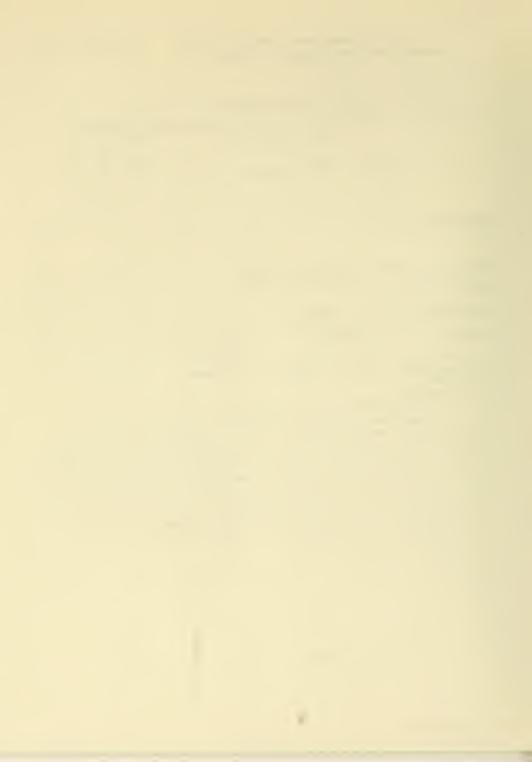


ZO1CP05469-02 LCMB

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TITEGENETHECT	विधित्रामानसम् साहस्य राष्ट्रा	usithe an hasemon to gettes						
PRINCIPAL INVEST	IGATOR (List other professions	al personnel below the Principal Investigator.) (Nama, titla, laboratory, and ins	titute affiliation)				
PI:	G. Kruh	Medical Staff Fellow	LCMB	NCI				
Others:	S. A. Aaronson	Chief	LCMB	NCI				
ounci s.		Visiting Associate	LCMB	NCI				
COOPERATING UNI								
		d Molecular Biology						
secophice of	the Chief							
INSTITUTE AND HO	Carethesda, Maryl	and 20892						
TOTAL MAN-YEARS	PROF	ESSIQNAG: OTH	ER: 0.0					
- ' '	n subjects 🗵 (t inors terviews	,	Neither					
SUMMARY OF WOR	RK (Use standerd unreduced ty	pe. 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.

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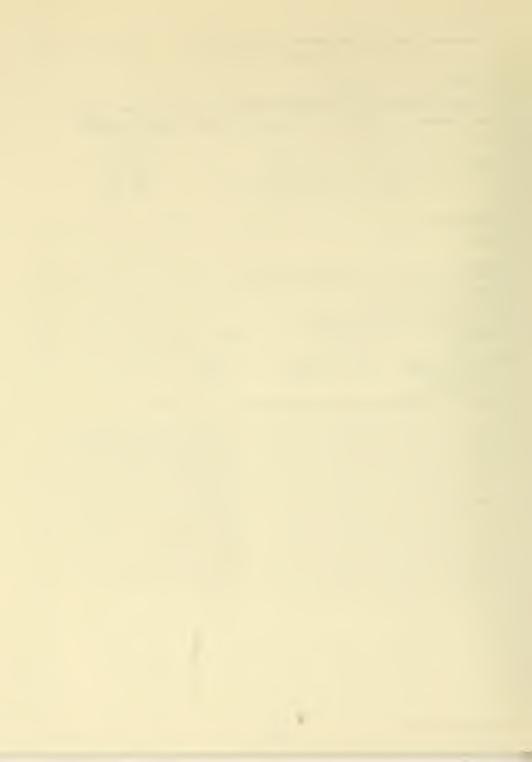


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PROJECT NUMBER

	NOTICE OF INT	RAMURAL RESEARCH	PROJECT	Z01C	Z01CP05472-02 LCMB	
PERIOD COVERE OCTOBER	P, 1986 to Sep	tember 30, 1987				
TITLE OF PROJECT	Characters or less.	Title must lit on one line between ation of Putative	Growth Factor R	eceptor Gen	e c- <u>erb</u> B-2	
PRINCIPAL INVES	STIGATOR (List other prof	essionel personnel below the Prin	cipal Investigetor.) (Name, tit	le, laboretory, and in:	stitute effilietion)	
PI:	S. A. Aarons	on Chief		LCMB	NCI	
Others:	O. Segatto P. P. Di Fio J. H. Pierce	Visiting Fell re Visiting Fell Research Micr	OW	LCMB LCMB LCMB	NCI NCI NCI	
None LAB/BRANCH Laborator		and Molecular Bio	logy			
SECTION Molecular	Biology Sect	ion			·····	
INSTITUTE AND L	Bethesda, Ma	ryland 20892				
TOTAL MAN-YEAR	s:	PROFESSIONAL:	OTHER: 0	.0		
CHECK APPROPR (a) Huma (a1) I	an subjects	∑ (b) Human tissues	☐ (c) Neither			
SUMMARY OF WO	ORK (Use standard unred	uced type. Do not exceed the spa	ce 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.



PROJECT NUMBER

	OTICE OF INT						Z01CP05473-0	D2 LCMB
October 1,	•							
Studies of	TITLE OF PROJECT (80 characters or less. Title must lit on one line between the borders.) Studies of Mechanisms of Pathogenesis of Animal Lentiviruses							
PRINCIPAL INVESTIGA	ATOR (List other pro	ntessional personnel	below the Prin	cipal Inves	tigator.) (Name, titi	le, laborat	ory, and institute affilia	tion)
PI:	S. R. Tr	onick	Chief, G	ene St	ructure Se	ction	LCMB	NCI
Others:	M. C. Wa S. A. Aa J. E. Da T. Kawak J. C. La	ronson hlberg ami	Visiting Chief Research Visiting Visiting	Micro Assoc	biologist iate		LCMB LCMB LCMB LCMB LCMB	NCI NCI NCI NCI
COOPERATING UNITS	(if any)						2016	
None								
Laboratory (of Cellular	and Moleci	ular Bio	logy				
Molecular B	iology Sect	ion						
NCI, NIH, BE	ction ethesda, Ma	ryland 208	92					
TOTAL MAN-YEARS:		PROFESSIONAL:			OTHER:	0	.0	
(a) Human s	(a1) Minors							
The mechanis						nia vi	rus (EIAV) i	s

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 $\underline{\text{E. coli}}$ and milligram quantities have been obtained which have made possible development of sensitive radioimmunoassays for EIAV.



PROJECT NUMBER

	NOTICE OF INTRAM	Z01	Z01CP05511-01 LCMB		
PERIOD COVERE October	1, 1986 to Septem	ber 30, 1987			
Purifica	CT (80 characters or less. Title tion and Characte	must fit on one line between the borders.) rization of Epithelial Cel	1 Mitogens		
PRINCIPAL INVE	STIGATOR (List other profession	nel parsonnel below the Principal Investigator.) (N	vame, title, laboretory, and ii	nstitute effiliation)	
PI:	S. A. Aaronson	Chief	LCMB	NCI	
Others:	J. S. Rubin P. W. Finch W. G. Taylor	Biotechnology Fellow Visiting Fellow Research Biologist	LCMB LCMB LCMB	NCI NCI NCI	
COOPERATING L	JNITS (if any)				
None					
Laborato	ry of Cellular and	d Molecular Biology			
SECTION					

Molecular Biology Section

NSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

0.0

CHECK APPROPRIATE BOX(ES) (a) Human subjects

(a1) Minors

(b) Human tissues (c) Neither

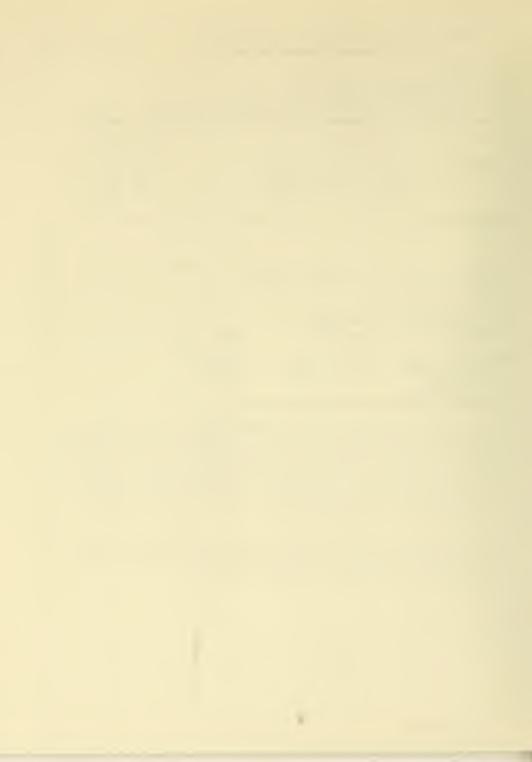
(a2) Interviews

SUMMARY OF WORK (Use stendard 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.

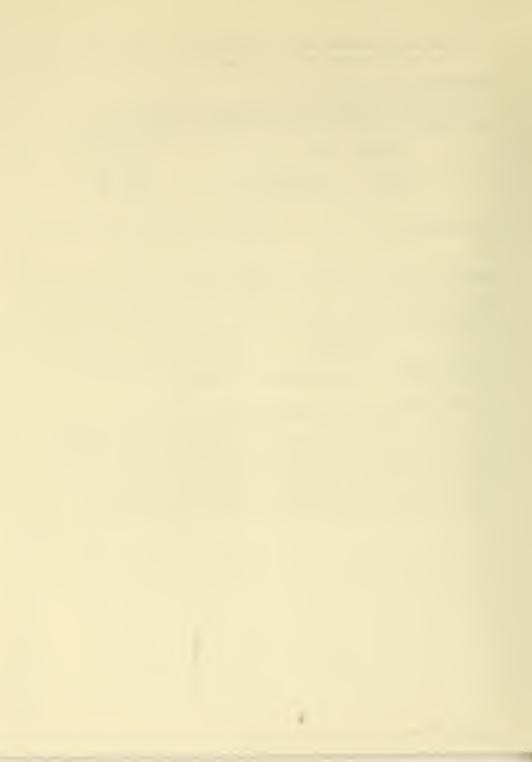
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PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT				Z01CP05512-01 LCMB			
October 1, 1986 to Sept							
Molecular Cloning of Ge	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 profe	essional personnal below the Principal In	vestigator.) (Name, title, labora	tory, and in	strtuta affiliation)			
PI: S. A. Aaronso	on Chief		LCMB	NCI			
Others: P. W. Finch	Visiting Fellow		LCMB	NCI			
J. S. Rubin	Biotechnology Fel	low	LCMB	NCI			
COOPERATING UNITS (if any)							
None							
LAB/BRANCH Laboratory of Cellular and Molecular Biology							
SECTION Molecular Biology Secti	on						
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892							
1.0	PROFESSIONAL:	OTHER:					
CHECK APPROPRIATE BOX(ES)	V						
☐ (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, pcDVl, 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.



PROJECT NUMBER

701CP05513-01 LCMB

PERIOD COVERED October 1, 1986 to September 30, 1987							
Me chan is	TITLE OF PROJECT (80 characters or less. Title myst lit on one line between the borders.) Mechanisms of Transformation Induced by <u>fgr</u> and Related Oncogenes						
PRINCIPAL INVES	TIGATOR (List other pro	fessional personnel below the Prin	cipal Investigetor.) (Name, title, labo	retory, and institute effiliatio	n)		
PI:	K. C. Robbin	s Chief, Molec	ular Genetics Section	on LCMB	NCI		
Others:	S. A. Aarons	on Chief		LCMB	NCI		
	S. R. Tronic	k Chief, Gene	Structure Section	LCMB	NCI		
	S. Katamine	Visiting Fel	low	LCMB	NCI		
	M. Cheah	Medical Staf	f Fellow	LCMB	NCI		
	T. Kawakami	Visiting Fel	low	LCMB	NCI		
Division of Hematology/Oncology, Washington Univ., St. Louis, Missouri (T. Ley)							
Laboratory of Cellular and Molecular Biology							
SECTION Molecular Genetics Section							
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892							
TOTAL MAN-YEAR	1.0	PROFESSIONAL: 0.5	OTHER:	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.)

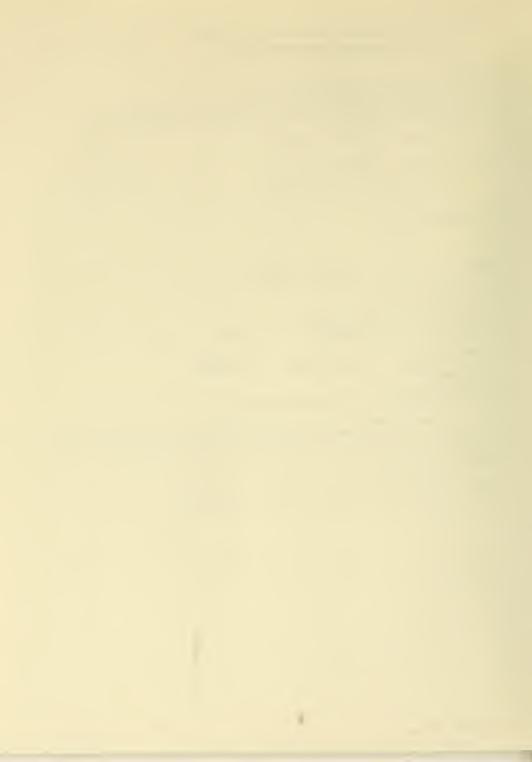
Efforts to determine normal functions for protein-tyrosine kinases encoded by human fgr and related proto-oncogenes have focused on the isolation of cDNA molecules representing their transcriptional units and complete coding sequences. We have isolated and sequenced human c-fgr and fyn cDNAs and have 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.

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PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT				Z01CP05514-01 LCMB		
PERIOD COVERED October 1, 1986 to September 30, 1987						
Analysis	of an Oncoger	. Title must fit on one line between the border ne Related to a Growth Fa	ictor and Its R	•		
PRINCIPAL INVES	STIGATOR (List other pro	fessional personnel below the Principal Invest	igator.) (Name, title, laboret	ory, 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 U	NITS (if any)					
None						
140/0041/01/		M 25.1				
Laborator	ry of Cellular	and Molecular Biology				
SECTION Molecular Biology Section						
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892						
TOTAL MAN-YEAR		PROFESSIONAL:	OTHER:	0.0		
	1.0	1.0		0.0		
CHECK APPROPR		∅ (b) Human tissues □	(c) Neither			
		(b) Human tissues	(c) Maithei			
☐ (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.						
(crosue species), respectively.						
Recently, we have isolated five genomic DNA clones homologous to the proto-						
oncogene family, which includes fms, kit and PDGF receptor.						



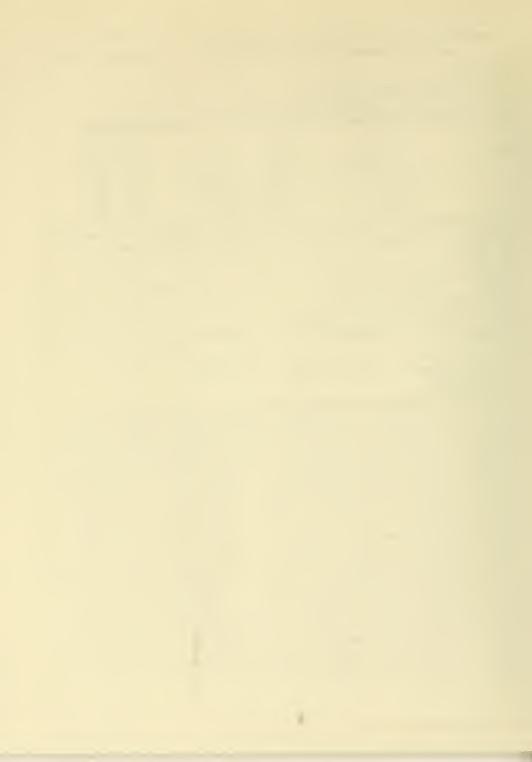
PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP04899-15 LM0

PERIOD COVERED						
October 1, 1986 to September 30, 1987						
TITLE OF PROJECT (80 characters or less. Titla must fit on one line between the borders.)						
		vian RNA Tumor '				
PRINCIPAL INVESTIGAT	Tor (List other pro	dessional personnel below th	e Pnncipal Inves Chief	tigator.) (Neme, title, leborete	ory, and institute LMO	affiliation) NC I
r1.	1. J. Fa	pas	Cirrer		Lino	1401
Others:	R. J. Fi	sher	Expert		LM0	NC I
	J. A. La	utenberger		taff Fellow	LMO	NCI
	D. K. Wa		Senior S	taff Fellow	LMO	NCI
	N. Sacch		Visiting	Associate	LMO	NCI
	N. K. Bh	at	Visiting		LMO	NCI
	S. Fujiw	ara	Visiting		LMO	NCI
COOPERATING UNITS	(if any)					
		Johns Hopkins Ur	niversity	School of Medi	cine, Bal	timore, MD
(E. Moudrianal	<pre>cis); Depa</pre>	rtment of Biolog	gy, Unive	rsity of Califo	rnia, Ber	keley, 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 retro-						
viral 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 evolu-						
tionarily 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 oncogenespecific 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 <u>ets</u> 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.



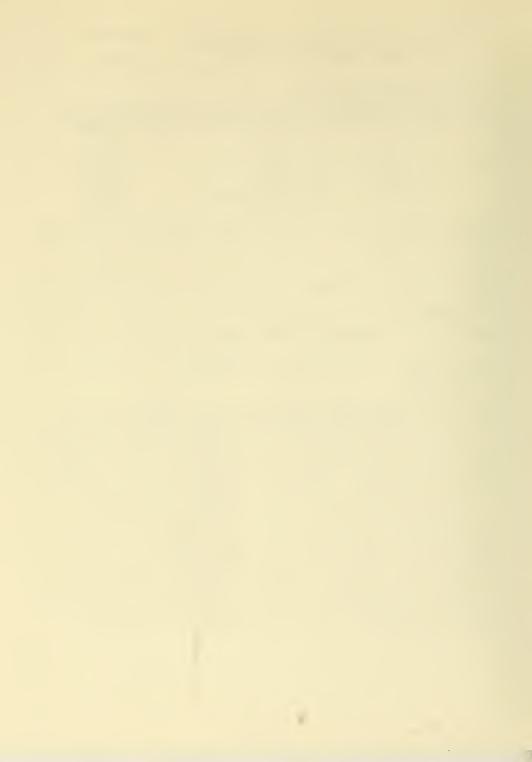
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PROJECT NUMBER

		TAMOTIAL TILOLATION	11100201	Z01CP04963-11 LM0
PERIOD COVERE		1 20 1007	-	
		ember 30, 1987		
		Title must fit on one line between		by p21 ras Oncogenes
		lessional personnel below the Print		
PI:	T. Y. Shih	Research Ch		LMO NC I
Others:	L. S. Ulsh	Microbiolog	ist	LMO NCI
	D. J. Clanto			LMO NCI
	P. Saikumar	Visiting Fe	llow	LMO NCI
	D. G. Blair	Supv. Resear	rch Chemist	LMO NCI
	Y. Lu	Visiting Fe	11ow	LMO NCI
COOPERATING U	,			
			yo, Tokyo, Japan (S	S. Hattori); NAPS, PRI,
Frederick,	MD (G. DuBoi	s)		
LAB/BRANCH				
	of Molecular	Oncology		
SECTION				
Office of			······································	
NCI, NIH,	Frederick, Ma	ryland 21701-1013 PROFESSIONAL:	OTUED:	
			OTHER:	
CHECK APPROPR	2.0	1.0	1.0)
(a) Huma		(b) Human tissues	(c) Neither	
☐ (a1) N		_ (b) Framan house	φ (σ) (νοιιποι	
☐ (a2) I				
SUMMARY OF WO	ORK (Use standard unred	luced type. Do not exceed the spe	ce provided.)	
The major	focus of this	project is to inve	estigate the molecu	ılar biology and bio-
chemistry	of the <u>ras</u> on	cogenes and the <u>ra</u>	<u>s</u> p21 proteins. Th	ne long-range objective
				induced by these genes
				p21 of H- and K-ras
				Harvey and Kirsten
				oteins in cells was
				ed p21 in vitro. The
present re	sults suggest	that these novel	ohosphorylations we	ere mediated by kinase

C. Structure-function of ras proteins were investigated by methods of sitedirected 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 prebound 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 protooncogene p21, suggesting its role in high oncogenicity of viral oncogenes.

or estable



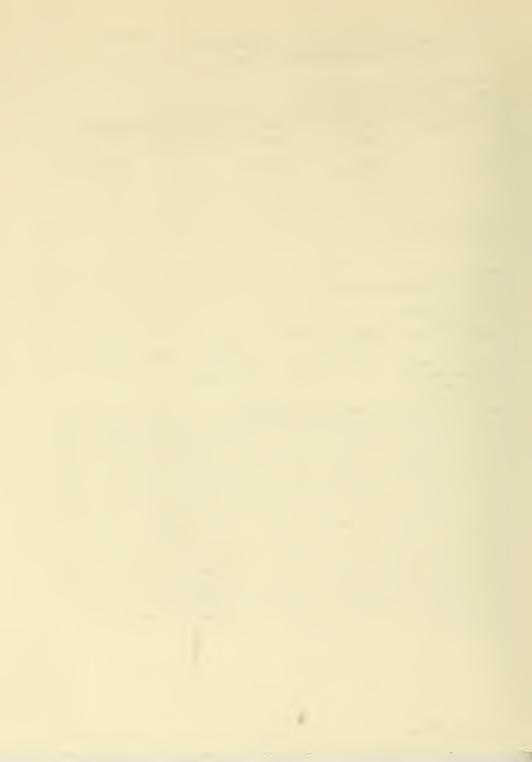
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04970-11 LM0

PERIOD COVERED					
October 1, 1986 to Sept	ember 30, 1987				
TITLE OF PROJECT (80 characters or les	s. Title must fit on one line be	tween the border	rs.)		
Biochemistry of Cellula	r Transformation	n by Aviar	n Tumor Viruses	3	
PRINCIPAL INVESTIGATOR (List other pr	ofessional personnel below th	e Principal Invest	igetor.) (Neme, title, lebore	tory, and institute affil	letion)
	Bader		Microbiologist		NCI
Others: D. A.	Ray	Chemist		LM0	NCI
		Chemist		LM0	NCI
COORTON TIME LINETO ALCON					
COOPERATING UNITS (d eny)					
LAB/BRANCH					
	01				
Laboratory of Molecular	uncology				
SECTION					
Office of the Chief					
INSTITUTE AND LOCATION					
NCI, NIH, Frederick, Ma		013			
TOTAL MAN-YEARS:	PROFESSIONAL:		OTHER:		
2.8	1.0			1.8	
CHECK APPROPRIATE BOX(ES)	_				
(a) Human subjects	(b) Human tissu	ues 🗓	(c) Neither		
(a1) Minors					
(a2) Interviews					
SUMMARY OF WORK (Use standard unre	duced type. Do not exceed the	he space provided	1.)		

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, pll0, 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 pllO associated with chromatin was found to be more highly phosphorylated than that in the nucleoplasm. Other studies indicate that pll0 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.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05120-08 LM0

PROJECT NUMBER

PERIOD COVERE	0						
		ember 30, 1987					
		s. Title must fit on one line be	tween the borde	rs.)			
		and Oncogene			ial and	Mammalian	Vectors
		ofessional personnel below th					
PI:		utenberger		earch Che		LMO	NC I
Others:	F. Wong-	Staal	Bio	logist		LTCB	NC I
	T. S. Pa		Chi	ef		LMO	NCI
	Z-Q. Che		Vis	iting Ass	ociate	LM0	NCI
	Research Prog	ram, Frederick, Delaware, Newa				ife and H	ealth
LAB/BRANCH							
	of Molecular	Uncology					
SECTION							
INSTITUTE AND L	esis Regulati	on Section					
	Frederick, Ma	ryland 21701-1	012				
TOTAL MAN-YEAR		PROFESSIONAL:	013	OTHER:			
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CHECK APPROPR		1.0					
(a) Huma (a1) N (a2) I	n subjects Minors nterviews	(b) Human tissu		(c) Neithe			
A protein	PRK (Use standard unred has been synt	hesized in E. c	e space provide	contains	HTLV-I a	ad dene se	equences.
The HTLV-I	gag gene was	placed into the	e mos gen	e codina	sequence	s in the	expressio
vector, pA	28, a derivat	ive of pJL6 dev	eloped in	our labo	ratory.	A 30 kDa	protein
can be det	ected by anti	-mos peptide an	tibody.	By use of	an incl	usion body	/ purifi-
cation pro	tocol, this p	rotein can be m	ade 50% p	ure witho	out the u	se of colu	ımn
		rotein is poten			diagnos	tic reager	nt since
it is reco	gnized by ant	ibodies in pati	ent serum	•			
specifical	ly binds a re	d in nuclear exegion on the LTR	near the	polyader	ylation	site. The	e speci-
addition	f unlabeled t	erified by demo .TR DNA as a com	nstratilly netitor	but not b	v nRR322	DNA lit	tle or
none of th	is activity w	as found in oth	er cell 1	ines test	ed, incl	uding MJ	leukemic

DNA sequences from the sea urchin, <u>Lytechinas variegatus</u>, 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.

T-cells, indicating that this phenomena is specific to the C10/MJ line.



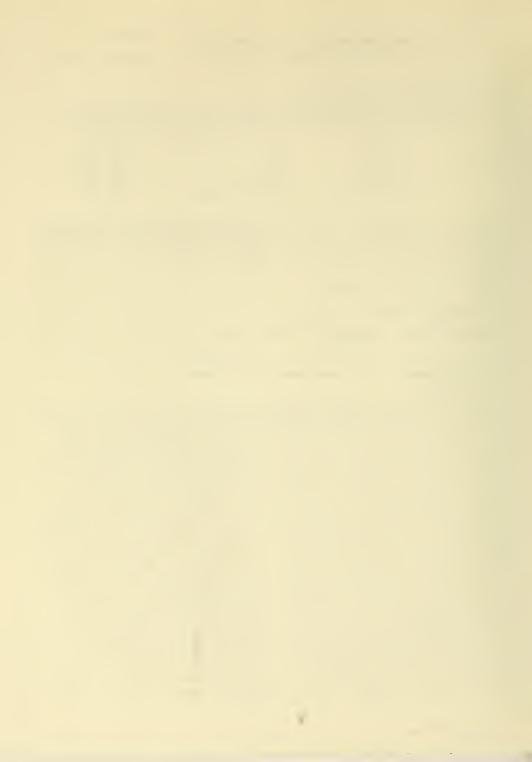
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05238-06 LM0

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PERIOD COVERED					
October 1, 1986 to Sept					
TITLE OF PROJECT (80 characters or less					.1
The Transforming Genes PRINCIPAL INVESTIGATOR (List other pro	or Acute Leukem	a viruse	s and their ce	Tular Hom	orogues
PI: D. K. Wat			taff Fellow	tory, and institute LMO	effiliation) NC I
D. K. Wat	3011	2611101 2	call lellow	LINU	INC I
Others: T. S. Pap	as	Chief		LMO	NC I
S. J. O'B		Chief		LVC	NCI
L. J. Pri		Biologis	t	LMO	NCI
	Beneden		searcher	LMO	NCI
					,,,,,
COOPERATING UNITS (if any) Develo	pmental Genetics	Lab., J	ohns Hopkins Ho	spital, B	altimore, M
(R. Reeves); Dept. Mole	cular Biology, l	J. Califo	rnia, Berkeley	, CA (P. H	. Duesberg)
Program Resources, Inc.	, Frederick, MD	(S. Redd)	y, S. Showalter	, M. J. S	mith); LBI-
Basic Research Program,	Frederick, MD ((A. Seth)			
LAB/BRANCH					
Laboratory of Molecular	Oncology				
SECTION					
Carcinogenesis Regulati	on Section				
INSTITUTE AND LOCATION	2 1 01701 1				
NCI, NIH, Frederick, Ma		113			
TOTAL MAN-YEARS:	PROFESSIONAL:		OTHER:		
1.9 CHECK APPROPRIATE BOX(ES)	0.9		1.0		
	(b) Human tissu	ес П	(c) Neither		
(a1) Minors	as (b) Haman book		(6) 110/11/01		
(a2) Interviews					
SUMMARY OF WORK (Use standard unred	fuced type. Do not exceed th	e space provided	d.)		
To provide an initial st	tep toward under	standing	the functional	relation	ship between
the onc genes of transfe	orming retroviru	ises and	their cellular	prototype	s, struc-
tural comparisons at the					
We have determined the	complete nucleot	ide sequ	ence of the chi	cken ets	gene and
compared it to the ets	gene of E26. E2	26 is a g	enetic hybrid w	ith seque	nces derive
from viral structural g	enes and parts o	of essent	ial cellular pr	oto-onc g	enes. The
chicken ets gene is pre	sent as a single	locus w	ith v-ets homol	ogous seg	uences

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 carboxytermini of p135 (gag-myb-ets transforming protein of E26) and the cellular protoets 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.



Z01CP05295-06 LM0

PROJECT NUMBER

NOTICE	OF INTRAMIDAL	RESEARCH PROJECT

PERIOD COVERED

PI:

October 1, 1986 to September 30, 1987

D. G. Blair

NCI, NIH, Frederick, Maryland 21701-1013

TITLE OF PROJECT (80 cherecters 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 personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Others: T. S. Papas LMO NC T LMO NC I K. J. Dunn Bio. Lab. Tech. (Micro.) Q. Yuan NC T Visiting Fellow LMO Visiting Fellow LMO NC I Y. Lu D. J. Clanton Senior Staff Fellow 1 MO NC I 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) Laboratory of Molecular Oncology SECTION Microbiology Section

Supv. Research Chemist

TOTAL MAN-YEARS: 2.75 CHECK APPROPRIATE BOX(ES)

INSTITUTE AND LOCATION

1.75

PROFESSIONAL:

1.0

(a) Human subjects (a1) Minors

(b) Human tissues

(c) Neither

OTHER:

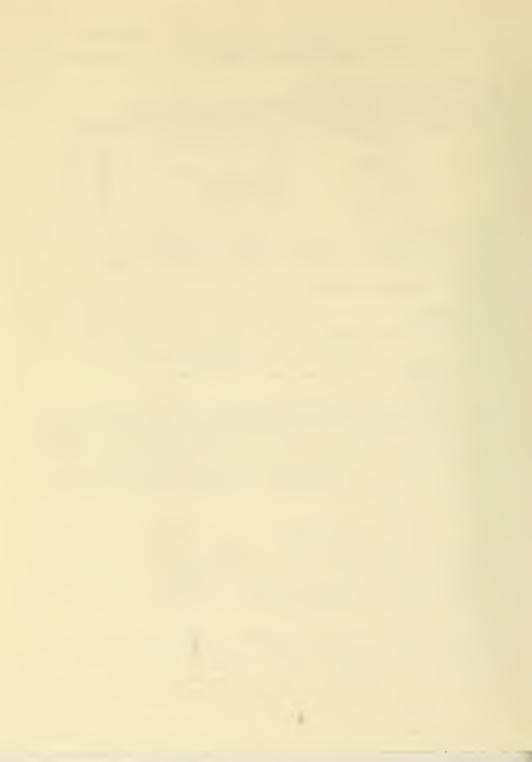
(a2) Interviews SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)
We have constructed a murine homolog (ME26) of the avian acute leukemia virus, E26, which replicates efficiently in murine cells and expresses transforming functions both in vitro and in vivo. The ME26 pl35 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.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

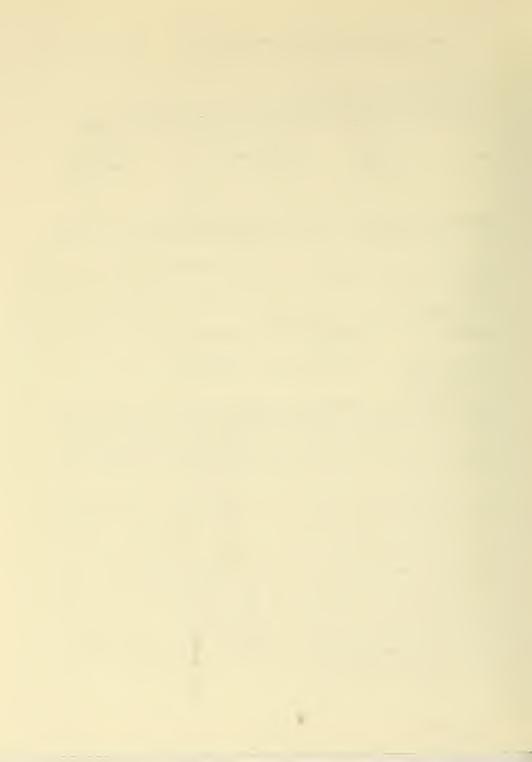
PROJECT NUMBER

701CP05440-03 LM0

SECTION Microbiology Section						
-						
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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.



PROJECT NUMBER

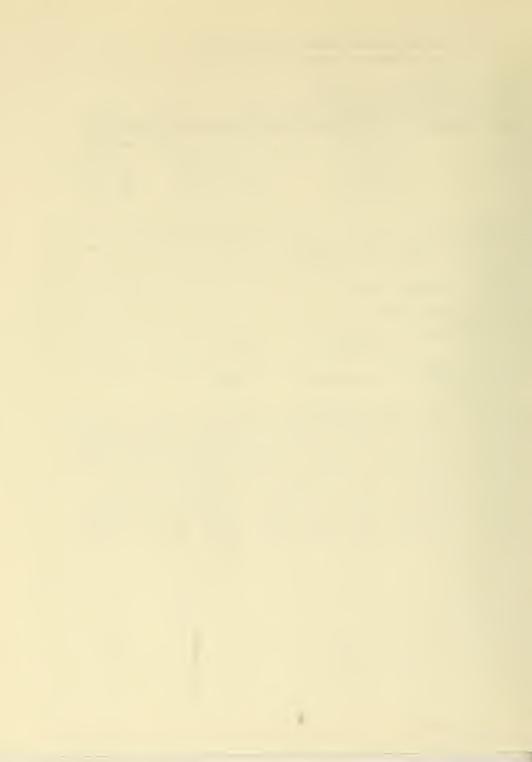
NOTICE OF INT	RAMURAL RESEARCH PRO-	JECT	Z01CP05441-03 LM0
PERIOD COVERED			
October 1, 1986 to Sept	ember 30, 1987		
TITLE OF PROJECT (80 cherecters or less		iers.)	
Characterization of the	Gene Products of the c	-myc Locus and t	the c-ets Locus
PRINCIPAL INVESTIGATOR (List other pro			
PI: R.	J. Fisher	Expert	LMO NCI
		= 11	
		Visiting Fellow	
		Visiting Fellow	
T.	S. Papas	Chief	LMO NCI
COOPERATING UNITS (# eny) Nucleic Acid and Protei MD (M. Zweig, G. DuBois		Program Resourc	ces, Inc., Frederick,
LAB/BRANCH	0 1		
Laboratory of Molecular	Uncology		
SECTION			
Transgenic Analysis Sec	tion		
INSTITUTE AND LOCATION			
NCI, NIH, Frederick, MD			
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:	
1.2	1.2	0	.0
CHECK APPROPRIATE BOX(ES)	F3		
(a) Human subjects (a1) Minors	(b) Human tissues	(c) Neither	

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

(a2) Interviews

The protein products of the \underline{ets} -1 and \underline{ets} -2 genes have been identified and characterized with polyclonal anti-peptide antibodies and with a monoclonal antibody prepared against a bacterially-expressed human \underline{ets} -2 protein. The \underline{ets} -1 protein is 52 kDa and found primarily in cells of lymphoid origin; the \underline{pto} \underline{ets} -2 protein was found to be widely distributed and located in the nucleus. In addition, a nuclear \underline{pto} and \underline{pto} were identified as \underline{ets} -2-related proteins which share a limited homology by two-dimensional peptide mapping. The nuclear \underline{pto} 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 \underline{ets} -2-related proteins. The next part of the work is to purify the native form of the \underline{ets} -2 protein in order to give insight into its function.

- Mariana Maria



NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05442-03 LM0

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.) Human ets Genes in Human and Cancer Genetics PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, leboratory, and institute effiliation) PI: N. Sacchi Visiting Scientist LMO NCI Other: T. S. Papas Chief LMO NCT COOPERATING UNITS (d env) 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) Laboratory of Molecular Oncology SECTION Carcinogenesis Regulation Section INSTITUTE AND LOCATION NCI, NIH, Frederick, Maryland 21701-1013 TOTAL MAN-YEARS: PROFESSIONAL: OTHER: 0.9 0.9 0.0

(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 requlation at a distance, may somehow alter their expression. The real "cancer genes" involved by the above-mentioned abnormalities, therefore, have to be identified.

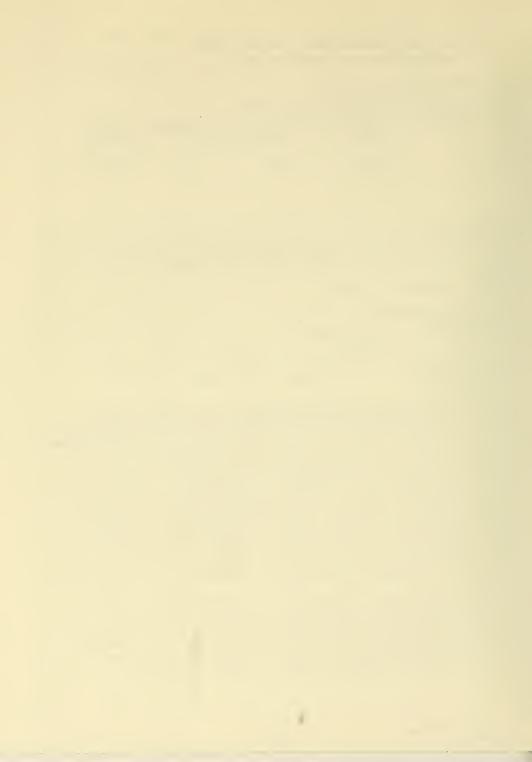
(c) Neither

(b) Human tissues

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.

CHECK APPROPRIATE BOX(ES) (a) Human subjects

(a1) Minors



PROJECT NUMBER

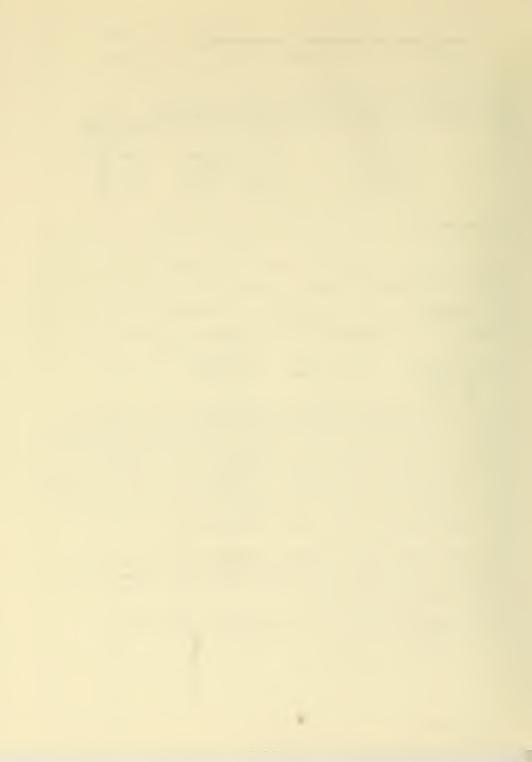
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NOTICE OF INTRAMURAL RESEARCH PROJECT

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October 1, 1986 to						
TITLE OF PROJECT (80 character						
Oncogene Expression		*				
PRINCIPAL INVESTIGATOR (List of				or.) (Name, title, labore		
PI: R.	J. Fi	sher	Expert		LMO	NC I
				c 11		NOT
	K. Bh			g Fellow	LM0	NCI
	Fujiw			g Fellow	LM0	NC I
	Ascio			n Chemist	LM0	NCI
1.	S. Pa	pas	Chief		LM0	NCI
				_		
COOPERATING UNITS (if any)						
LAB/BRANCH						
Laboratory of Molec	ular	Oncology				
SECTION OF MOTEC	ulai	oncorogy				
Office of the Chief	:					
INSTITUTE AND LOCATION						
NCI, NIH, Frederick	Mar	yland 21701-1013				
TOTAL MAN-YEARS:		PROFESSIONAL:	01	HER:		
1.5	1	1.5		0.0)	
CHECK APPROPRIATE BOX(ES)						
(a) Human subjects		(b) Human tissues	□ (c) Neither		
(a1) Minors		_ ,,				
(a2) Interviews						
SUMMARY OF WORK (Use stands	rd unredu	ced type. Do not axceed the spa	ce provided.)			
Analysis of c-ets of	ene e	xpression during o	cell pro	liferation an	nd differe	entiation
indicate that (i) t	he et	s-1 and ets-2 gene	es are a	ctivated by	serum add	ition to
quiescent fibroblas	t cel	Is before DNA synt	thesis;	(ii) the inc	rease in t	the level of
ets mRNAs is due to	an i	ncrease in the tra	anscript	ion of these	genes and	d stabiliza-
tion of their mRNA;						
not the ets-1 mRNA,	leve	1 increases before	DNA sy	nthesis; (iv	the ets	-1 and ets-2
genes are different	ially	regulated; (v) in	n vivo e	ts-2 gene exp	ression	is regulated
mainly at the post-	-trans	criptional level;	(vi) ad	dition of TP/	A to HL60	cells
appears to stabiliz	e bot	h ets-1 and ets-2	mRNAs;	and (vii) sub	ocellular	fractiona-
tion indicates that	: 56 k	Da protein is loca	alized i	n the nucleus	, whereas	s 55 kDa
ets-1 protein local						
These results sugge	est th	at the ets-2 gene	product	s accumulate	well before	ore 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.



PROJECT NUMBER

Z01CP05483-02 LM0

NOTICE OF INTRAMURAL RESEARCH PROJECT

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.) (Neme, title, leboretory, and institute affiliation)
PI: D. L. Court Research Biologist LMO NCI
Others: H. E. Takiff Guest Researcher LMO NCI

R. J. Fisher Expert LMO NC I T. A. Patterson Biotechnology Fellow I MO NCT S-M. Chen Guest Researcher LMO NC I T. L. Wigle Biologist LMO NCT

COOPERATING UNITS (If env) 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)

LAB/BRANCH

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

CHECK APPROPRIATE BOX(ES)

(a) Human subjects

(c) Neither

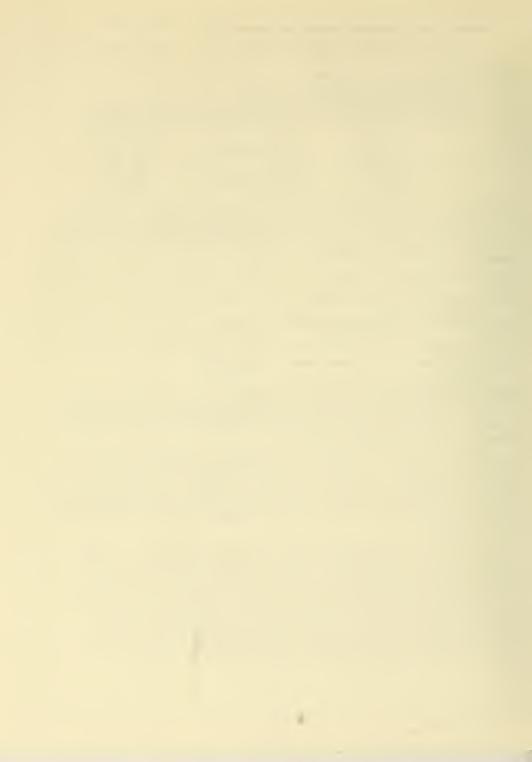
0.0

(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 $\underline{\mathbf{E}}$. $\underline{\mathbf{coli}}$. 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 <u>int</u> gene of phage λ is transcribed from two promoters yielding different mRNA transcripts. <u>Int</u> expression from one is reduced by RNaseIII; from the other, expression is <u>enhanced</u>. 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.

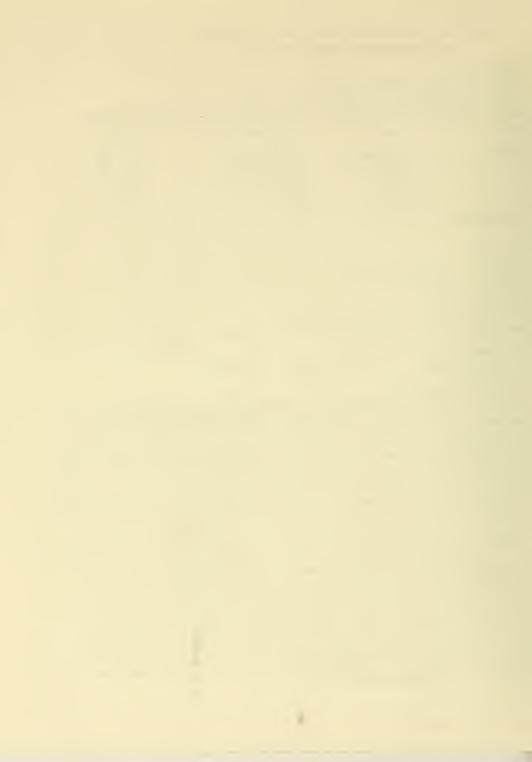


NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05484-02 LM0

October 1, 198	36 to September 30, 198	7	
	characters or less Title must lit on one line ets in Sea Urchin and		
PRINCIPAL INVESTIGAT	OR (List other professional personnel beid	w the Principal Investigator) (Name, title, lab	oratory and institute affiliation)
PI:	ZQ. Chen	Visiting Associate	LMO NCI
Others:	J. A. Lautenberger S. Fujiwara R. J. Fisher R. Ascione T. S. Papas	Research Chemist Visiting Fellow Expert Research Chemist Chief	LMO NCI LMO NCI LMO NCI LMO NCI LMO NCI
COOPERATING UNITS (if any)	_	
Laboratory of	Molecular Oncology		
Office of the	Chief		
NCI, NIH, Fred	on derick, Maryland 21701	-1013	
TOTAL MAN-YEARS. 1.8	PROFESSIONAL 1.8	OTHER	0.0
CHECK APPROPRIATE I (a) Human su (a1) Minor (a2) Interv	bjects (b) Human t	issues 🗓 (c) Neither	

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.



PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05485-02 LM0

PERIOD COVERED						
		ember 30, 1987				
	•	. Title must fit on one line be				
		al Antibodies to				
	IGATOR (List other pro	ofessional personnal below th	a Principal Inves	tigator.) (Name, titla, labor		
PI:	R. J. Fish	er	Expert		LMO	NC I
Others:	S. Fujiwar	a	Visiting	Fellow	LMO	NCI
	N. K. Bhat		Visiting	Fellow	LMO	NCI
	T. S. Papa	s	Chief		LMO	NC I
COOPERATING UN	ITS (if any)					
BRI-Basic F	esearch Prog	ram, Frederick,	MD (A. S	eth)		
LAB/BRANCH						
	of Molecular	Oncology				
SECTION						
	sis Regulati	on Section				
INSTITUTE AND LO						
	rederick, Ma		013	,		
TOTAL MAN-YEARS		PROFESSIONAL:		OTHER:		
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(a) Human		(b) Human tissu	jes 🗆	(c) Neither		
(a1) M						
	terviews					
C-myc and C	-ets (1 and	duced type. Do not exceed the 2) genes are ce	e space provide llular ho	mologues of th	ne oncogenes	carried
by the avia	n myelocytom	atosis virus MC:	29 and th	e avian acute	leukemia vir	us E26.
		nes are suspect				
		malignancy. Pro				
		was planned for				
		on of these prod				

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 $\overline{53}$ 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.

(中央)を引き



PROJECT NUMBER

Z01CP05515-01 IMO

NOTICE OF INTRAMURAL RESEARCH PROJECT PERIOD COVERED October 1, 1986 to September 30, 1987 TITLE OF PROJECT (80 characters or lass. 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 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) LAR/RRANCH Laboratory of Molecular Oncology

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.) The replication-defective avian erythroblastosis virus, E26, induces a mixed erythroid/myeloid leukemia in chickens. E26 includes elements from two protooncogenes, 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 polyadenylation 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 <u>erg</u> gene and analysis of the <u>erg</u> 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.



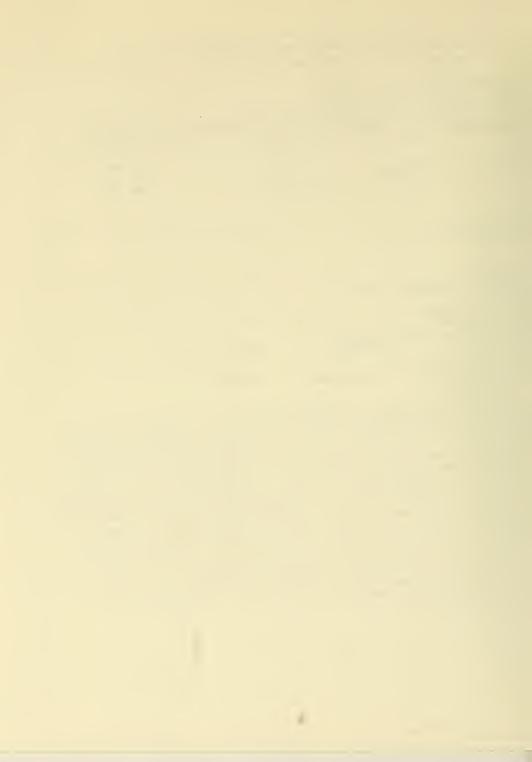
PROJECT NUMBER

	NOTICE OF INTRAMURAL F	RESEARCH PROJECT	Z01CP05516-01 LM0			
	October 1, 1986 to September 30,	1987				
	TITLE OF PROJECT (80 characters or less. Title must fit on o	ne line between the borders)				
	Characterization of Normal and On	cogenic ras Proteins				
	PRINCIPAL INVESTIGATOR (List other professional personnel	below the Principal Investigator.) (Neme, title, lebore	atory, and institute affiliation)			
	PI: T. Y. Shih	Research Chemist	LMO NCI			
	Others: P. Saikumar	Visiting Fellow	LMO NCI			
ı		Senior Staff Fellow	LMO NCI			
ľ		Microbiologist	LMO NCI			
			LIIO NOI			
	COOPERATING UNITS (if any)					
ì	LAB/BRANCH					
l	Laboratory of Molecular Oncology					
l	SECTION					
Ļ	Office of the Chief					
l	INSTITUTE AND LOCATION	3				
	NCI, NIH, Frederick, MD 21701-101	.3				
	TOTAL MAN-YEARS: PROFESSIONAL:	OTHER:				
	1	.0	1.0			
	CHECK APPROPRIATE BOX(ES) (a) Human subjects					
	SUMMARY OF WORK (Use standard unreduced type. Do not a	xceed the space provided.)				
	To understand the molecular princi	ples involved in the cellula	r transformation by			
	ras oncogenes has been the major f	ocus of this project. The u	inderstanding of the			
	structure-function relationship of	ras protein is important in	this regard. As a			
	necessary prefude, we have channed	V-H-ras IINA at two pocition	or with made			
	crons by orrgonucleotide-directed	MULAGenesis. The v-ras prot	ain differe from the			
	ecitata proto-oncodene product at	The amino acid positions 12	and 50 Ho have			
	oprained murants 15k/591 (edulvale	nt to v-ras), 12R/59A 12R/5	OS 120/50T 120/500			
	and 120/39A (equivalent to c-ras).	We compared some of the hi	ochomical proportion			
	or these proteins, especially quan	ine nucleotide binding nucl	entide exchange and			
	dirase activities. Our preliminar	V results indicate that sino	le noint mutations			
ľ	ercher at positions 12 or 59, prod	uce oncodenic activation ref	lected in their			
	GTPase activity (lowered). Position 59 is important in the nucleotide exchange					

transducing role of p21.

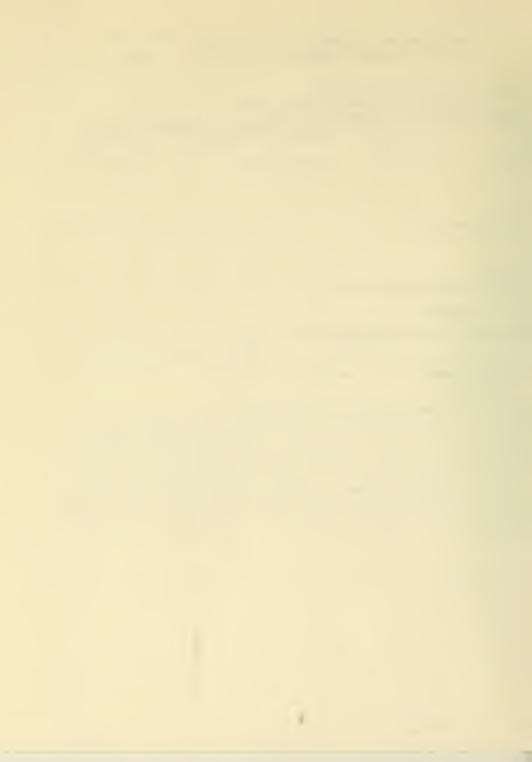
of ras proteins. Threonine or serine are required for higher rate of exchange. Alamine at position 59 renders a poor exchange of nucleotides. Thus, altered amino acids at positions 12 and 59 sustain the v-ras protein in an activated state. Our studies are continuing in the direction of understanding the signal-

PENDAGE.



PROJECT NUMBER

NOTICE OF IN			
	TRAMURAL RESEARC	H PROJECT	Z01CP05517-01 LM0
PERIOD COVERED			
October 1, 1986 to Sept	ember 30, 1987		
TITLE OF PROJECT (80 cheracters or les	s. Title must fit on one line between	en the borders.)	
Changes in Transcription	n Induced by myc D	rotain	
PRINCIPAL INVESTIGATOR (List other pr	rofessionel personnel below the Pri	ncipel Investigetor.) (Name, title, la	boratory, and institute affiliation)
PI: J. P. Bad	er Resear	ch Microbiologist	LMO NC I
Others: M. Ohtsuk	a Visiti	ng Fellow	LMO NCI
COOPERATING UNITS (if any)			
LAB/BRANCH			
Laboratory of Molecular	Oncology		
SECTION			
Office of the Chief			
NCI, NIH, Frederick, Mai	nuland 21701 1012		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:	
1.0	1.0		0.0
CHECK APPROPRIATE BOX(ES)			0.0
(a) Human subjects	(b) Human tissues	(c) Neither	
(a1) Minors			
(a2) Interviews			
SUMMARY OF WORK (Use standard unred	uced type. Do not exceed the spe	ce provided.)	
Cells transformed by the	aviali MC29 Virus	and related viruse	s assume a charac-
	loananco which diff		
transformed by other vir	pearance which diff	ers from the change	es seen in cells
cransformed by other vir	ruses or other agen	its. We are interes	ctod in identifui-
transformed by other vir transcriptional changes these myc-containing vir	ruses or other agen which occur specif ruses, which may di	its. We are interestically as a result	sted in identifying of infection with
transcriptional changes these myc-containing vir all cells transformed to	ruses or other agen which occur specif ruses, which may di o malignancy Rat	its. We are interestically as a result ffer from general of the second s	sted in identifying of infection with changes which occur in
transcrimed by other vir transcriptional changes these <u>myc</u> -containing vir all cells transformed to virus, and clones of tra	ruses or other agen which occur specifruses, which may dio malignancy. Rat	its. We are interestically as a result ffer from general cembryo cells were	sted in identifying of infection with changes which occur in infected with SV40
transformed by other virtranscriptional changes these myc-containing vir all cells transformed to virus, and clones of tracconstructs containing the	ruses or other agen which occur specificuses, which may di malignancy. Rat unsformed cells wer	its. We are interestically as a result ffer from general embryo cells were es superinfected with a lyadonylator moss.	sted in identifying of infection with changes which occur in infected with SV40 th murine retrovirus
transformed by other virtranscriptional changes these myc-containing vir all cells transformed to virus, and clones of tracconstructs containing thand cDNA libraries were	ruses or other agen which occur specificuses, which may di malignancy. Rat insformed cells were myc oncogene. P	its. We are interestically as a result ffer from general embryo cells were es superinfected with olyadenylated messoned in bactarioph	sted in identifying of infection with changes which occur in infected with SV40 th murine retrovirus enger RNA was isolated,
transformed by other virtranscriptional changes these myc-containing vir all cells transformed to virus, and clones of tracconstructs containing thand cDNA libraries were repression of mRNAs by m	ruses or other agen which occur specificuses, which may di malignancy. Rat insformed cells were myc oncogene. P	its. We are interestically as a result ffer from general embryo cells were es superinfected with olyadenylated messoned in bactarioph	sted in identifying of infection with changes which occur in infected with SV40 th murine retrovirus enger RNA was isolated,
transformed by other virtranscriptional changes these myc-containing vir all cells transformed to virus, and clones of tracconstructs containing thand cDNA libraries were repression of mRNAs by m	ruses or other agen which occur specificuses, which may di malignancy. Rat insformed cells were myc oncogene. P	its. We are interestically as a result ffer from general embryo cells were es superinfected with olyadenylated messoned in bactarioph	sted in identifying of infection with changes which occur in infected with SV40 th murine retrovirus enger RNA was isolated,
transformed by other virtranscriptional changes these myc-containing vir all cells transformed to virus, and clones of tracconstructs containing thand cDNA libraries were repression of mRNAs by m	ruses or other agen which occur specificuses, which may di malignancy. Rat insformed cells were myc oncogene. P	its. We are interestically as a result ffer from general embryo cells were es superinfected with olyadenylated messoned in bactarioph	sted in identifying of infection with changes which occur in infected with SV40 th murine retrovirus enger RNA was isolated,
transformed by other virtranscriptional changes these myc-containing vir all cells transformed to virus, and clones of tracconstructs containing thand cDNA libraries were repression of mRNAs by m	ruses or other agen which occur specificuses, which may di malignancy. Rat insformed cells were myc oncogene. P	its. We are interestically as a result ffer from general embryo cells were es superinfected with olyadenylated messoned in bactarioph	sted in identifying of infection with changes which occur in infected with SV40 th murine retrovirus enger RNA was isolated,
transformed by other virtranscriptional changes these myc-containing vir all cells transformed to virus, and clones of tracconstructs containing thand cDNA libraries were repression of mRNAs by m	ruses or other agen which occur specificuses, which may di malignancy. Rat insformed cells were myc oncogene. P	its. We are interestically as a result ffer from general embryo cells were es superinfected with olyadenylated messoned in bactarioph	sted in identifying of infection with changes which occur in infected with SV40 th murine retrovirus enger RNA was isolated,
transformed by other virtranscriptional changes these myc-containing vir all cells transformed to virus, and clones of tracconstructs containing thand cDNA libraries were repression of mRNAs by m	ruses or other agen which occur specificuses, which may di malignancy. Rat insformed cells were myc oncogene. P	its. We are interestically as a result ffer from general embryo cells were es superinfected with olyadenylated messoned in bactarioph	sted in identifying of infection with changes which occur in infected with SV40 th murine retrovirus enger RNA was isolated,
transformed by other virtranscriptional changes these myc-containing vir all cells transformed to virus, and clones of tracconstructs containing thand cDNA libraries were repression of mRNAs by m	ruses or other agen which occur specificuses, which may di malignancy. Rat insformed cells were myc oncogene. P	its. We are interestically as a result ffer from general embryo cells were es superinfected with olyadenylated messoned in bactarioph	sted in identifying of infection with changes which occur in infected with SV40 th murine retrovirus enger RNA was isolated,
transformed by other virtranscriptional changes these myc-containing vir all cells transformed to virus, and clones of tracconstructs containing thand cDNA libraries were repression of mRNAs by m	ruses or other agen which occur specificuses, which may di malignancy. Rat insformed cells were myc oncogene. P	its. We are interestically as a result ffer from general embryo cells were es superinfected with olyadenylated messoned in bactarioph	sted in identifying of infection with changes which occur in infected with SV40 th murine retrovirus enger RNA was isolated,
transformed by other virtranscriptional changes these myc-containing vir all cells transformed to virus, and clones of tracconstructs containing thand cDNA libraries were repression of mRNAs by m	ruses or other agen which occur specificuses, which may di malignancy. Rat insformed cells were myc oncogene. P	its. We are interestically as a result ffer from general embryo cells were es superinfected with olyadenylated messoned in bactarioph	sted in identifying of infection with changes which occur in infected with SV40 th murine retrovirus enger RNA was isolated,
transformed by other vir transcriptional changes these myc-containing vir all cells transformed to virus, and clones of tra constructs containing th and cDNA libraries were repression of mRNAs by m investigation.	ruses or other agen which occur specificuses, which may di malignancy. Rat insformed cells were myc oncogene. P	its. We are interestically as a result ffer from general embryo cells were es superinfected with olyadenylated messoned in bactarioph	sted in identifying of infection with changes which occur in infected with SV40 th murine retrovirus enger RNA was isolated,
transformed by other virtranscriptional changes these myc-containing vir all cells transformed to virus, and clones of tracconstructs containing thand cDNA libraries were repression of mRNAs by m	ruses or other agen which occur specificuses, which may di malignancy. Rat insformed cells were myc oncogene. P	its. We are interestically as a result ffer from general embryo cells were es superinfected with olyadenylated messoned in bactarioph	sted in identifying of infection with changes which occur in infected with SV40 th murine retrovirus enger RNA was isolated,
transformed by other virtranscriptional changes these myc-containing vir all cells transformed to virus, and clones of tracconstructs containing thand cDNA libraries were repression of mRNAs by m	which occur specificuses, which may divide myc oncogene. Programmer may constructed and cluyc using these lib	its. We are interestically as a result ffer from general embryo cells were es superinfected with olyadenylated messoned in bactarioph	sted in identifying of infection with changes which occur in infected with SV40 th murine retrovirus enger RNA was isolated,



PROJECT	NUMBER	

701CP05101-09 LMV

NOTICE OF I	INTRAMURAL	RESEARCH	PROJECT
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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 personnal below the Principal investigator.) (Name, title, laboratory, and institute affiliation)

PI: Gilbert Jay

Chief, Cell Physiology Section

NCT LMV

Others:

Steven Hinrichs Medical Staff Fellow Michael Nerenberg Medical Staff Fellow Kazuhiko Koike Visiting Fellow

NCT 1 MV LMV NCT 1 MV NCI

COOPERATING UNITS (if any)

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

LAB/BBANCH

Laboratory of Molecular Virology

SECTION

Cell Physiology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: PROFESSIONAL:

CHECK APPROPRIATE BOX(ES)

(b) Human tissues (c) Neither

OTHER: 0

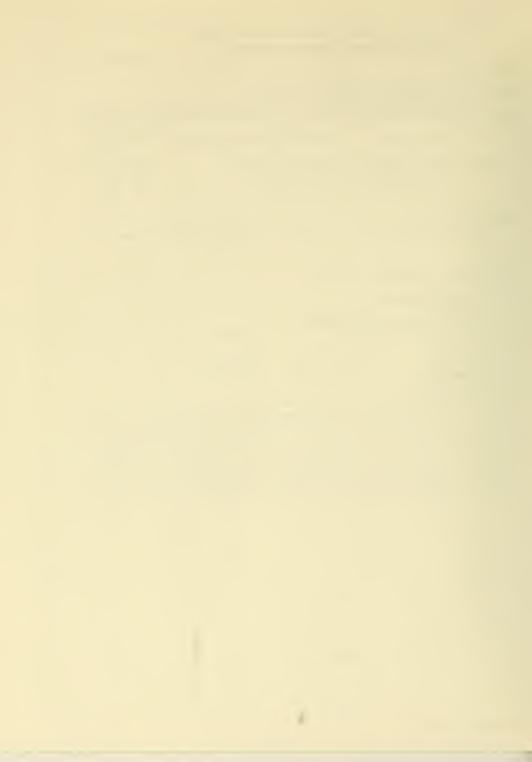
(a) Human subjects (a1) Minors

(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.

- ALES - 6785/6-



PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05216-07 LMV

				1	
PERIOD COVERED					
		September 30, 19			
		must fit on one line between the	e borders.)		
<u>Ras</u> Onco	gene Regulation	in Yeast			
PRINCIPAL INVESTI	GATOR (List other profession	nal personnel below the Principa	al Investigator.) (Name, title,	laboratory, and institu	ute affillation)
PI:	Ravi Dhar	Visiting Scie	entifist	LMV	NCI
Others:	T.L.V. Sreenat	h Visiting Fell	OW	LMV	NCI
	Richard Koller			LMV	NCI
		Ĭ			
COORERATING UNI	TC //4				
COOPERATING UNI	15 (if any)				
None					
LAB/BRANCH					
	ny of Moloculan	Vinology			
Laboratory of Molecular Virology SECTION					
Virus Tumor Biology Section					
INSTITUTE AND LO		CTON			
	, Bethesda, Mar	vland 20892			
TOTAL MAN-YEARS		FESSIONAL:	OTHER:		
	3.0	2.0	1.0		
CHECK APPROPRIA			1.0		
(a) Human		(b) Human tissues	(c) Neither		
☐ (a1) Mi		(-, -, -, -, -, -, -, -, -, -, -, -, -, -	(-,		
_ ` `	erviews				
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)					

Expression of the rasl and ras2 genes of Saccharomyces cerevisiae has been examined at the transcriptional and translational levels. In cells grown with glucose as carbon source, rasl mRNA and rasl 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 Gl-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.

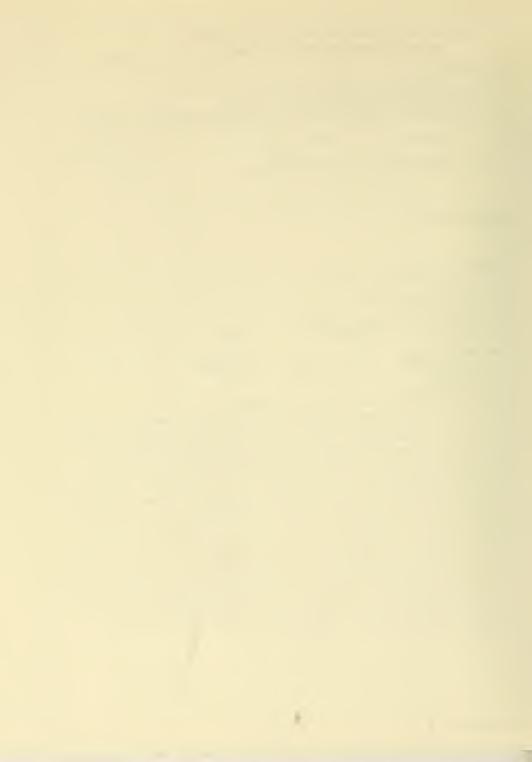
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PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT			Z01CP05217-07 LMV		
October 1, 1986 through September 30, 1987					
Studies on the Regulat	ss. Title must fit on one line between the borders.) tion of SV40 Gene Expression				
PRINCIPAL INVESTIGATOR (List other p	professional personnel below the Principal Investigator.) (Name, title, labor	atory, and in	nstitute effiliation)		
P.I.: John Brady	Expert	LMV	NC I		
Others: Kamel Khalili Jeffrey Green	. The string is a	LMV LMV	NC I NC I		
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: 0.7	PROFESSIONAL: OTHER: 0				
CHECK APPROPRIATE BOX(ES) (a) Human subjects (a1) Minors (a2) Interviews	☐ (b) Human tissues ☒ (c) Neither				
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)					

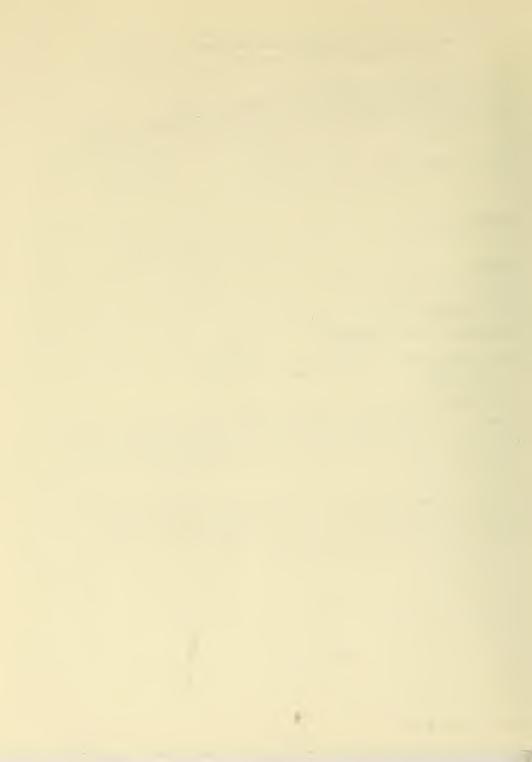
Simian virus 40 (SV40) has two early transcriptional units that are transcribed from a region near the origin of replication. The early-early (EE) transcription unit, whose RNA encodes a large T-antigen and small t-antigen, predominates at early times post infection. The late-early (LE) transcription 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 potential 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.



PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT 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 effiliation)
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 (# any)
Department of Biology, The Johns Hopkins University (George Scangos)
LAB/BRANCH Laboratory of Molecular Virology
SECTION
Cell Physiology Section NSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892
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.)
We have cloned and analyzed cDNA sequences derived from a family of
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 possessions.
cicularly with regard to the regulation of their expression in both normal and
concer ceris.
We have studied the expression and function of the human interleukin-2 receptor.
can bind interleukin-2 efficiently, and may function to regulate the inter-
Accion becween interregional and its call surface recentes by using DNA
nediated gene transfer, we have demonstrated that the interleukin-2 receptor can function effectively in nonlymphoid cells.
distance,



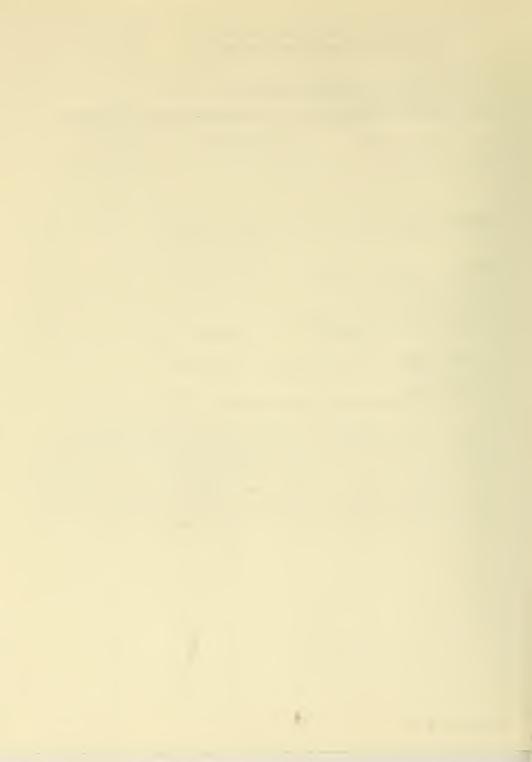
PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05254-06 LMV

PERIOD COVERED					
October 1, 1986 thro	ough September 30, 1987				
	less. Title must fit on one line between the	ne borders.)			
Regulation of Gene E	XD CESSION r professional personnel below the Princip	al Investigator.) (Neme, title,	laboratory, and institute affiliation)		
			,		
PI: Kuan-Teh Je	ang Medical St	aff Fellow	LMV NCI		
Others: John Brady	Expert		LMV NCI		
COOPERATING UNITS (if any)					
	nstitute, Boston, MA (David Livingsto	n)		
LAB/BRANCH	7 14 2				
Laboratory of Molecu	ilar Virology				
	Section				
Virus Tumor Biology INSTITUTE AND LOCATION	3,0,1,0,1				
NCI, NIH, Bethesda,					
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:			
1.4 CHECK APPROPRIATE BOX(ES)	1.4		0		
(a) Human subjects (a1) Minors (a2) Interviews	☐ (b) Human tissues				
SUMMARY OF WORK (Use standard to	nreduced type. Do not exceed the space	provided.)			
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.					

en vance

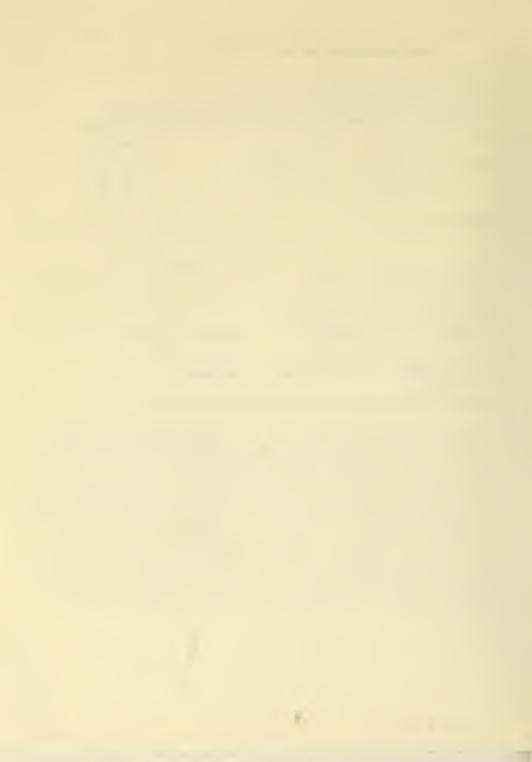


PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT							01CP	05354-05 LMV
	PERIOD COVERED October 1, 1986 through September 30, 1987							
Studies	OT (80 ch On the	eracters or less Activat	Title must fit on one line ed Form of the	between the borde Human Pro	oto-oncogene	, с-На	-ras	
PRINCIPAL INVE	STIGATOR	(List other pro	fessional personnel below	the Pnncipal Inves	tigetor.) (Name, title, I	laboratory,	and ins	trtute effilietion)
PI:	John	Brady	Expert				LMV	NCI
Others:	Mary	Pozzatti McCormic Liotta		esearcher Staff Fello	w		LMV LMV LP	NCI NCI
COOPERATING U	JNITS (# e	nv)						
None		,						
Laborato	ry of	Molecula	r Virology					
Virus Tu	mor Bi	iology Se	ction					
NCI, NIH			ryland 20892					
TOTAL MAN-YEAR	RS: 2.2		PROFESSIONAL: 2.2		OTHER:	0		
CHECK APPROPE (a) Huma (a1) (a2)	an subj	ects	(b) Human tis	sues 🗵	(c) Neither			
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.								

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 <u>ras</u> oncogene alone was observed; however, a 10-fold increase in the transformation frequency was obtained when <u>ras</u> was cotransfected with the adenovirus E1A gene. We have examined cell lines transformed by the <u>ras</u> oncogene alone, and by <u>ras</u> plus E1A and have observed a striking difference in their metastatic potential as assayed in nude mice. Specifically, the <u>ras</u> 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 <u>ras</u> 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 <u>ras</u> 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.

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PROJECT NUMBER

DEPART	MENT OF HEALTH	AND HUMAN SE	RVICES - P	UBLIC HE	ALTH SERVICE					
	NOTICE OF INT	FRAMURAL F	ESEARC	H PRO	JECT		ZOICE	P05355	-05 LMV	
	, 1986 throug									
Regulatio	on of Immune S	urveillance	e Agains	st Tumo	or Cells					
PRINCIPAL INVES	TIGATOR (List other pro	ofessional personnel	below the Pr	incipal Inve	stigator.) (Neme, til	de, laborat	ory, and ins	trtute affille	tion)	Ī
PI:	Gilbert Jay		Chief,	Cell F	Physiology	Secti	on	LMV	NCI	
Others:	Roberta Reyn				robiologist	:		LMV	NCI	
	Takayuki Yos	птока	Visitir	ig reii	IOW			LMV	NCI	
COOPERATING U	NITS (if any)									Г
(Sidney S	t of Pharmaco trickland)	logy, State	e Univer	rsity	of New York	at S	tony Bi	rook		
AB/BRANCH										Ī
Laboratory of Molecular Virology										
SECTION										
	iology Sectio	n								
NSTITUTE AND LO										
	Bethesda, Ma		392							
OTAL MAN-YEAR	S:	PROFESSIONAL:			OTHER:					ĺ

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

(b) Human tissues

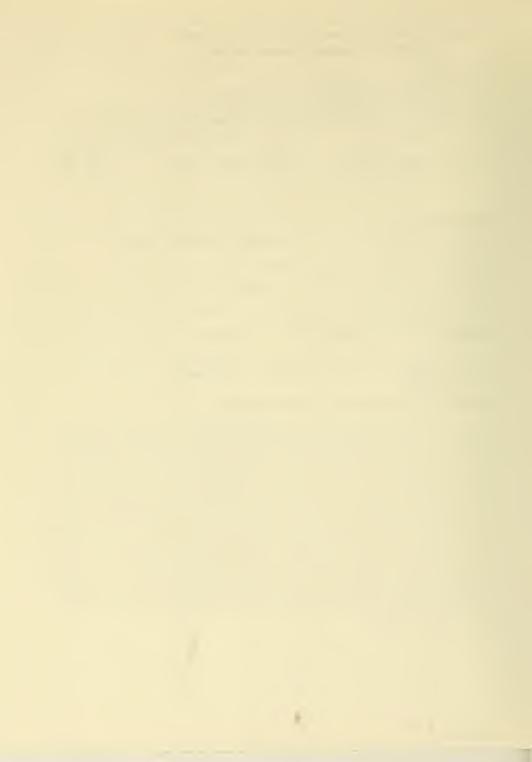
CHECK APPROPRIATE BOX(ES) (a) Human subjects

> (a1) Minors (a2) Interviews

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 selfnonself 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.

277

(c) Neither



PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

701CP05390-04 LMV

NCT

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 Jav Chief, Cell Physiology Section

Others:

Jonathan Vogel Lian-Sheng Chen

Medical Staff Fellow Visiting Fellow

1 MV NCI I MV NCI

COOPERATING UNITS (if any)

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

LABBERANCH Laboratory of Molecular Virology

SECTION Cell Physiology Section

INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS PROFESSIONAL . 1.7

OTHER: 1.7

CHECK APPROPRIATE BOX(ES) (a) Human subjects

(a1) Minors

(b) Human tissues

eth-project-

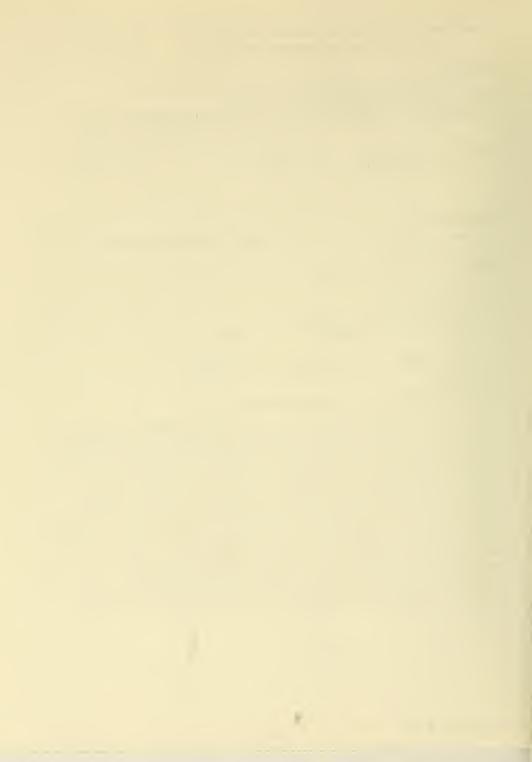
X (c) Neither

n

(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 DNĂmediated 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 (Adl2) tumors was observed with intramuscular injections of interferon. Interestingly, Adl2 tumor cells treated with interferon can immunize mice against untreated Ad12 tumors.



PROJECT NUMBER

701CP05391-04 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 1 MV NC I Others: Lionel Feigenbaum Microbiologist LMV NC I Kamel Khalili Visiting Fellow LMV NC I COOPERATING UNITS (if any) National Institute of Neurological and Communicative Disorders and Stroke, NIH (Eugene Major) LAB/BRANCH Laboratory of Molecular Virology Virus Tumor Biology Section NCI, NIH, Bethesda, Maryland 20892 TOTAL MAN-YEARS: PROFESSIONAL: OTHER: 0.5 2.0

CHECK APPROPRIATE BOX(ES) 1.5

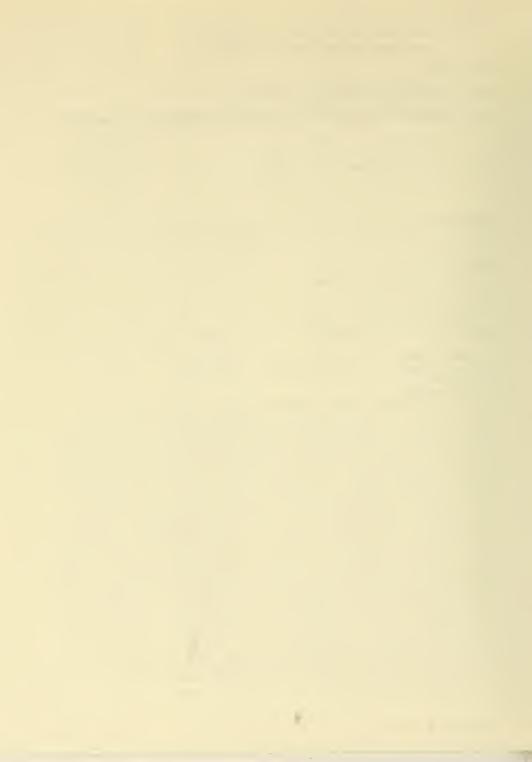
X (c) Neither

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

(a) Human subjects (a1) Minors (a2) Interviews

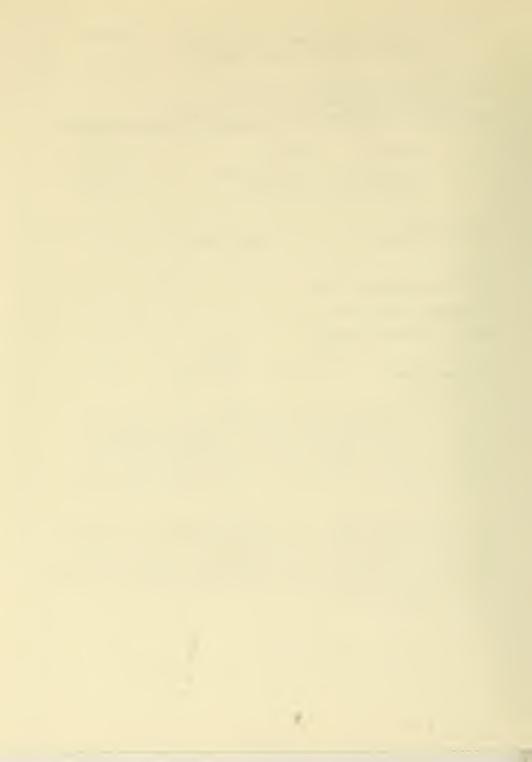
(b) Human tissues

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 antiqen (T-antiqen), 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 speciesspecific factor, presumably a component of DNA polymerase, which is found in a wide range of primate cells. We further demonstrated that simian virus 40 Tantigen has sufficient homology to efficiently substitute for the analogous JCV protein in initiating viral DNA replication. We have used primer extension and S₁ 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.



PROJECT NUMBER DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT Z01CP05392-04 LMV PERIOD COVERED October 1, 1986 through September 30, 1987.

TILE 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 I MV NC T Others: Mary Loeken Guest Researcher LMV NC T 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 Virus Tumor Biology Section INSTITUTE AND LOCATION NCI_NIH_Bethesda, Maryland 20892
TOTAL MAN-YEARS: PROFESSIONAL: OTHER: 1.7 CHECK APPROPRIATE BOX(ES) (b) Human tissues (c) Neither (a) Human subjects (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 ElA 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. of oppos



PROJECT NUMBER

DEPARTMEN	TO OF HEALTH AND HUMAN SE	HVICES - PUBLIC HEALTH SERVICE		
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Effects o	of JC Virus Early Regi	on in Transgenic Mice		
PRINCIPAL INVESTIG	ATOR (List other professional personnel	below the Principal Investigator) (Name, title, labo	ratory, and institute ai	Milletion)
PI:	John Brady	Expert	LMV N	CI
Others:	Judy Small	Guest Researcher	LMV N	CI
	Lionel Feigenbaum		LMV N	
	Jeffrey Green	Biotechnology Fello		
	Kamel Khalili	Visiting Fellow	LMV N	
			2	•
COOPERATING UNITS	(if any)			
Departmen	t of Biology, The Joh	ns Hopkins University, Balt	imore, MD (G. Scangos
LAB/BRANCH				
Laborator	y of Molecular Virolo	y		
SECTION				
Virus Tum	or Biology Section			
		20892		
TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER:		
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(a) Human subjects

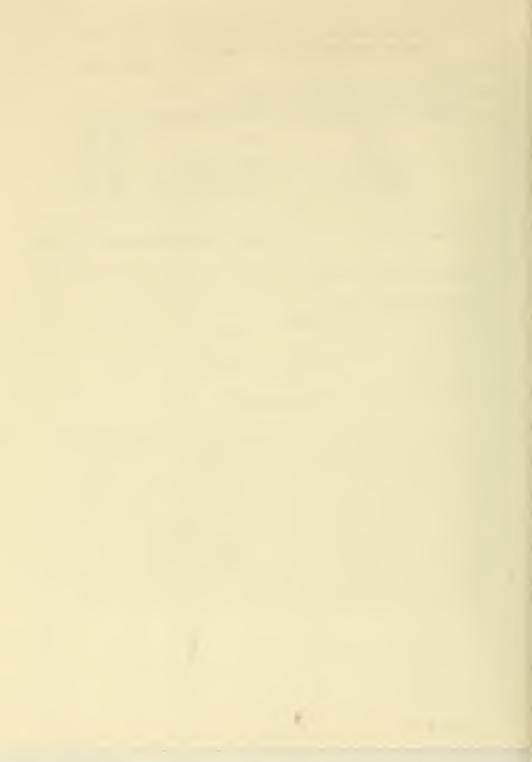
(a1) Minors (a2) Interviews

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.

(b) Human tissues (c) Neither

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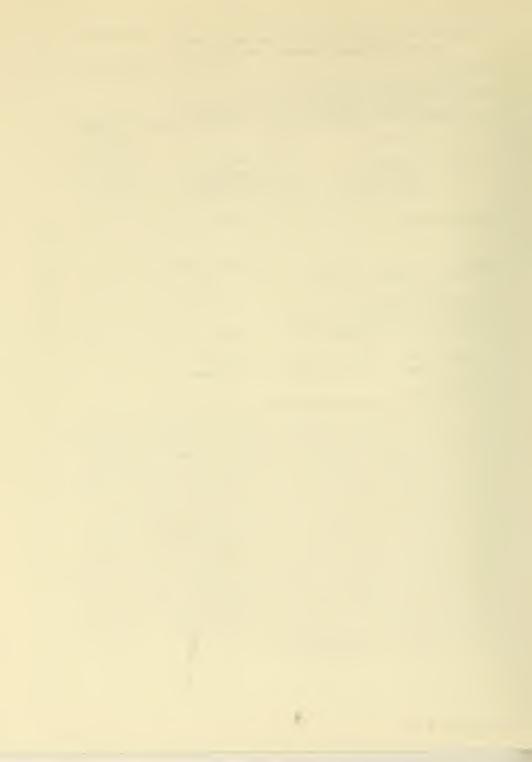


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PRINCIPAL INVESTIGA	ATOR (List other pro	fessional personnel bei	low the Pnncipa	al Investi	gator.) (Name. title,	laboratory, and ins	titute affilietion)	1
PI:	John Brady		Expert			LMV	NCI	
Others:	Imre Boros		Visiting	a Fel	1ow	LMV	NCI	
7 311.51.51	Chou-zen G		Guest Re			LMV	NCI	
	Kuan-Teh J		Medical	Staf	f Fellow	LMV	NCI	
		renberg			f Fellow	LMV	NCI	
COOPERATING UNITS	(d any)							
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None								
None								
LAB/BRANCH								
Laborato	ry of Molec	ular Virolog	y					
SECTION								
	nor Biology	Section						
INSTITUTE AND LOCA			000					
	, Bethesda,	Maryland 20	892					
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(a2) Inter								
SUMMARY OF WORK	lilisa standard unred	uced type. Do not exci	anang ant has	hehiven	1			

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.



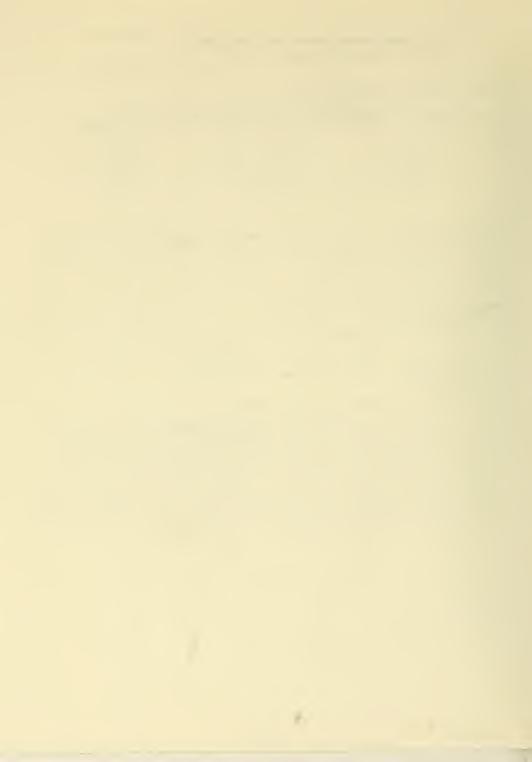
PROJECT NUMBER

701CP05534-01 LTCB

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	igocytes and Accessory C						
PRINCIPAL INVESTIGATOR (List other pro-			oratory, and institute affiliation)				
PI: M. Popovic	Visiting Scie	ntist	LTCB NCI				
Others: R.C. Gallo	Chief		LTCB NCI				
S. Gartner	Senior Staff	Fellow	LTCB NCI				
A. Minassian	Guest Researc	her	LTCB NCI				
H. Buchow	Guest Researc	her	LTCB NCI				
COOPERATING UNITS (if eny)							
Institute for Tropical	Disease, Hamburg, Germa	ny (P. Racz);	Karolinska Institute,				
Stockholm, Sweden (EM	1. Fenyo); Temple Univer	sity, Philade	lphia, PA (H. Uschner);				
Cornell University, NY	, NY (S. Pahwa)						
LAB/BRANCH							
Laboratory of Tumor Cel	ll 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 standard 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.

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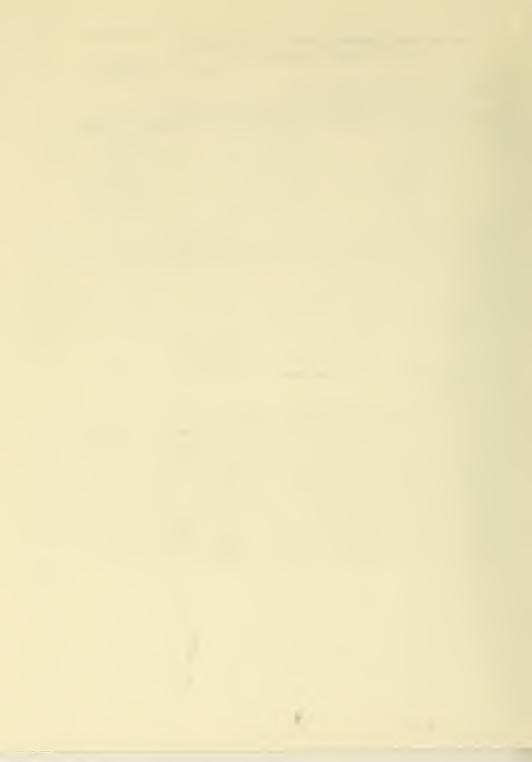
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	reatment, Prevention and		SP			
PRINCIPAL INVESTIGATOR (List other pro	ofessional personnel below the Principal Inves	tigator.) (Name, title, labora	tory, and institute affiliation)			
PI: P.S. Sarin	Research Chemi	st	LTCB NCI			
Others: R.C. Gallo	Chief		LTCB NCI			
Y. Taguchi	Visiting Fello		LTCB NCI			
M. Civeira	Guest Research	ner	LTCB NCI			
C.C. Gajdusek			CNSS NINCDS			
C.J. Gibbs	Deputy Chief		CNSS NINCDS			
P.R. Johnson	Visiting Scien	itist	CNSS NINCDS			
COOPERATING UNITS (if any)						
	rsity Medical Center, Wa					
	f); Worcester Fdn. for E					
	tern University, Chicago	o, IL (R. Letsi	nger)			
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Laboratory of Tumor Cel	1 Blology					
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:				
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(a1) Minors (a2) Interviews						
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			HIV-1 replication in			
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 syndr	ome (AIDS). Antisense o	ligonucleotides	s have also been			
found to be effective	in blocking human immuno	deficiency viru	us (HIV-1)			
replication. A syncyt	ia assay has been develo	ped and is beir	na utilized to			
measure the effect of	these drugs in HIV-1 rep	lication. Anti	bodies made against			
a 30 amino acid HIV-1	p17 synthetic peptide (H	GP30) were four	d to inhibit			
syncytia formation as	well as HIV-1 replication	n in H9 and Mol	t3 cells. HIV-1			
inoculation studies in	chimpanzees indicate the	e development d	f 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

Assistant.

further.



PROJECT NUMBER

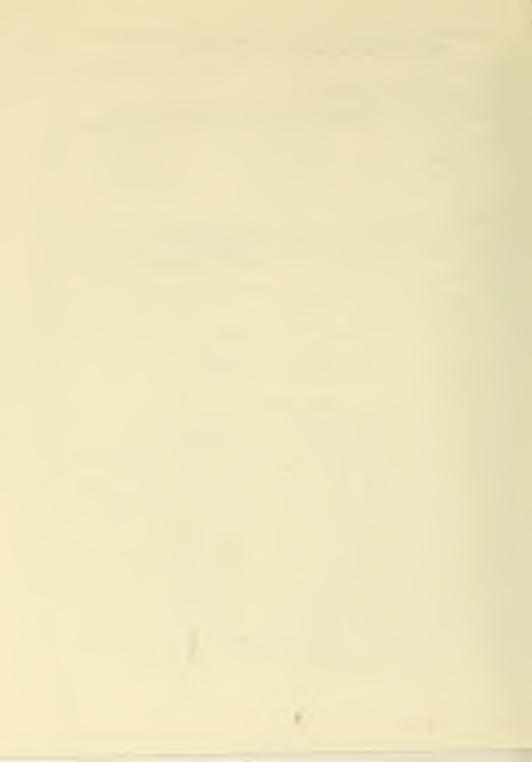
Z01CP05536-01 LTCB

PERIOD COVERED October 1, 1986 to September 30, 1987 TITLE OF PROJECT (80 characters or lass. 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 NCT M. Reitz Research Chemist LTCB NCI R Moss Chief LVD. NIAID J. Goedert Medical Officer EEB. NCI COOPERATING UNITS (# 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/BBANCH 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

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-1infected 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.

(a1) Minors
(a2) Interviews



PROJECT NUMBER

Z01CP05537-01 | TCR

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.) Immunopathogenesis of Human RNA and DNA Viruses PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute attiliation, PI: W.C. Saxinger Research Microbiologist LTCB NCI Others: R.C. Gallo Chief	
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunopathogenesis of Human RNA and DNA Viruses PRINCIPAL INVESTIGATOR (Ust other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation, PI: W.C. Saxinger Research Microbiologist LTCB NCI	
Immunopathogenesis of Human RNA and DNA Viruses PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation, PI: W.C. Saxinger Research Microbiologist LTCB NCI	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation PI: W.C. Saxinger Research Microbiologist LTCB NCI	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation PI: W.C. Saxinger Research Microbiologist LTCB NCI)
PI: W.C. Saxinger Research Microbiologist LTCB NCI	
Others: R C Gallo Chief	
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	

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

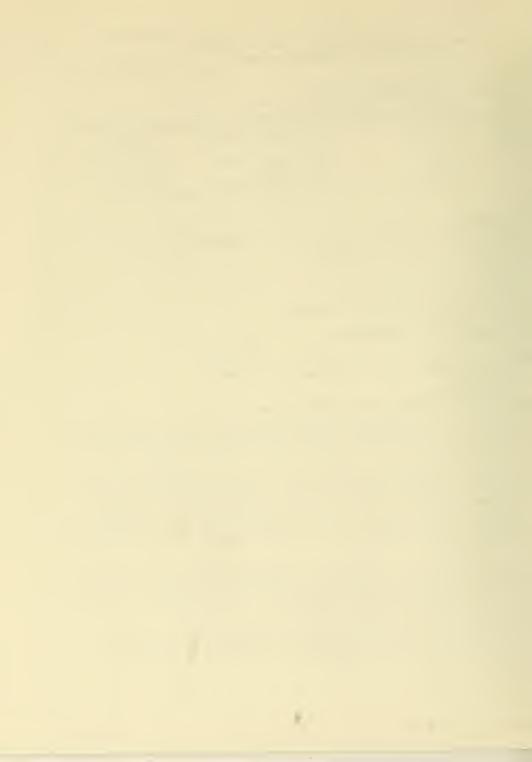
HIV serological testing: Work on the development of an improved serological test has been successfully concluded. The competition ELISA test is superior in both sensitivity and specificity to licensed tests, including the Western blot.

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.

PHS 6040 (Rev. 1/84)



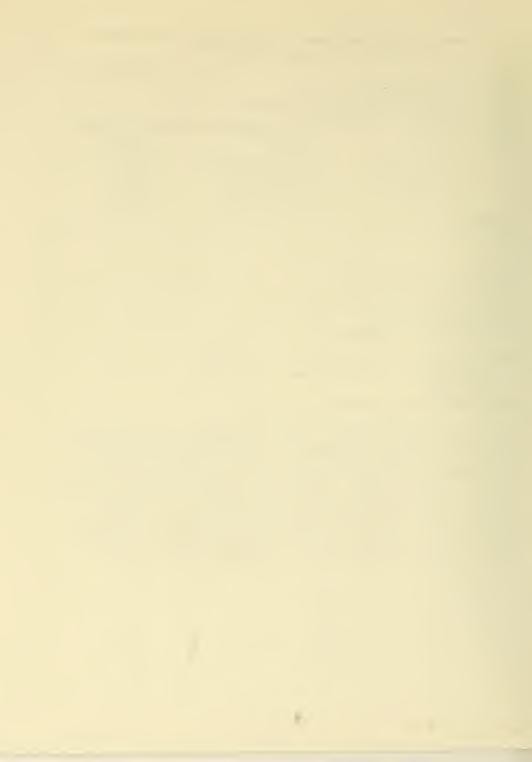
PROJECT NUMBER

Z01CP05538-01 LTCR

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oc cober 1	1986 to Sep	tember 30,	1987				
	CT (80 charecters or less and Function			the borde	rs.)		
PRINCIPAL INVE	STIGATOR (List other pro	fessional personnel					
	M. Reitz		Research	Chem	ist	LTCB	
Others:	R.C. Gallo		Chief			LTCB	NCI
	F. Wong-Staal		Research	Micr	obiologist	LTCB	NCI
	M. Robert-Gur	off	Research	Biol	ogist	LTCB	NCI
	M. Popovic		Visiting	Scie	ntist	LTCB	NCI
	G. Franchini		Guest Res			LTCB	
	HG. Guo		Visiting	Scien	ntist	LTCB	
			3			2.05	
NONE	NITS (if eny)						
LAB/BRANCH							
Laborator	y of Tumor Ce	ll Biology					
SECTION	J						
Molecular	Genetics of L	Hematopoiet	ics Cells	5			
INSTITUTE AND L							
NCI, NIH,	Bethesda, MD	20892					
TOTAL MAN-YEAR	RS:	PROFESSIONAL:			OTHER:		
	4.0		2.0		2.0		
CHECK APPROPE	RIATE BOX(ES)		-				
(a) Huma	an subjects	X (b) Huma	n tissues		(c) Neither		
(a1) I	Minors	` ′			(-,		
☐ (a2) I	nterviews						
SUMMARY OF WO	DRK (Use standard unred	uced type. Do not e	xceed the space	provided	1)		

Nucleotide sequence of the genome of simian T-lymphotropic virus type III from African green monkeys (STLV-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.

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PROJECT NUMBER

Z01CP05539-01 LTCB

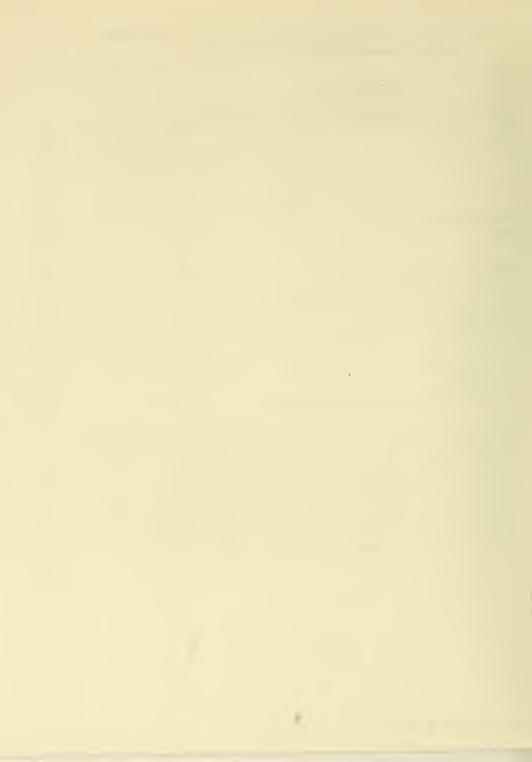
		2010/03339-01 [10]
PERIOD COVERED		
October 1, 1986 to Se	ptember 30, 1987	
TITLE OF PROJECT (80 characters or le	ss. Title must fit on one line between the borders.)	
Mapping of the Regula	tory Elements of Human Retroviruses	
PRINCIPAL INVESTIGATOR (List other p	professional personnel below the Principal Investigator.) (Name, title, li	aboratory, and institute affiliation)
1 PI: 5. Arya	Research Biologist	LTCB NCI
Others: R.C. Gallo	Chief	LTCB NCI
F. Wong-Staa	Research Microbiologist	LTCB NCI
		ETEB NOT
COOPERATING UNITS (if any)		
NONE		
LAB/BRANCH		
Laboratory of Tumor Ce	ll Biology	
SECTION		
Molecular Genetics of	Hematopoietic Cells	
INSTITUTE AND LOCATION		
NCI, NIH, Bethesda, MD	20892	
TOTAL MAN-YEARS	PROFESSIONAL: OTHER:	
_ 2.0	1.0	
CHECK APPROPRIATE BOX(ES)	1.0	
(a) Human subjects		
(a1) Minors	_ (-)	

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

(a2) Interviews

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 transactivators such as human T-lymphotropic virus-1 (HTLV-I) and oncogenic DNA viruses. DNA sequencing and functional mapping of the long terminal repeats (LTR), $3 \cdot total$ 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.

busania



PROJECT NUMBER

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			ZUICPU/140-U4 LICB				
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, leboratory, and institute affiliation)							
PI: R.C. Gallo	Chief		LTCB NCI				
Others: S.Z. Salahuddi	in Cancer Expert		LTCB NCI				
S. Nakamura	Visiting Scien	ntist	LTCB NCI				
K. Krohn	Visiting Scien	ntist	LTCB NCI				
A. Ranki	Guest Research	Guest Researcher LTCB NCI					
P.S. Sarin	Research Chem	ist	LTCB NCI				
W.C. Saxinger	Research Micro	obiologist	LTCB NCI				
M. Robert-Guro	off Research Biolo	ogist	LTCB NCI				
COOPERATING UNITS (if eny)							
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)							

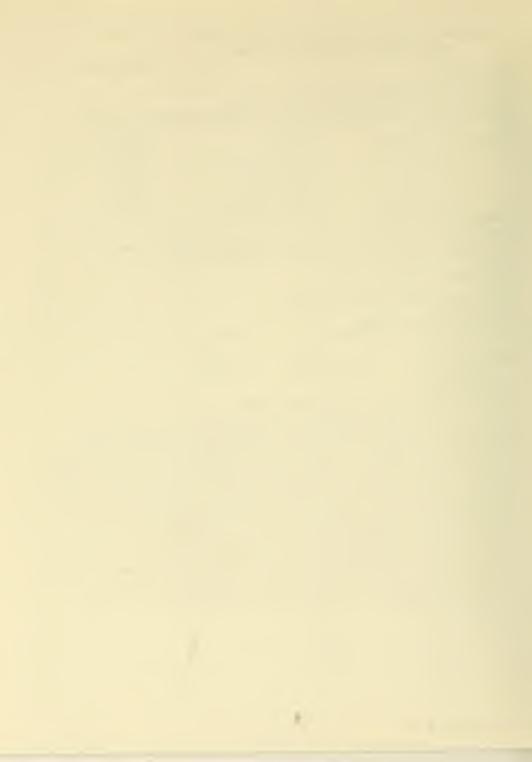
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

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

The cell biology studies have been focused on: (1) the role of human Tlymphotropic 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 monocytemacrophage 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 <u>in vitro</u> 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.

to made to

(a1) Minors
(a2) Interviews



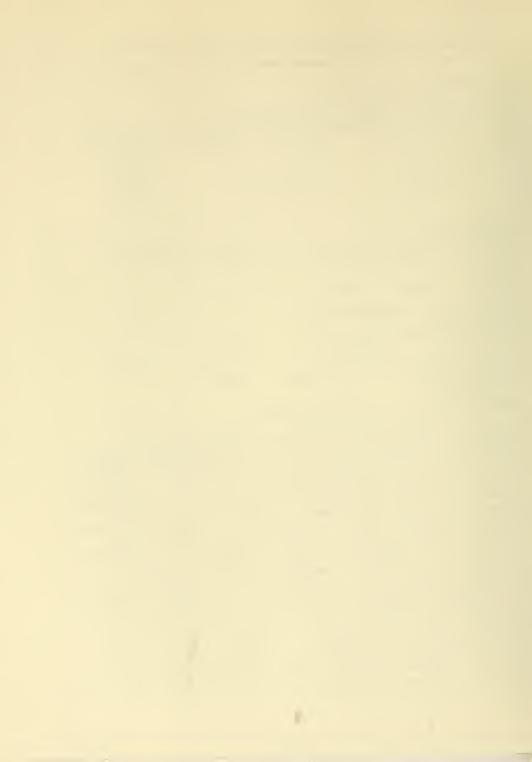
PROJECT NUMBER

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.) (Nama, title, laboratory, and institute affiliation) F. Wong-Staal Research Microbiologist LTCB NCI Others: R.C. Gallo Chief LTCB NCT S. Josephs Research Chemist LTCB NCI B. Starcich Visiting Associate LTCB NCT A. Aldovini Visiting Fellow LTCB NCT E. Collalti Visiting Fellow LICE 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. Nonavama); NICHD, Bethesda, MD (W. Leonard) LAB/BRANCH Laboratory of Tumor Cell Biology 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 X (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.



PROJECT NUMBER

Z01CP00543-09 LTVB

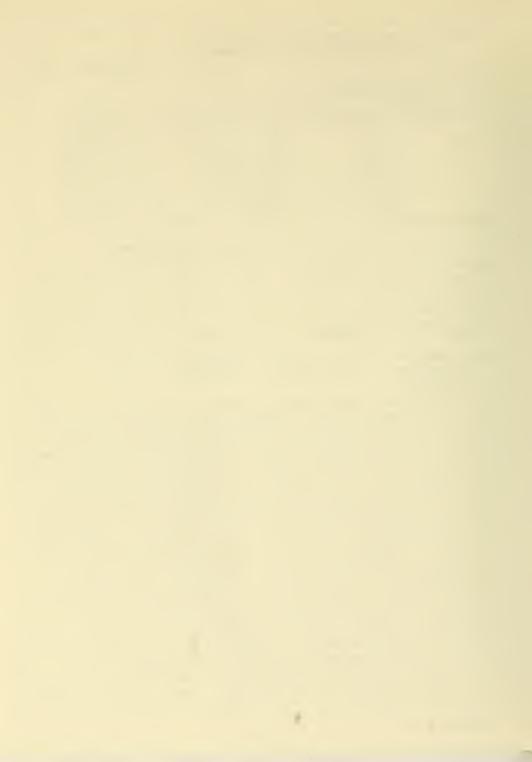
PERIOD COVERED							
October 1, 1986 to Sep							
TITLE OF PROJECT (80 charecters or less. Title must lit on one line between the borders.)							
Characterization of the Papillomaviruses							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Neme, title, leboratory, end institute affiliation)							
PI: P. M. How	ley Chief		LTVB	NCI			
Others: B. Spalho	lz Senio	r Staff Fellow	LTVB	NCI			
V. Lindgr		Guest Researcher		NCI			
P. Lamber	t Guest	Guest Researcher		NCI			
P. Hermon	at Guest	Guest Researcher		NCI			
M. Sippol	a-Thiele Visit	Visiting Fellow		NCI			
A. McBrid	e Visit	Visiting Fellow		NCI			
J. Byrne	Biolo	Biologist		NCI			
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.)							
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The papillomaviruses are a group of small ONA 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 boyine 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

369

binding site. PHS 6040 (Rev 1/84)

GPO 914-918



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, leboratory, and institute effiliation)

PI:

P. M. Howley

Chief

LTVB NCI

Others:

J. C. Byrne

Biologist

LTVB NCI

0.0

COOPERATING UNITS (if any)

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

0.1

LAB/BRANCH

Laboratory of Tumor Virus Biology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: PROFESSIONAL:

0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues

(c) Neither

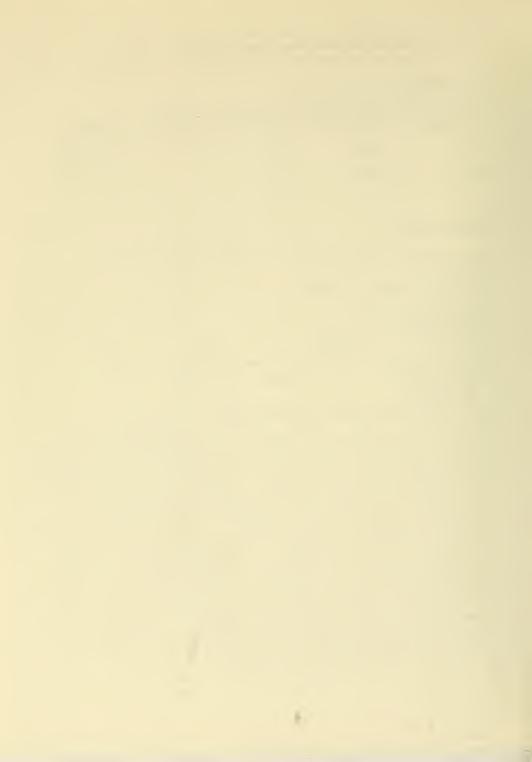
OTHER:

(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 enhancerdependent. 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.



PROJECT YUMBER

701CP00565-05 LTVB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 cherecters 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, leboratory, and institute affiliation.)

PI: R. Schlegel Chief. CRT Section LTVB NCI A. Burkhardt Guest Researcher Others: LTVB NCT V. Bubb Visiting Fellow LTVB NCT Y. 7hang 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. (Or. 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: 1.0

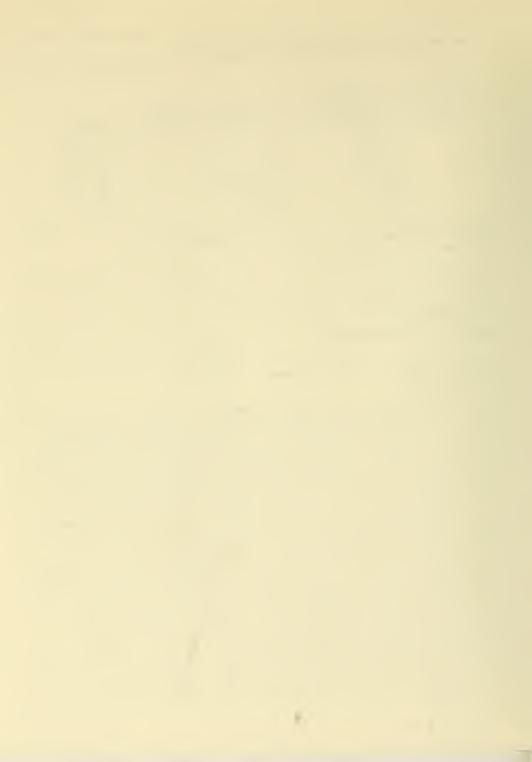
CHECK APPROPRIATE BOX(ES)

☐ (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.



PROJECT NUMBER

701CPD0898-04 | TVB

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.) (Neme, title, laboratory, and instituta affiliation.) P. M. Howley Chief 1 TVR NCT C. C. Baker Senior Investigator LTVB NCI W. Phelps Others: Guest Researcher LTVB NCT K. Munger Visiting Fellow LTVB NCT C. Yee Biologist LTVB NCI J. Byrne Biologist LTVB NCI

COOPERATING UNITS (# 80V)

Frederick Cancer Research Facility, NCI (Mike Braun and Matt Gonda)

LAB/BRANCH

Laboratory of Tumor Virus Biology

Viral Oncology Section

INSTITUTE AND LOCATION

NCI. NIH. Bethesda, MD 20892

TOTAL MAN-YEARS: PROFESSIONAL: OTHER: 2.5 1.4

CHECK APPROPRIATE BOX(ES) (a) Human subjects

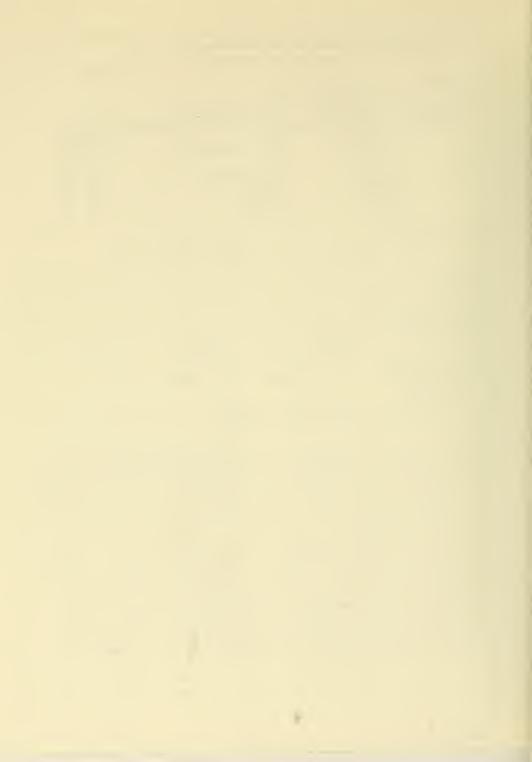
(b) Human tissues (c) Neither

(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 \underline{ras} in the transformation of primary rat embryo cells.



PAOJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT Z01CP05420-03-LTVB PERIOD COVERED October 1, 1986 to September 30, 1987 TITLE OF PROJECT (80 characters or less. Title must lit on one line between the borders.) Transformation by Polyomaviruses PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute effilietion) PI: J. B. Bolen Senior Staff Fellow LTVB NCT Others: S. Amini Visiting Fellow LTVB NCT Biologist V. DeSeau LTVB NCI J. O'Shaughnessy Medical Staff Fellow MB NCT COOPERATING UNITS (M any)
Department of Molecular and Cellular Biology, Pennsylvania State University, University Park, Pennsylvania (D. Shalloway) LAB/BBANCH Laboratory of Tumor Virus Biology Cellular Regulation and Transformation Section INSTITUTE AND LOCATION NCI, NIH, Bethesda, MD 20892 TOTAL MAN-YEARS: PROFESSIONAL . OTHER 1.5 1.0 0.5

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

(b) Human tissues

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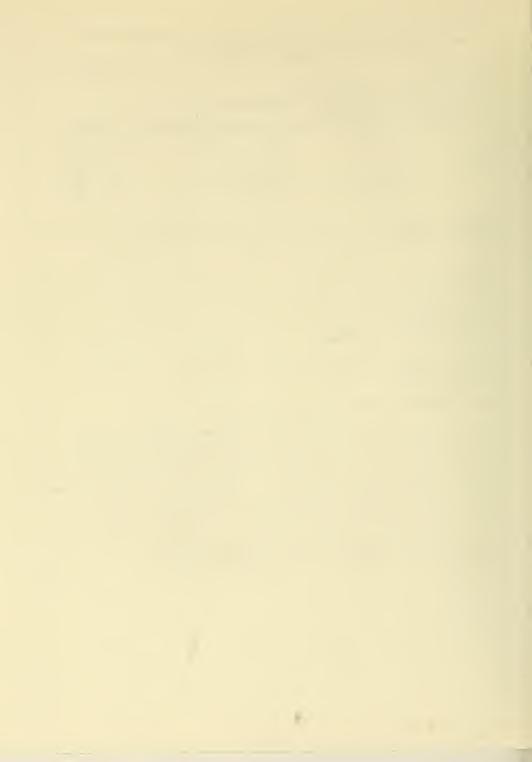
(a) Human subjects

(a1) Minors
(a2) Interviews

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, pp60c-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.

(c) Neither

the expectations



PROJECT NUMBER

Z01CP05481-02-LTVB

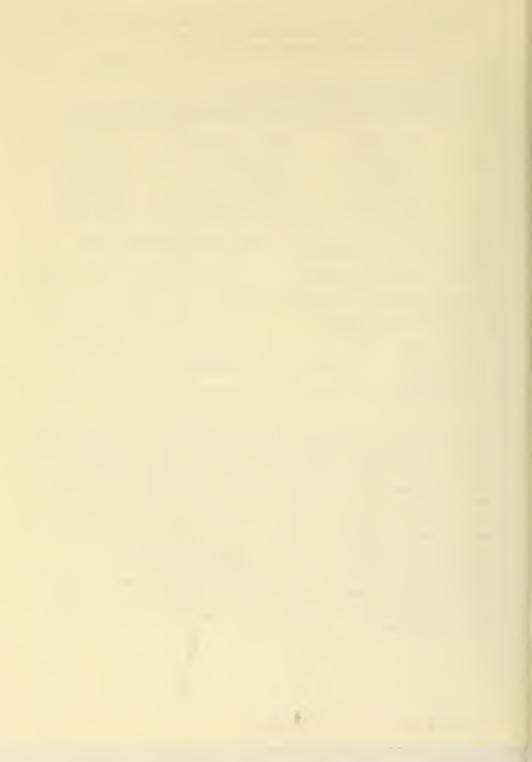
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PRINCIPAL INVESTIG	ATOR (List other pri	ofessional personnal be	elow the Pnncipal Inv	vestigetor.) (Name, title, labo	oratory, and institute aff	liliation)
PI:	J. B. Bol	en	Senior Sta	iff Fellow	LTVB	NCI
			W			
Others:	S. Amini		Visiting F		LTVB	NCI
	A. Veille	•	Guest Rese	archer	LTVB	NCI
	G. DeSeau		Biologist		LTVB	NCI
	N. Rosen		Senior Inv		MB	NCI
	J. O'Shau	ghnessy	Medical St	aff Fellow	MB	NCI
COOPERATING UNITS	(if any)					
				on University M	ledical Cente	r,
Washington.	D. C. (A.	M. Schwartz)				
LAB/BRANCH						
Laboratory C	of Tumor Vi	rus Biology	. <u></u>		-	
SECTION						
Cellular Reg		d Transforma	tion Sectio	n		
INSTITUTE AND LOCA						
NCI, NIH, Be	thesda, MD					
TOTAL MAN-YEARS:		PROFESSIONAL:		OTHER:		
	3.0		2.5		0.5	
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(a1) Mine					,	
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SUMMARY OF WORK	(Usa standard unred	duced type. Do not ext	ceed the space provi	ded.)		
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				erentiation.		
functional s	tate of pr	oto-oncogene	-encoded pr	oteins in huma	ın malignanci	es

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. pp60c-arc. This protein is the normal cellular nomolog of the Rous sarcoma virus oncogene, v-src. The transforming potential of pp60v-arc and mutated species of pp60c-arc 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 pp60c-arc. Our results show that while pp60c-arc protein kinase activity is low in most types of human tumors, significant elevation of pp60c-arc kinase activity can be found in all human tumors of neural origin, several sarcomas, all human breast and all colon carcinomas tested.

PHS 6040 (Rev 1/84)

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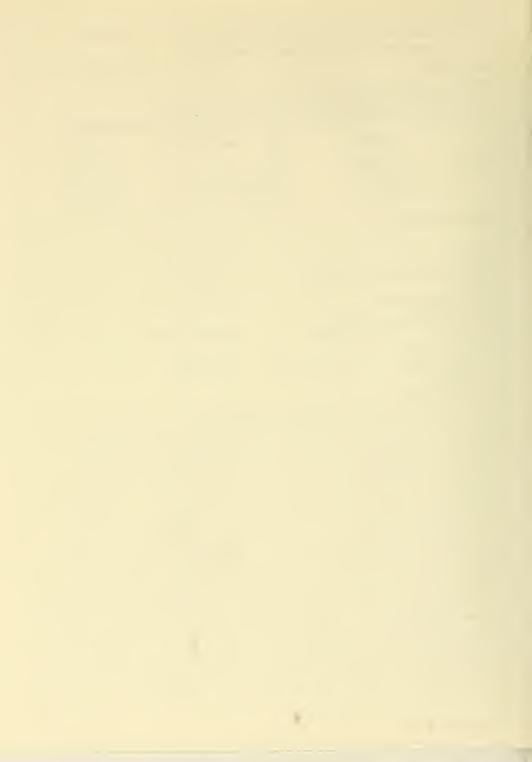


PROJECT NUMBER

701CP05482-02 LTVB

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					1.71/0	
PI:	C. C. B	aker	Senior Inve	stigator	LTVB	NCI
Others:	P. M. H	owlev	Chief		LTVB	NCI
others.	L. M. C		Biotechnolo	TV Fellow	LTVB	NCI
	U. Linz		Visiting Fe		LTVB	NCI
	J. S. N		Biologist	1 TOW	LTVB	NCI
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COOPERATING UN	ITS (if any)		· · · · · · · · · · · · · · · · · · ·			
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None						
LAB/BRANCH						
Laboratory	of Tumor	Virus Biology				
SECTION						
Viral Onco	logy Secti	on				
INSTITUTE AND LO	CATION					
NCI, NIH,		MD 20892				
TOTAL MAN-YEAR		PROFESSIONAL:		OTHER:		
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virus. An	understan	ding of the t	ranscriptiona	l regulation of	the papil	loma-

viruses 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.



PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT 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) Senior Staff Fellow ! TVB NCT PI: J. B. Bolen NCI P. M. Howley Chief LTVB J. Pyper Guest Researcher LTVB NCT Others: S. Mackem Medical Staff Fellow I P NCI COOPERATING UNITS (if any) None Laboratory of Tumor Virus Biology SECTION Cellular Regulation and Transformation Section INSTITUTE AND LOCATION NCI. NIH. Bethesda, MD 20892 TOTAL MAN-YEARS: PROFESSIONAL: OTHER: 0.0 0.8 CHECK APPROPRIATE BOX(ES)

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

(b) Human tissues

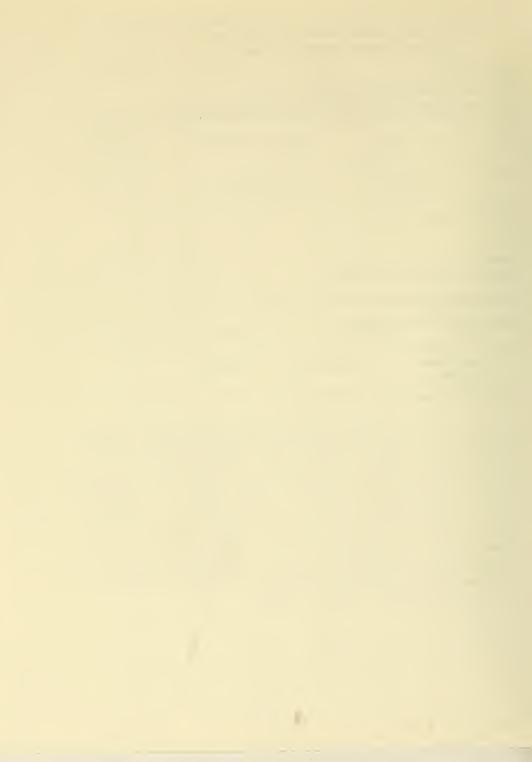
(a) Human subjects

☐ (a1) Minors ☐ (a2) Interviews

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 papillomavirues, 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.

(c) Neither

property.

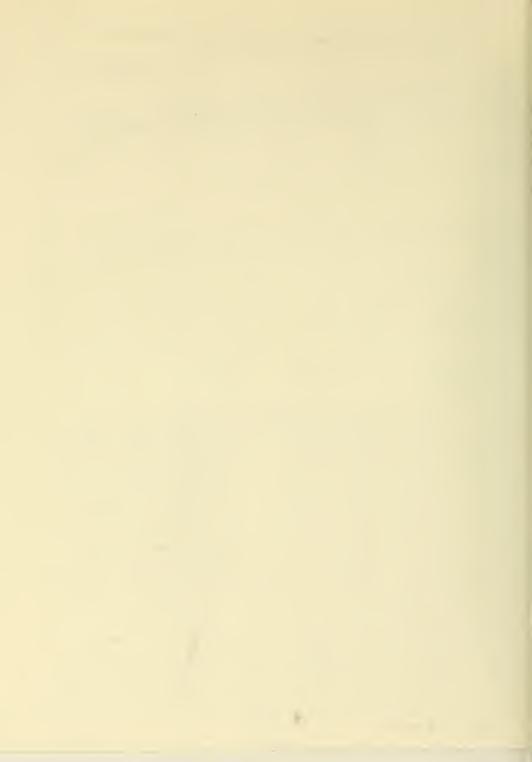


DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01CP05330-05 LVC
PERIOD COVERED October 1, 1986 to September 30, 1987	
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Urinary Transforming Growth Factors (TGFs) in Human Neoplasia	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, labore	tory, and institute affiliation)
PI: Kurt J. Stromberg Medical Director	LVC NCI
Others: None	
COOPERATING UNITS (# any) Division of Endocrinology, Vanderbilt University, Nashville, Department of Biochemistry, George Washington University, Was 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:	1.7
CHECK APPROPRIATE BOXIES) ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither	

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 HNW 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 TGFalpha 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.

(a1) Minors (a2) Interviews



NOTICE OF INTRAMURAL RESEARCH PROJECT

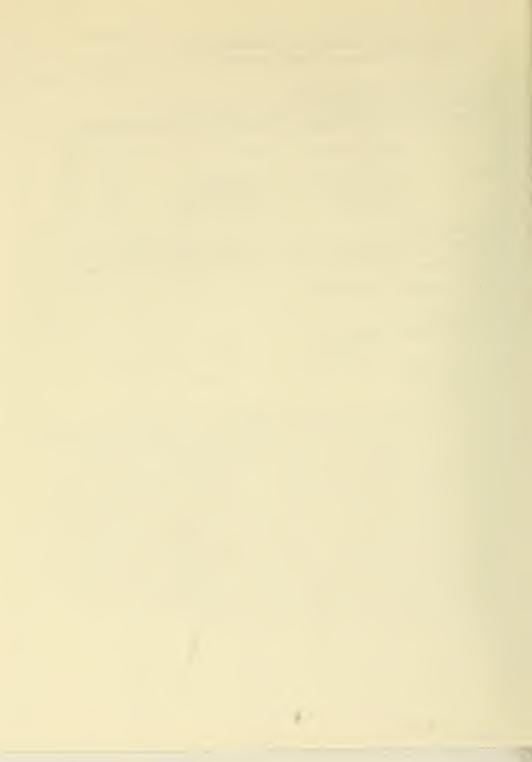
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The Genetic	Structure of Natural Popul	ations of Past and Prese	:nt	
PRINCIPAL INVESTIGA	ATOR (List other professional personnel below the	e Principal Investigator.) (Name, title, laborato	ry, end institute effiliati	ion)
D.T.	C1 1 1 01D :	01 : 6	1.1/0	NOT
PI:	Stephen J. O'Brien	Chief	LVC	NCI
Others:	Janice S. Martenson	Microbiologist	LVC	NCI
others:	Mary A. Eichelberger	Microbiologist	LVC	NCI
	Lisa Forman	Guest Researcher	LVC	NCI
	Hector Seuanez	Visiting Scientist	LVC	NCI
	neccor seguinez	Visiting Sciencist	LVC	NCI
COOPERATING UNITS	(if eny)	* * *		
Laboratory o	of Clinical Studies, ALC, N	IH. Bethesda, MD (D.Gold	lman): Nation	al
Zoological P	ark, Washington, DC (M.Bus	h. D.E. Wildt): National	Museums of K	enva.
Nairobi, Ken	ya (R.Leakey); PRI, Freder	ick. MD(W.Modi.D.Gilbert	.D.Janczewsk	i)
LAB/BRANCH				
Laboratory o	f Viral Carcinogenesis			
SECTION				
Genetics Sec				
INSTITUTE AND LOCA	····			
NCI, NIH, Fr	ederick. Maryland 21701-1	013		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:		
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	derived from distance mat			
	genetic distance. A corre			
	enetic variation and the ph			
	ies of lions. Genetic vari			
	cies were examined. A comp			
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occurred during carnivore evolution has been achieved.

speciation events. A reconstruction of cytological rearrangements which had



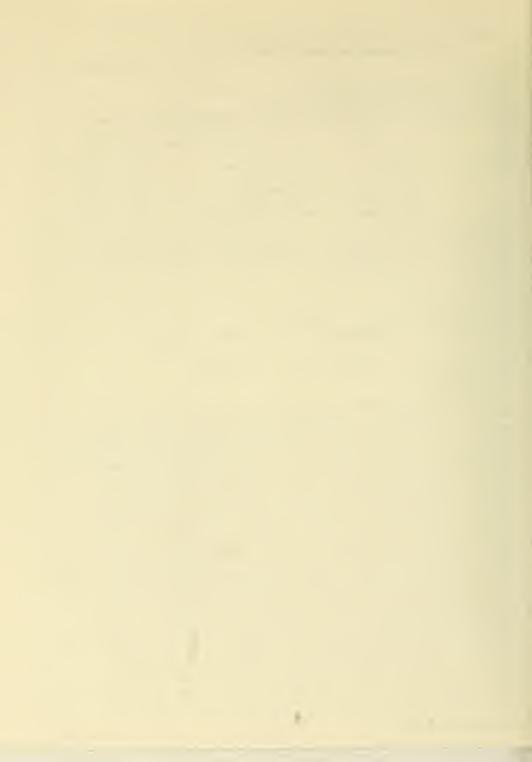
PROJECT NUMBER

701CP05382-04 LVC

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	d in Preneoplastic P				
PRINCIPAL INVESTIGATO	R (List other prolassional personnel b	elow tha Principal Invest	igator.) (Name, titla, leboratory,	end institute et	filiation)
PI:	Nancy H. Colburn	Chief, Cell	Biology Section	LVC	NCI
0.1	7.1.0				
Others:	John Seed	Special Volu		LVC	NCI
	W. Karol Dowjat	Visiting Fel		LVC	NCI
	Cao Ya	Guest Resear	cher	LVC	NCI
	Michael Antecol	Visiting Fel	low	LVC	NCI
	Glenn A. Hegamyer	Health Science	ce Officer	LVC	NCI
COOPERATING UNITS (if	any)				
Hunan Med. Co	llege, Hunan, China	(KT. Yao); (Cancer Res. Lab	Univ. W	. Ontario.
Canada (D. Der	nhardt); Dept. Radia	tion Oncology,	, Univ. Arizona N	Med. Sch.	. Tucson.
AR (T. Bowden)); PRI, Frederick, M	D (R. Garrity)		
LAB/BRANCH			·		
Laboratory of	Viral Carcinogenesi	s			
SECTION					
Cell Biology S	Section				
INSTITUTE AND LOCATIO	N				
NCI, NIH, Fred	derick, Maryland 21	701–1013			
TOTAL MAN-YEARS:	PROFESSIONAL:		OTHER:		
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(a2) Intervi	ews				
CHAMADY OF WORK (III	a standard unraduced type. Do not a	second the season provider	1)		

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, 12-0tetradecanoylphorbol-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. PHS 6040 (Rev 1/84)

420



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

Nancy H. Colburn

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

Chief. Cell Biology Section

Special Volunteer

NCI

Others: Bonita M. Smith John Seed

Special Volunteer

NCI LVC LVC NCT

COOPERATING UNITS (if any)

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

LAR/RRANCH

PT:

Laboratory of Viral Carcinogenesis

Cell Biology Section
INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS PROFESSIONAL: 3.2

1.5

CHECK APPROPRIATE BOX(ES) (a) Human subjects

(b) Human tissues (a1) Minors

(c) Neither

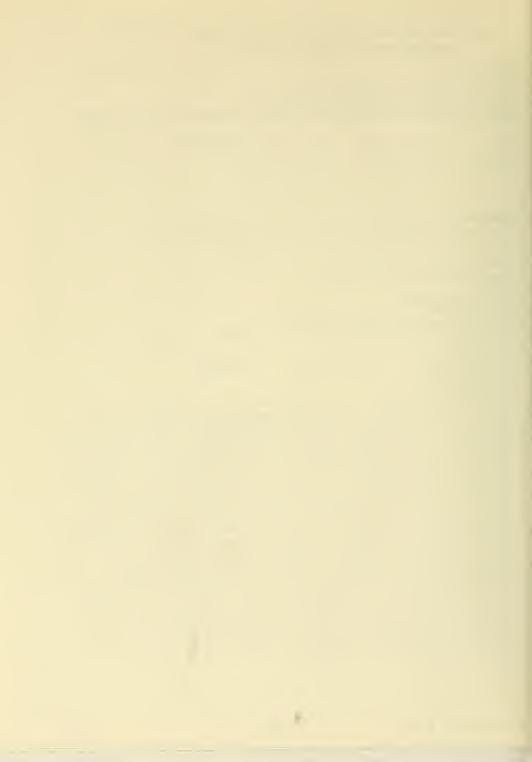
1.7

OTHER:

(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 \bar{C} (PKC) and the subsequent loss of PKC activity may be on the signal transduction pathway for 12-0-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, TPAstimulated 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.

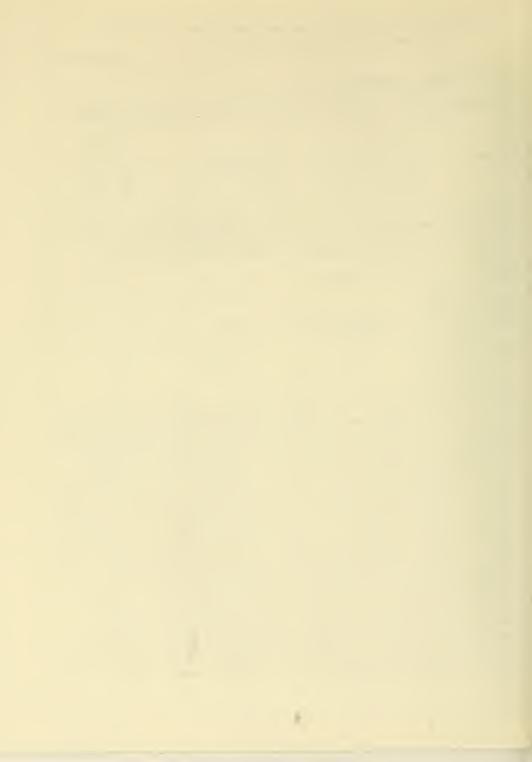


PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

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) Stephen J. O'Brien PI: Chief LVC NCI Others: LVC NCI Janice S. Martenson Microbiologist Mary A. Eichelberger Microbiologist LVC NC T Takis S. Papas NCT Chief LMO Dennis K. Watson Senior Staff Fellow OM 1 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) Laboratory of Viral Carcinogenesis SECTION Genetics Section INSTITUTE AND LOCATION NCI, NIH, Frederick, Maryland 21701-1013 OTHER: 0.70.6 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 5g 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.



PROJECT NUMBER

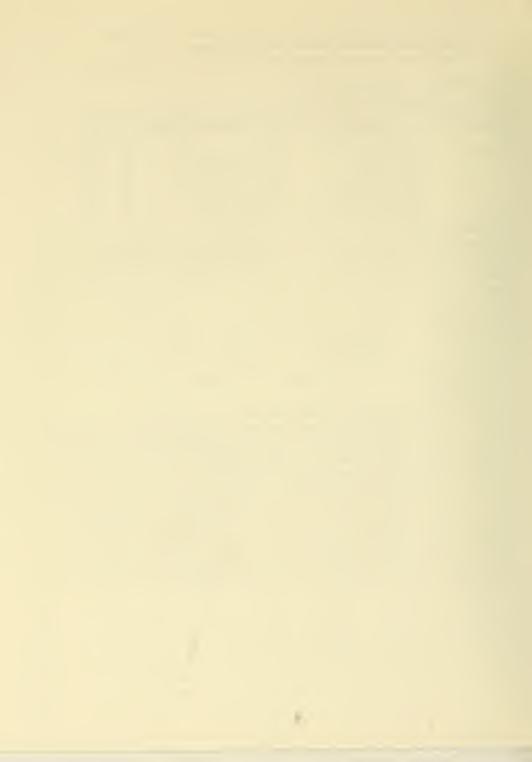
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October 1, 1986	to Sept	ember 30, 1987					
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PRINCIPAL INVESTIGATOR	(List other pro	fessional personnel below the	Principal Inves	tigator.) (Name, tit	le, laboratory, a	ind institute e	effiliation)
PI:	Stephen	J. O'Brien	Chief			LVC	NCI
Others:	David D	erse	Senior	Staff Fel	llow	LVC	NCI
	Naoya Y	uhki	Visiti	ng Fellow		LVC	NCI
	James W	. Casey		Staff Fel	llow	LVC	NCI
	Raoul E	. Benveniste	Medica	1 Officer		LVC	NCI
	Hector	Seuanez	Visiti	ng Scienti	ist	LVC	NCI
	Janice	S. Martenson	Microb	iologist		LVC	NCI
		Eichelberger	Microb	iologist		LVC	NCI
COOPERATING UNITS (if e	ny)						
		A. Gilbert, W. S.					
Sterling, VA (W	. G. Nas	h); Univ. of CA,	San Die	go, CA (J.	S. O'Br	ien); N	IIAID, NIH,
	. Kozak)	: National Zoolog	<u>gical Pa</u>	rk, Washir	ngton, DC	(D. E.	_Wildt)
LAB/BRANCH							
Laboratory of V	<u>iral Car</u>	cinogenesis					
SECTION							
Genetics Section							
INSTITUTE AND LOCATION							
NCI. NIH. Frede	rick. Ma	ryland 21701-10	13	OTHER:			
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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, bandfor-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.

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of other



PROJECT NUMBER

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PI:	Stephen J. C)'Brien	Chief		LVC	NCI		
011	D. 23 F 1127	1.14	C . 1 V 1		LVC	NCT		
Others:	Janice S. Ma		Special Volunt Microbiologist		LVC	NCI NCI		
	ballice 5. Ma	ii censon	microbiologist	•	LVC	NOI		
COOPERATING UN	TO CL.							
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(a2) Interviews

(b) Human tissues

(a) Human subjects

(a1) Minors

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 (anima) 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.

X (c) Neither



PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05401-03 LVC

October 1, 1986 to Sep	tember 30, 1987				
TITLE OF PROJECT (80 characters or less Structure and Transcri	s. Title must lit on one line betw ptional Regulatio	een the borders.) n of the Bo	vine Leuken	nia Virus	
PRINCIPAL INVESTIGATOR (List other pr	ofessional personnel below the I	Principal Investigator.) (Name, title, labora	tory, and institut	e affiliation)
PI: James W.	Casey S	enior Staff	Fellow	LVC	NCI
Others: David D.	Derse S	enior Staff	Fellow	LVC	NCI
COOPERATING UNITS (if any)	F	(14 0 1)			
Program Resources, Inc	. Frederick, MU	(M. Gonda):	University	of Calif	fornia.
Davis, CA (M. Thurmond (G. Cockerell)); Colorado State	University	• Fort Coll	ins. CO	
LAB/BRANCH					
Laboratory of Viral Ca	rcinogenesis				
SECTION					
Viral Leukemia and Lym	ohoma Section				
INSTITUTE AND LOCATION					
NCI, NIH, Frederick, M.	aryland 21701-10	13			
TOTAL MAN-YEARS:	PROFESSIONAL:	ОТН	ER:		
1.0	0.5			0.5	
CHECK APPROPRIATE BOX(ES)	_				
(a) Human subjects	(b) Human tissue	s 🗵 (c)	Neither		
(a1) Minors					
(a2) Interviews					

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

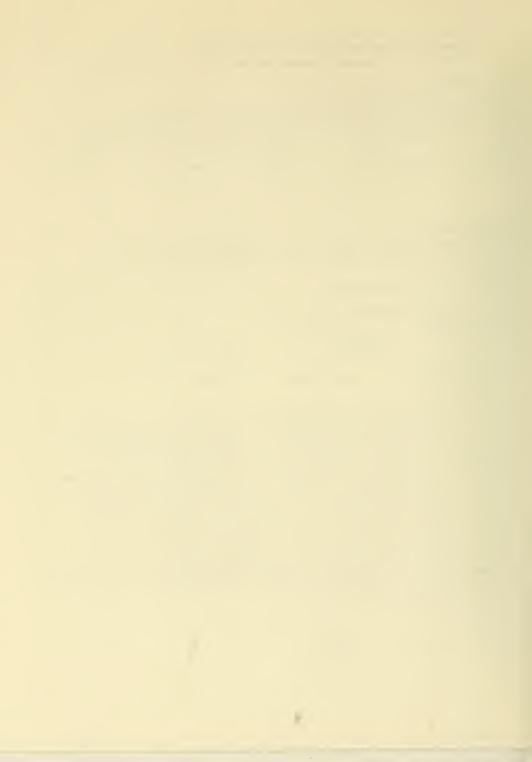
The structure and expression of deletion-type and full-length bovine leukemia virus (BLV) proviruses have been examined from tumors of infected cattle. sheep. and established cell lines. A deletion-type provirus present in a bovine lymphoid cell line (NBC-13) has been cloned, sequenced, and shown to arise by a recombination event between two 10 base-pair direct repeats. Different deletiontype 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 Blymphocytes occurred in one animal and transcripts originating from the pX region of the provirus were detected.

PHS 6040 (Rev 1/84)

ME-NEPROSE

449

GPO 914-918



PROJECT NUMBER

1 VC

1.0

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05414-04 LVC

NCT

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

Senior Staff Fellow

PRINCIPAL INVESTIGATOR (List other professional porsonnel below the Principal Investigator) (Name, title, leboretory, and institute effiliation)

PI: Raoul E. Benveniste Medical Officer LVC NCI

Others: Gisela Fanning-Heidecker Staff Fellow LVC NCI

COOPEHATING 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)

Laboratory of Viral Carcinogenesis

David Derse

SECTION

Viral Leukemia and Lymphoma Section

INSTITUTE AND LOCATION

NCI, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS: PROFESSIONAL: OTHER: 1.6 0.6

CHECK APPROPRIATE BOX(ES)

(a) Human subjects

ubjects (b) Human tissues

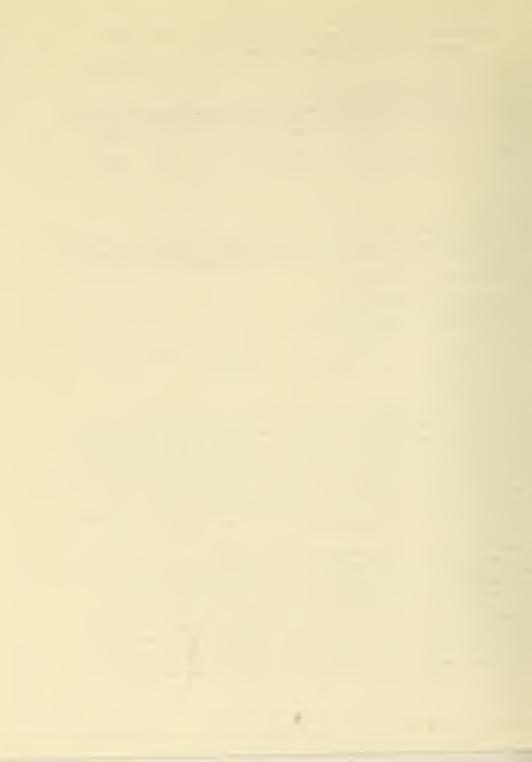
(c) Neither

(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.



PHOJECT NUMBER

NOTICE OF INTRAMURAL RES		
		Z01CP05417-03 LVC
PERIOD COVERED		
October 1, 1986 to September 30, 19	87	
TITLE OF PROJECT (80 characters or less. Title must fit on one li	ne between the borders)	
Molecular Characterization of raf C	incogenes in Normal and Tum	or Cells
PRINCIPAL INVESTIGATOR (List other professional personnel bell PI: Ulf R. Rapp	ow the Principal Investigator) (Name, title, laborat	ory, and institute affiliation)
PI: Ulf R. Rapp	Chief, Viral Pathology Sec	tion LVC NCI
Others: Thomas W. Beck		
The state of the s	Biotechnology Fellow	LVC NCI
	Staff Fellow	LVC NCI
Walter Kolch	Guest Researcher	LVC NCI
	Senior Staff Fellow	LVC NCI
Takayasu Matsugi	Visiting Fellow	LVC NCI
Berton Zbar	Chief, Cellular Immunity Se	ection LI NCI
COOPERATING UNITS (if any)		
None		
LAB/BRANCH		
Laboratory of Viral Carcinogenesis		
Viral Pathology Section		
NCI, NIH, Frederick, Maryland 2170		
TOTAL MAN-YEARS: PROFESSIONAL	OTHER	
1.8	1.5	0.3
CHECK APPROPRIATE BOX(ES)		
(a) Human subjects (b) Human t	issues (c) Neither	

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

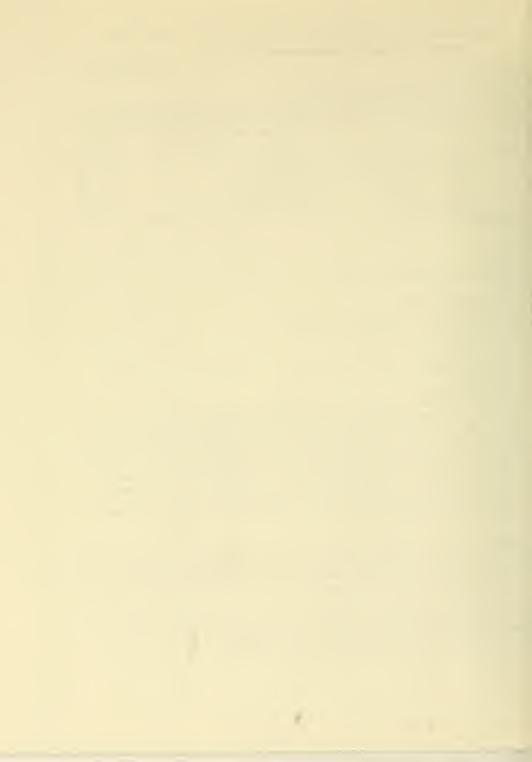
Two active oncogenes related to v-raf have been identified in both mouse and man. c-raf-1 has been localized to mouse chromosome 6 and to human chromosome 3p25 near sites specifically altered in small cell lung carcinoma (SCLC), familial renal cell carcinoma, mixed parotid gland tumors, and ovarian cancer. 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-<u>raf</u>-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.

PHS 60-10 (Rev 1/84)

(a1) Minors
(a2) Interviews

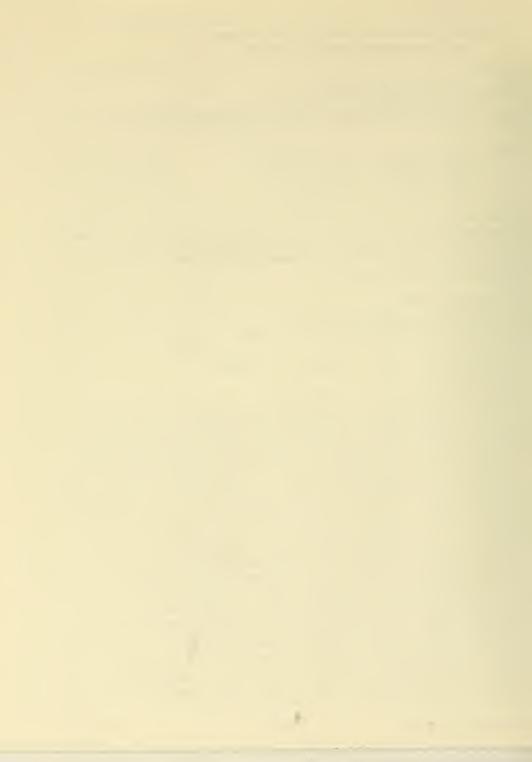


PROJECT NUMBER

			<u>Z01</u>	CP05418-03	3L.V.C
PERIOD COVERED		- · · · · · · · · · · · · · · · · · · ·			
October 1. 1986 to September TITLE OF PROJECT (80 characters or less. Title must	30. 1987				
Role of raf and myc Oncogene	s in Transform	ation In Vivo	and In Vitro		
PRINCIPAL INVESTIGATOR (List other professional per	ersonnel below the Principa	al Investigator.) (Name, tit	le, leboratory, end insti	ute effilietion)	
PI: Ulf R. Rapp	Chief, Vira	1 Pathology Se	ection LVC	NCI	
Others: John L. Cleveland	Senior Staf	f Fellow	LVC	NCI	
Mahmoud Huleihel	Visiting Fe	llow	LVC	NCI	
Robert Nalewaik	Microbiolog	ist ,	LVC	NCI	
Michael Potter	Biologist		LG	NCI	
COOPERATING UNITS (# eny) Program Resources, Inc., Fre MD (J. Pierce); NIAID, NIH, Frederick, MD (J.N. Ihle); N LABBBRANCH Laboratory of Viral Carcinog	Bethesda, MD (IDR, NIH, Beth	H.C. Morse); [Bionetics Res	NIH, Bethe	esda,
SECTION					
Viral Pathology Section INSTITUTE AND LOCATION					
INSTITUTE AND LOCATION					
NCI, NIH, Frederick, Marylan	d 21701-1013				
TOTAL MAN-YEARS: PROFESS	SIONAL:	OTHER:			
1.3	0.8		0.5		
(a1) Minors	Human tissues	☐ (c) Neither			
(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.

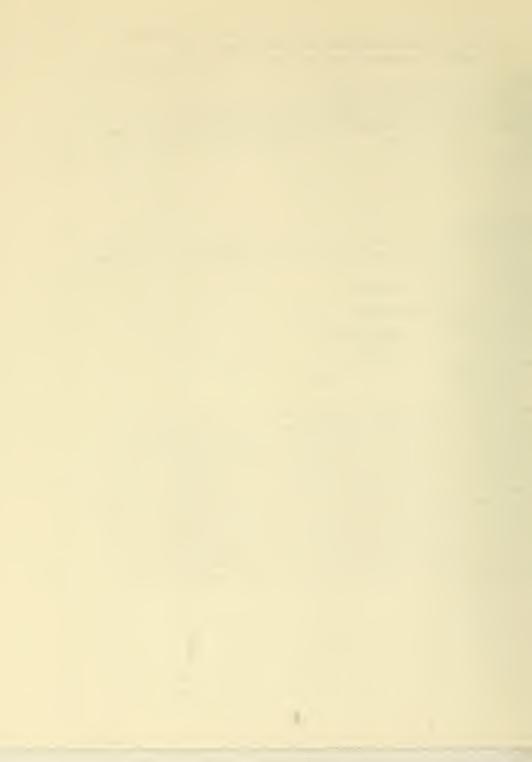


PROJECT NUMBER

DEPARTMENT OF HEALTH A	AND HUMAN SERVICES - PUBLIC HEA	LIH SERVICE	
NOTICE OF INT	RAMURAL RESEARCH PROJE	CT	
			Z01CP05490-02 LVC
October 1, 1986 to Sept			
Molecular Basics of Ler	s. Title must fit on one line between the border ntiviral Transcriptional	Trans-activati	ion
PRINCIPAL INVESTIGATOR (List other pro	ofessional personnel below the Principal Invest	igator.) (Neme, title, labore	etory, end institute affiliation)
PI: James W. C		aff Fellow	LVC NCI
Others: None			
COOPERATING UNITS (if eny)			
	. Frederick, MD (M. Gond	a). Promotina	Danasanah Tura
Frederick MD (N. Rice)	; Texas A & M University	d); blonetics	Research, Inc.,
Treder text Tib (III Kree)	, Texas A a M offiversity	, correge stat	ion, ix (J. Edwards)
LAB/BRANCH			
Laboratory of Viral Car	cinogenesis		
SECTION			
Viral Leukemia and Lymp	homa Section		
NCI, NIH, Frederick, Ma	ryland 21701-1013		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:	
0.9	0.5		0.4
CHECK APPROPRIATE BOX(ES)			
(a) Human subjects	☐ (b) Human tissues 区	(c) Neither	
(a1) Minors			
(a2) Interviews			
	duced type. Do not exceed the space provided		
The lentivirus, equine	infectious anemia virus	(EIAV), displa	ys a highly
restricted cell type pr	eference both in vitro a	nd <u>in vivo</u> . A	dditionally, like
other members of the le	ntivirus family, EIAV is	subject to an	tigenic variation as
infection To further	at occur in envelope gly understand the restricti	coproteins dur	ing the course of
provinal changes that o	ccur during pathogenesis	ve nost range	and correlate
sequenced an FIAV provi	rus. Comparison of the	gag and nol ge	nos of FIAV with the
human immunodeficiency	virus and the visna virus	s clearly esta	blishes that FIAV is
genetically related and	equally divergent from	these two dist	inct lentiviruses
Additionally, we have p	erformed DNA-mediated tr	ansfection ana	lysis and viral
infectivity assays of D	NA isolated from a produc	ctively infect	ed dog cell line
(tiAV cf-2). Results f	rom these experiments in	dicate that so	me proviruses
harbored in this cell a	re biologically active.	We have molecular	ularly cloned 23 of
these proviruses using	lambda vectors and are co	urrently assay	ing each for

東部マセキロア

infectivity.



PROJECT NUMBER

Z01CP05491-02 LVC

P	ERIC	DD C	OV	ER	ED

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 effiliation)

PI: John L. Cleveland Senior Staff Fellow

LVC NCI

Others: Ulf R. Rapp Mahmoud Huleihel Chief, Viral Pathology Section

LVC NCT I VC NCI

Visiting Fellow

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. Llovd, M. Dean)

Laboratory of Viral Carcinogenesis

Viral Pathology Section INSTITUTE AND LOCATION

NCI. NIH, Frederick, Maryland 21701-1013

1.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (a1) Minors

(c) Neither

0.1

OTHER:

(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 cellprogrammed 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 y-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 $10 \, \overline{\text{t}}$ imes 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.



PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PRO	JECT	Z01CP05492-	-02 LVC
PERIOD COVERED			
October 1, 1986 to September 30, 1987			
TITLE OF PROJECT (80 cherecters or less. Title must fit on one line between the box	ders.)		
Activation of raf Oncogenes PRINCIPAL INVESTIGATOR (List other professional personnal below the Principal Inv	astigator) (Nama title Jahan	ton, and institute official	
	iral Pathology S		NCI
Others: Mahmoud Huleihel Visiting	Fellow	LVC	NCI
,	taff Fellow	LVC	NCI
Gisela Fanning-Heidecker Staff Fe		LVC	NCI
Robert Nalewaik Microbio	logist	LVC	NCI
Michael Potter Biologis	t	LG	NCI
COOPERATING UNITS (if eny)			
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		
	(c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided in the standard unreduced type.)	ded.)		
A 1.6-Kb cDNA (A-raf) has been isolated from encodes part of a protein related to the v-rahas 85% homology to raf in a central protein porated into a retrovirus, the resulting gag-formation in vitro and induces tumors in newb complete 2453- nucleotide sequence of the hum cDNA library. When the 5' deleted fragment of murine retrovirus, the resulting gag-A-raf further contents of the contents of t	f oncogene. Its of 100 amino aci A- <u>raf</u> fusion ger orn mice. Later an A- <u>raf</u> gene fr f the cDNA is ir	amino acid sed ds. When inco e causes trans- on we isolated om a human T-co corporated into	quence r- d the ell o a

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 \underline{qaq} -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 \underline{qag} -A-raf fusion gene causes transformation in vitro and in vivo. Whereas, the full-lengths of c-raf-l 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-l and incorporated them into a murine retrovirus, the resulting \underline{qaq} -c-raf-l 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 $\overline{14}$ 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-l. These mutants will be incorporated into retroviral vectors for expression.

the tradition



PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

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 effiliation) Raoul E. Benveniste Medical Officer NCI

Gisela Fanning-Heidecker Staff Fellow NCI Others: LVC

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

(a2) Interviews

NCI. NIH. Frederick. Maryland 21701-1013

TOTAL MAN-YEARS PROFESSIONAL: OTHER: 0.9 0.6 CHECK APPROPRIATE BOX(ES)

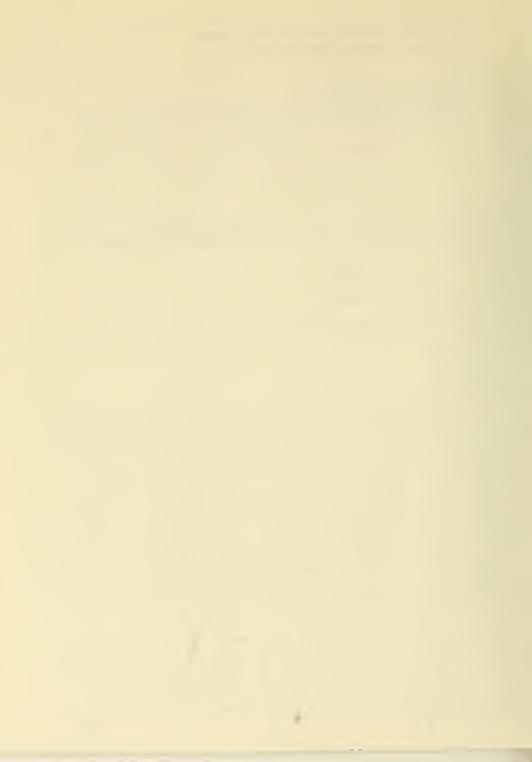
(a) Human subjects (b) Human tissues (c) Neither (a1) Minors

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 electro-phoresis (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 IqG. 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.

483



PROJECT NUMBER

Z01CP05528-01 LVC

PERIOD COVERED

October 1, 1986 to September 30, 1987

David Derse

TITLE OF PROJECT (80 characters 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.)

Senior Staff Fellow

IVC NCT

Others:

PI:

Stephen J. O'Brien

Chief

IVC NCT

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Viral Carcinogenesis

Genetics Section INSTITUTE AND LOCATION

NCI. NIH. Frederick, Maryland 21701-1013

0.8 CHECK APPROPRIATE BOX(ES)

(a) Human subjects

(b) Human tissues (a1) Minors

X (c) Neither

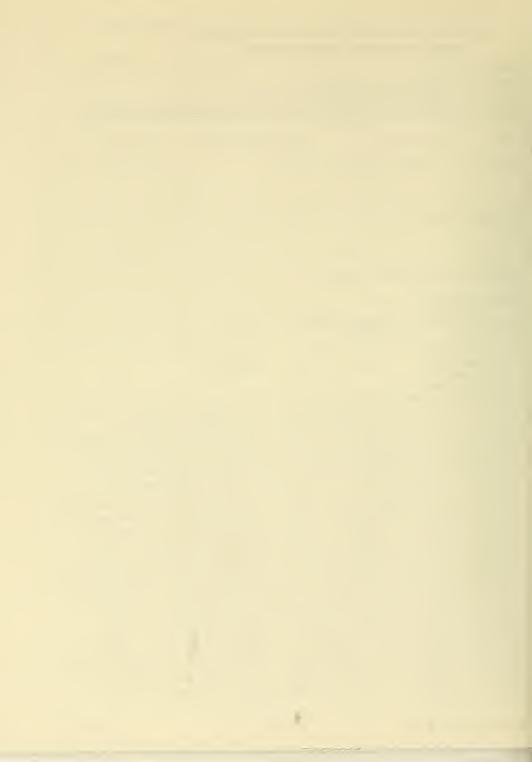
OTHER:

0.4

(a2) Interviews

SUMMARY OF WORK (Use standard 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 cisacting elements in the provinal 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 drugresistant 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. 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.



PROJECT NUMBER

					Z01CP055	29-01 LVC
PERIOD COVERED						
October 1, 1986	to Septe	mber 30, 1987				
TITLE OF PROJECT (80 chare	ecters or less.	Title must fit on one line	between the border	s.)		
Genetic and Mole	cular Or	ganization of	the MHC i	the Domestic	Cat	
PRINCIPAL INVESTIGATOR (List other profe	essional personnel balow	the Principal Invest	getor.) (Nema, title, labora	itory, and institute a	ffiliation)
PI:	Stephen	J. O'Brien	Chief		LVC	NCI
Others:	Naoya Yu	ıhki	Visiting	Fellow	LVC	NCI
		J. Cevario	Biologia		LVC	NCI
COOPERATING UNITS (If any Program Resource Frederick, MD (A	s, Inc.,		D (C. A. W	inkler); Bione	tics Resear	ch, Inc.,
LAB/BRANCH						
Laboratory of Vi	ral Card	inogenesis				
SECTION						
Genetics Section						
INSTITUTE AND LOCATION						
NCI, NIH, Freder		V	1013			
TOTAL MAN-YEARS:		PROFESSIONAL:	2	OTHER:	0 2	
		1.3	0		0.3	
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X (c) Neither

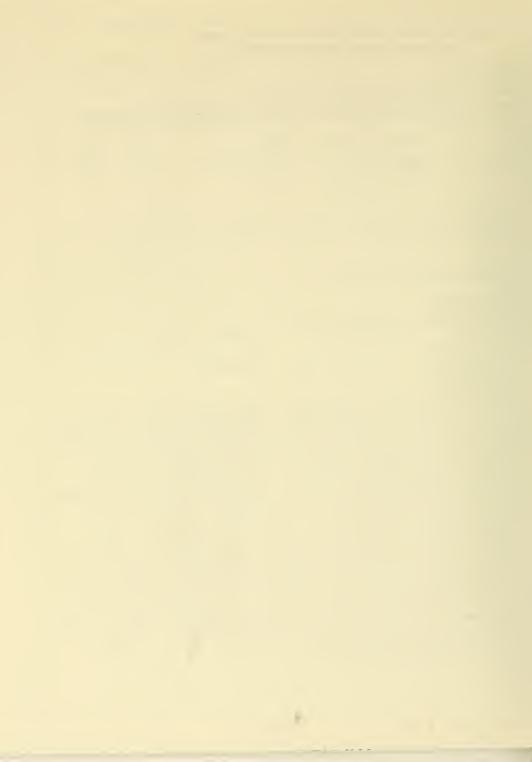
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(b) Human tissues

(a) Human subjects

(a1) Minors
(a2) Interviews

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.

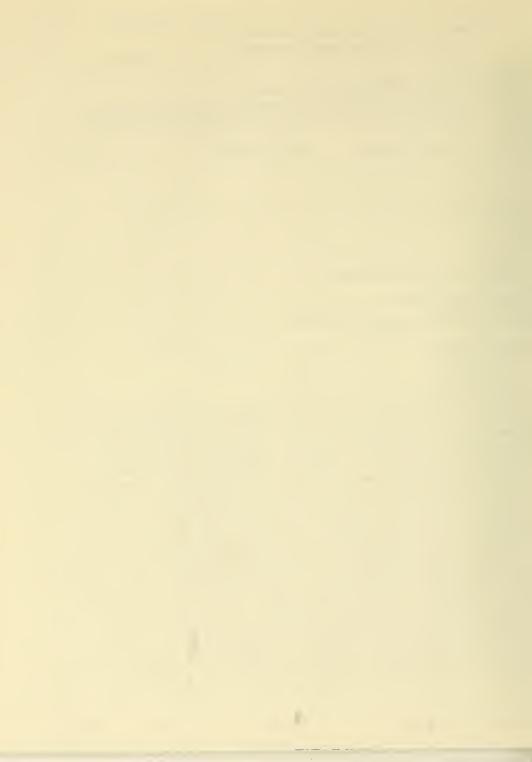


PROJECT NUMBER

Z01CP05530-01 LVC

PERIOD COVERED October 1, 1986 to September 30, 1987 TITLE OF PROJECT (80 characters or less. Title must lit 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, end institute affiliation) PI: Ulf R. Rapp Chief. Viral Pathology Section Others: Gunamani Sithanandam Guest Researcher LVC NCT COUPERATING UNITS (if any) None LAR/BRANCH Laboratory of Viral Carcinogenesis SECTION Viral Pathology Section INSTITUTE AND LOCATION NCI, NIH, Frederick, Maryland 21701-1013 TOTAL MAN-YEARS: OTHER PROFESSIONAL: 1.2 0.2 CHECK APPROPRIATE BOXIES) (a) Human subjects X (c) Neither (b) Human tissues (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 <u>in vitro</u>. Endogenous ecotropic type C virus induced by iododeoxyuridine from the nontransformed 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 <u>in vitro</u> 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, Cl 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.



PROJECT NUMBER

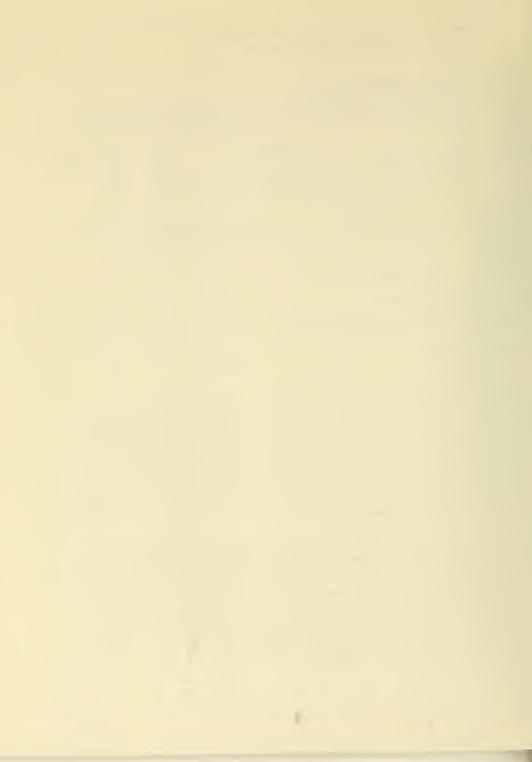
Z01CP05531-01 LVC

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 pursonnel below the Principal Investigator.) (Name, title, laboratory, end institute affiliation) PI: Ulf R. Rapp Chief. Viral Pathology Section LVC NCI Others: Walter Kolch Guest Researcher LVC NCT John L. Cleveland Senior Staff Fellow LVC MCT Mahmoud Huleihel Visiting Fellow LVC NCT Thomas Beck Biotechnology Training Fellow LVC NCI G. Fanning-Heidecker Staff Fellow LVC MCI 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 Viral Pathology Section INSTITUTE AND LOCATION NCI, NIH, Frederick, Maryland 21701-1013 TOTAL MAN-YEARS PROFESSIONAL OTHER 1.4 0.2 CHECK APPROPRIATE BOXIEST (a) Human subjects X (c) Neither (b) Human tissues (a1) Minors (a2) Interviews SUMMAR: 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.

PHS 6040 (Rev 1/84)



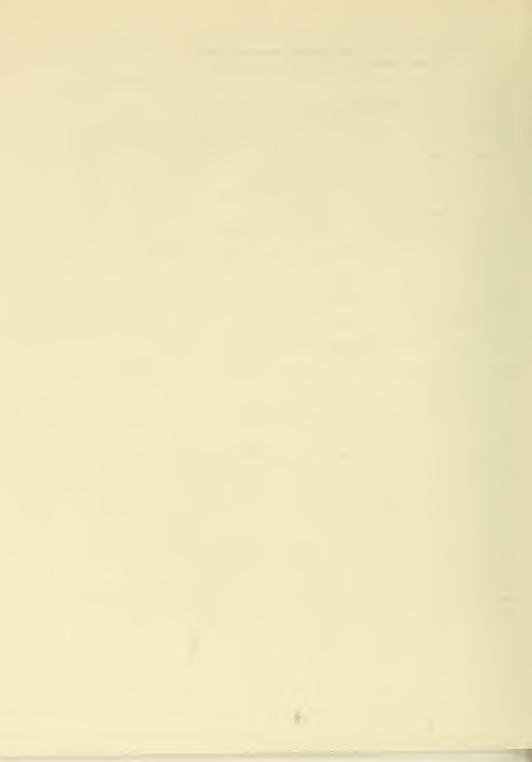
PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01CP05	532-01 LVC				
PERIOD COVERED						
October 1, 1986 to September 30, 1987						
TITLE OF PROJECT (80 cheracters 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, labore	tory, end institute	effiliation)				
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 Institu (HF. Kung)	te, Frede	rick, MD				
LAB/BRANCH						
Laboratory of Viral Carcinogenesis						
SECTION						
Viral Pathology Section						
INSTITUTE AND LOCATION						
NCI, NIH, Frederick, Maryland 21701-1013						
TOTAL MAN-YEARS: PROFESSIONAL: OTHER:						
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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.)

- end- o-Chapter

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 <u>in vitro</u> and <u>in vivo</u>. (2) <u>raf</u> proteins are cytoplasmically located protein kinases related to the <u>src</u> 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 TGFalpha 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.



PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

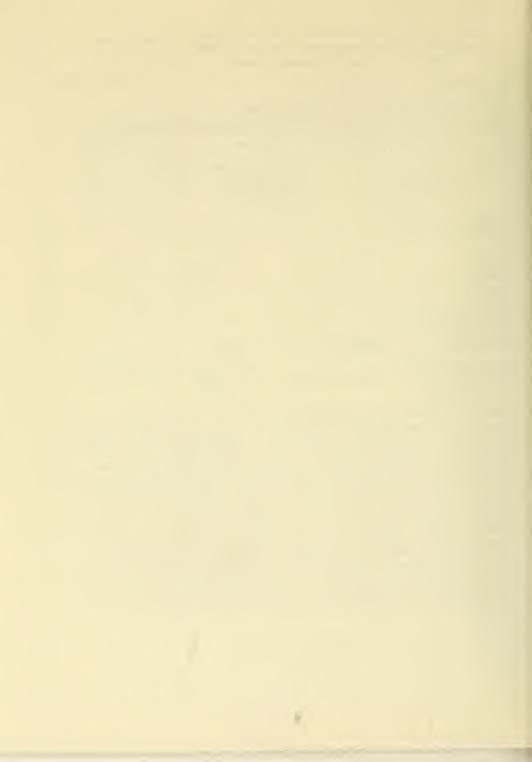
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PRINCIPAL INV	ESTIGATOR (List other pr	olessionel personnel bel	ow the Principal Inve	stigetor.) (Neme, title, lebo	oretory, end insti	tute effili	etion)	
PI:	Gisela Fannin	g-Heidecker	Staff Fell	ow		LVC	NCI	
Others:	Ulf R. Rapp		Chief. Vir	al Pathology S	Section	LVC	NCI	
concr 3.	Mahmoud Hulei	ha l	Visiting F				NCI	
	Thomas Beck	iie i		ogy Training F			NCI	
	Robert Nalewa	- L				LVC	NCI	
	Robert Marewa	1 K	Microbiolo	gist		LVC	MCI	
COOPERATING	UNITS (if any)							
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None								
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LAB/BRANCH								
Laborato	ry of Viral Ca	rcinogenesis						
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NCI, NIH	Frederick, M	aryland 2170	11-1013					
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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.

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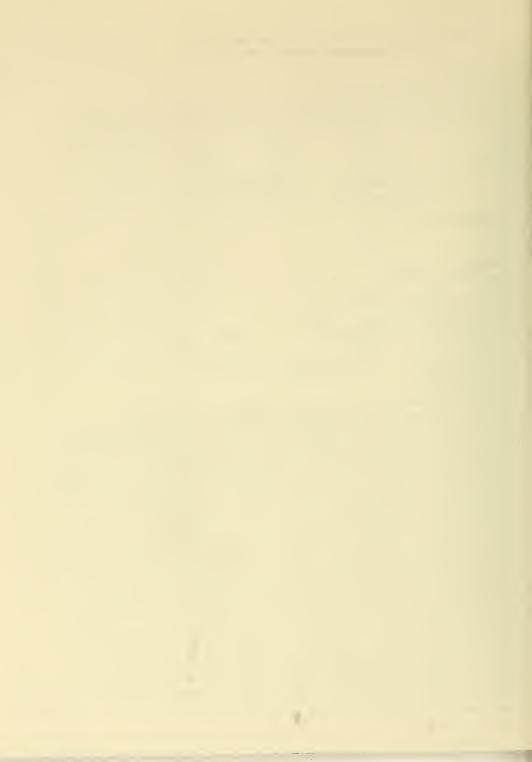


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PRINCIPAL INVEST	TIGATOR (List other pr	ofessional personnel	below the Principal I.	nvestigator) (Name.	title, laboratory, a	and institut	e affiliation)
PI:	J. A. 0	iPaolo	Chief		LE	NCI	
Others:	J. Doni L. A. F N. C. F	irisi	Visitin	Staff Fello g Fellow ologist	OW LE LE LE		
	C. Wood			Staff Fello	ow LE	NC I	
LAB/BRANCH			• • • • • • • • • • • • • • • • • • • •				
Laboratory	of Biology						
SECTION							
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	Bethesda, Mar		2				
TOTAL MAN-YEARS	S.	PROFESSIONAL:		OTHER:	1.0		
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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.



Z01CP04673-16 LB

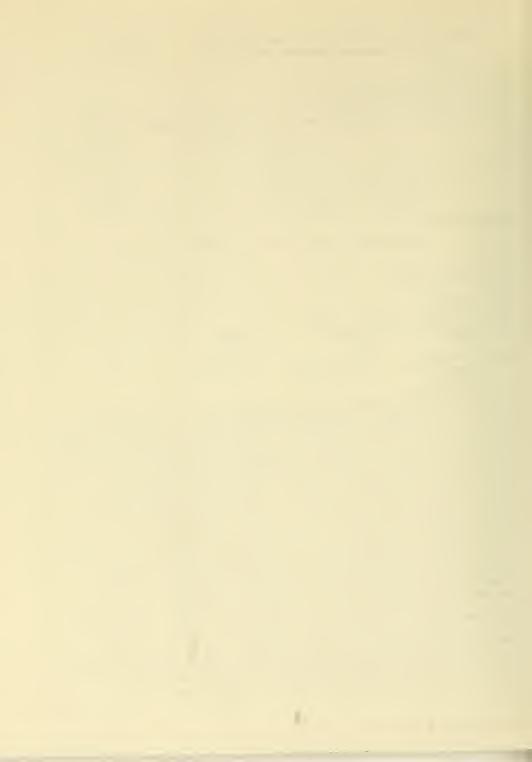
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TITLE OF PROJECT (80 cherecters or less The Immunobiology of Ca	Title must fit on one line between the borders.)	
PRINCIPAL INVESTIGATOR (List other pro	olessional parsonnal below the Principal Investigator.) (Nama, title	, leboratory, and institute effiliation)
PI: C. H. Evans	Chief, Tumor Biology Section	n LB NCI
Others: S.C. Barnett B.A. Gelleri P. Furbert-H P. D. Baker A. C. Wilson	Visiting Fellow arris IRTA Fellow Microbiologist	LB NCI LB NCI LB NCI LB NCI LB NCI
COOPERATING UNITS (If any) Laboratory of Neurophys	iology, NINCDS, NIH (P. A. Sheehy,	J.L. Barker)
LAB/BRANCH Laboratory of Biology		
SECTION Tumor Biology	,	
NCI, NIH, Bethesda, Mar	yland 20892	
TOTAL MAN-YEARS: 5.5	PROFESSIONAL: OTHER: 1.0	
CHECK APPROPRIATE BOX(ES) (a) Human subjects (a1) Minors (a2) Interviews	☑ (b) Human tissues ☐ (c) Neither	
Lymphokines, interleuki bioregulatory macromole are being studied to de inhibitory activities. course of this project, Anticarcinogenic action Inhibition of tumor cel irreversible due to inc to cytolytic destructio target cell interaction directly cytolytic for	fuced type. Do not exceed the space provided.) ns, and other immunological hormonicules of lymphocytes, macrophages, fine their effective anticarcinoger. Leukoregulin, a lymphokine recent can prevent carcinogenesis and inlis direct, irreversible and occur: I growth is primarily reversible by reased susceptibility of preneoplasm by natural killer cells resulting. Leukoregulin at very high concertumor cells. The direct-acting and potent than the tumor cell inhibition.	and other leukocytes, nic and tumor cell growth ly isolated during the hibit tumor cell growth. s without cytotoxicity. ut can become stic and neoplastic cells g from leukoregulin ntrations is also ticarcinogenic activity

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

tumor and other abnormal cell proliferation by this immunologic hormone.

and possibly signifying its central role in immunological homeostasis.

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



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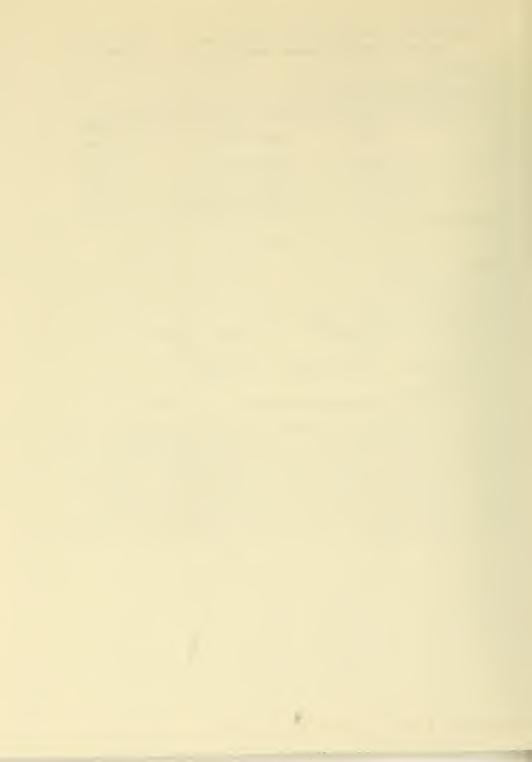
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PRINCIPAL INVESTIGATOR	R (List other professional personnel b	below the Principal Investigator.) (Neme, title, labore	tory, and institute a	Hilletion)
PI:	N. C. Popescu	Microbiologist	LB	NCI
Others:	S. Amsbaugh J. A. DiPaolo M. Kraus G. Kruh R. C. King	Microbiologist Chief Visiting Associate Medical Staff Fellow Senior Staff Fellow	LB LB LCMB LCMB LCMB	NCI NCI NCI NCI NCI
	Medical Center (Y.	T. Chen)		
Lab/BRANCH Laboratory of B	iology			
SECTION Somatic Cell Ge	netics Section			
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TOTAL MAN-YEARS:	PROFESSIONAL: 0.9	OTHER: 0.5		
CHECK APPROPRIATE BO (a) Human subj (a1) Minors		tissues		

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

(a2) Interviews

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.

per prempres



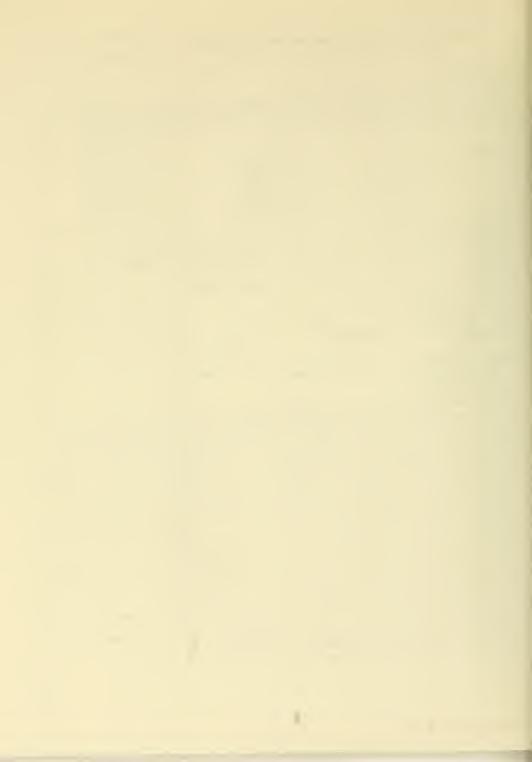
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04504-15 CCTP

October 1, 1986	to September 30,	1987			
Model Systems fo	cters or less. Title must fit on a r the Study of Cl	ne line between the bord nemical Carcin	ers.) nogenesis at the	Cellular	Level
PRINCIPAL INVESTIGATOR (L. PI: S. H.	st other professional personnel Yuspa	below the Principal Inve Chief	stigetor.) (Neme, title, leboret	LCCTP	NC I
M. Po D. Ro J. St	op rickland eenhalgh	Research Cher Research Cher Microbiologis Research Cher Visiting Fel Guest Research	nist st nist low	LCCTP LCCTP LCCTP LCCTP LCCTP LCCTP	NCI NCI NCI NCI NCI NCI
Center, Baltimor Bowden); Alton J	tories, Rockville e, MD (R. Tucker) ones Cell Science); University	of Arizona, Tuc	son, AZ (
LAB/BRANCH Laboratory of Ce	llular Carcinoger	nesis and Tumo	or Promotion		
SECTION In Vitro Pathoge	nesis Section				
NCI, NIH, Bethes	da, Maryland 208	392			
TOTAL MAN-YEARS: 9.0	PROFESSIONAL:		OTHER:		
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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 <u>in vivo</u> and <u>in vitro</u> 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.

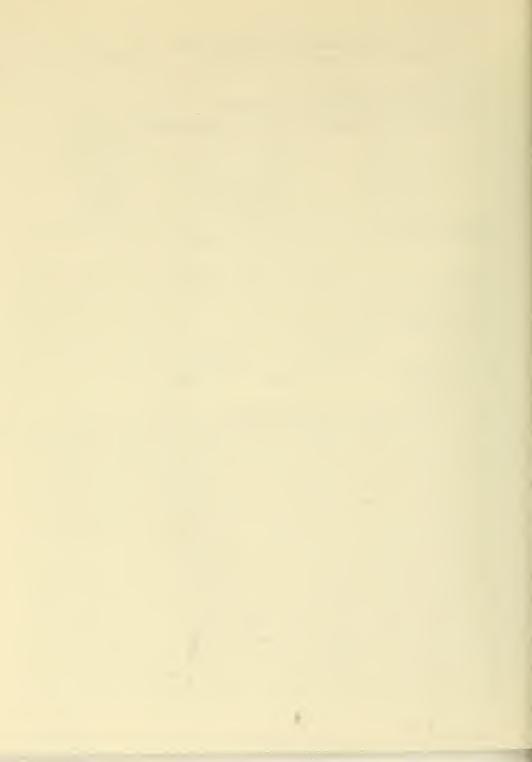


PROJECT NUMBER

Z01CP04798-17 CCTP

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PRINCIPAL INVESTIGATOR (List other p	rofessional personnel below the Principal Inv	estigator) (Name, title, labora	story, and institute affiliation)
PI: L. M. De Luc	a Research	Chemist	LCCTP NCI
Others: K. E. Creek	Staff Fel		LCCTP NCI
S. Kato	Visiting		LCCTP NC1
E. M. McDowe	in in the po		LCCTP NGI
D. Joel	IRTA Appo		LCCTP NCI
R. Sinha	Volunteer		LCCTP NCI
COOPERATING UNITS (if any)			
ImmuQuest, Rockville,	MD (R. Shores and E. F.	Spangler)	
LAB/BRANCH			
Laboratory of Cellula	r Carcinogenesis and Tum	or Promotion	
SECTION			
Differentiation Contr	ol Section		
INSTITUTE AND LOCATION			
NCI, NIH, Bethesda, M	aryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:	
7.0	5.0	2.0	
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(a) Human subjects	(b) Human tissues	(c) Neither	
(a1) Minors			
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	educed type. Do not exceed the space provide		
	uses apparent hyperplasi		
	er tracheal epithelium <u>i</u>		
	cheal epithelial cells o		
	arget cell for retinoid		
	tory (mucous) cell. In		
secretory cells flatten	ed and their capacity to	divide was gre	atly 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.



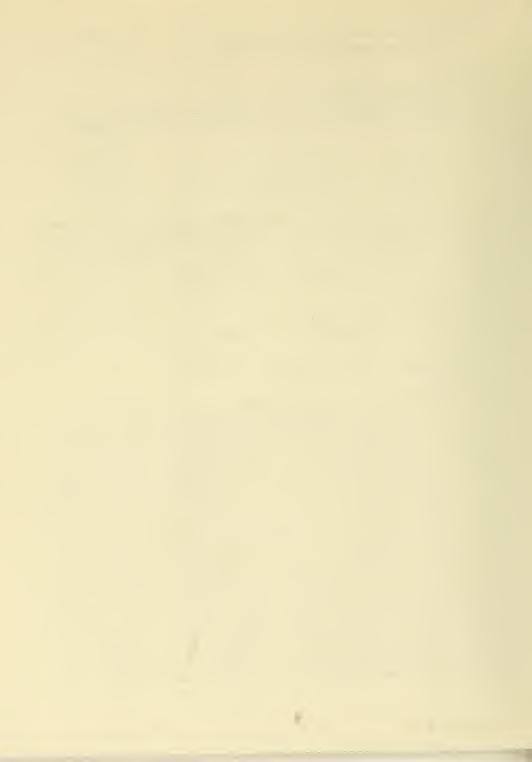
NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05177-06 CCTP

October 1, 1986	to September 3	0, 1987			
Use of Immunolog	gical Technique	•	action of Carcinog		
PRINCIPAL INVESTIGATOR PI: M. C. F		sonnel below the Principal Inve earch Chemist	stigetor) (Neme, titla, laboratory,		filiation) NC I
Others: S. H. Y O. Oliv E. Reec C. Litt R. Ozol	vero Fog I Spe cerst Res s Chi	arty Fellow cial Assistant fo earch Chemist ef		LCCTP DCT LMCP MB	NCI NCI NCI NCI
TX (J. M. Hunt); Huitfeldt); CIII	NCTR, Jeffers , Res. Triangl	on,AR (F. A. Bela e Park,NC (J. Swe	Univ. of Texas Med and); National Hos enberg); U. of Iow sy); McArdle, Madi	p.,Oslo, a, Iowa	Norway (H. City,IA
	ellular Carcino	genesis and Tumor	Promotion		
SECTION					
In Vitro Pathoge					
INSTITUTE AND LOCATION		30003			
NCI, NIH, Bethes	professi	20892	OTHER:		
3.25	PROFESSI	1.75	1.5		
CHECK APPROPRIATE BOX (a) Human subjet (a1) Minors (a2) Interview	ects 🗵 (b) H	luman tissues	(c) Neither		
		o not exceed the space provid			
and consequences substituted with (cis-DDP) have be chemical procedu	s of <u>in vitro</u> a n 2-acetylamino peen analyzed b ures developed	nd <u>in vivo</u> DNA mo fluorene (AAF) ar y quantitative in to localize adduc	have probed the nodification. Bio od <u>cis</u> -diamminedic. In the cist of the cis	logical hloropla immunoh epatic D	samples tinum II isto- NA of rats
diet. A compute one from which a slowly. Studies ferent cell type	er-derived phar adducts are rem s initiated to es within the l	macokinetic model oved rapidly and identify the two iver and DNA asso	ring 4 subsequent proposed two gen another from which compartments have being the district of the control of th	omic com h they a investi or less	partments, re removed gated dif- tightly-
localization of concentrations if foci induced by 231 nucleated pe times during cou	AF-DNA adducts in periportal r several differ eripheral blood urses of <u>cis</u> -DD	in livers of rategions and no addent protocols. (cell DNA samples r therapy. Add	s fed AAF demonst ducts detectable in is-DDP-DNA adduct from cancer pation act accumulation, otal cumulative do	rated hi n preneo s were m ents at in posit	gh adduct plastic easured in multiple ive samples

models.

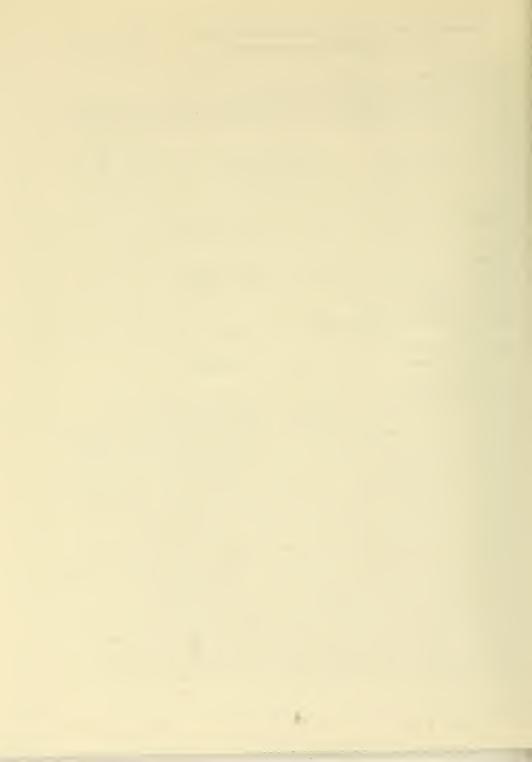
relatively slow adduct removal. Disease response data on 55 ovarian cancer patients indicated that individuals with high adduct levels have a high rate of complete response to therapy, and many individuals who do not respond also do not form adducts. Adduct persistence was demonstrated in many tissues 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



Z01CP05178-06 CCTP

PERIOD COVERED October 1, 1986, to September 30, 1987							
TITLE OF PROJECT (80 cherecters or less. Title must lit 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. Strickla	and Res	earch Chem	iist	LCCTP	NC I	
Others:	S. H. Yuspa	Chi			LCCTP	NCI	
	H. Hennings	Res	earch Chem	nist	LCCTP	NCI	
	A. Koceva-Chyl				LCCTP	NCI	
	•						
	D. Greenhalgh	Vis	iting Fell	OW	LCCTP	NCI	
COOPERATING UNITS (if any)							
		Inc., Rockvil	la MD (F	F Spanglor)			
Limitaques	t Laboratories,	. Inc., ROCKVII	ie, MD (E.	r. 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 BOXIES)							
(a) Human subjects (b) Human tissues (c) Neither							
(a) Minors							
□ (a2)	Interviews						

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the spece 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 12-0tetradecanovlphorbol-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 papillomaforming SENCAR cells and vice-versa should be helpful in elucidating mechanisms of sensitivity to promotion.



PROJECT NUMBER

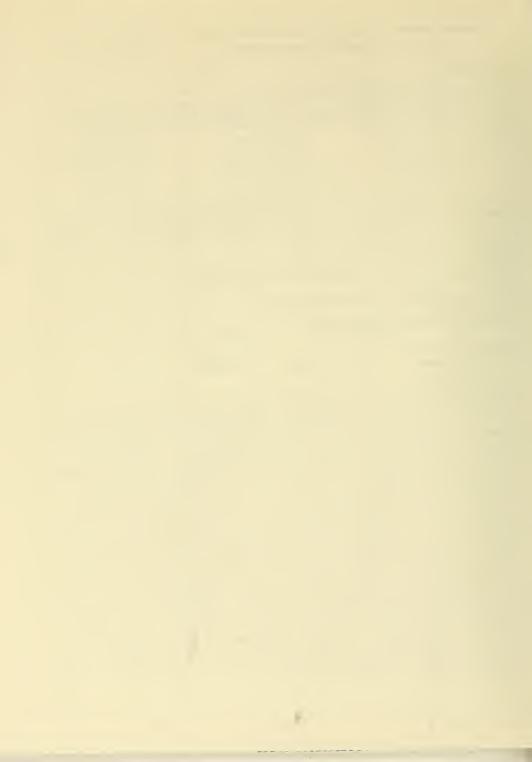
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05270-06 CCTP

October 1, 1986 to Sep	tember 30, 1987						
TITLE OF PROJECT (80 charecters or lass. Title must lit on one line between the borders.) Molecular Mechanism of Action of Phorbol Ester Tumor Promoters							
PRINCIPAL INVESTIGATOR (List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)							
PI: Peter M. Blum		LCCTP NCI					
Others: M. Dell'Aquil	a 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 Internat	ional Fellow LCCTP NCI					
COOPERATING UNITS (# 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 C3H1OT1/2 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 snown 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.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05445-03 CCTP

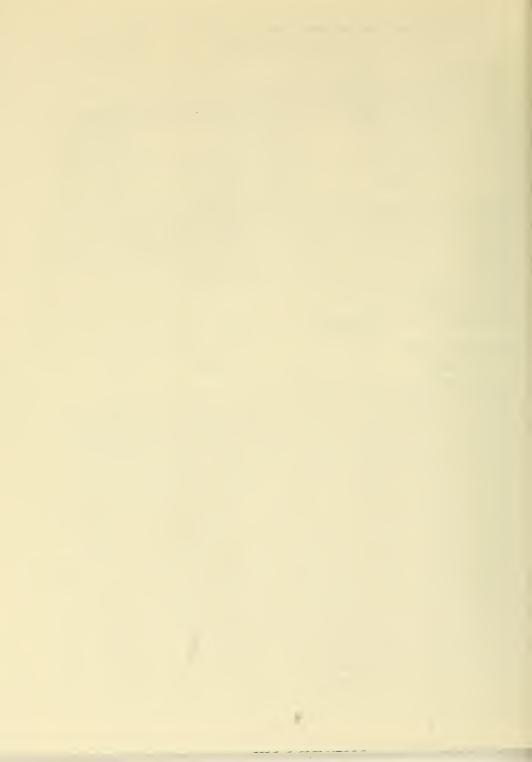
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PRINCIPAL INVES	STIGATOR (List other pro		the Principal Invas Microbiolo		oratory, and institute affiliat	tion) NCI
Others:	S. H. Yuspa		Chief	9136	LCCTP	NCI
others.	H. Nakazawa		Visiting F	01100	LCCTP	NCI
	T. Mehrel		Visiting F		LCCTP	NCI
	D. Rosentha					
	L. De Luca			ogy Fellow	LCCTP	NCI
			Research C		LCC.TP	NCI
	P. Steinert		Visiting S		DB	NCI
	S. Chung		Senior Sta		LEC	NCI
					. Spangler); U	
					r for Drugs an	
Biologics,	Bethesda, MU	('J. Ridge);	Baylor Col	lege of Med.,	Houston, TX (J. Clark)
U. of Wash	i., Seattle, W	A (C. Fisher);	Univ. of	Oslo, Oslo, N	orway, (H. Hui	tfeldt)
LAB/BRANCH						
Laboratory	of Cellular	Carcinogenesis	and Tumor	Promotion		
SECTION		•				
In Vitro P	athogenesis S	ection				
INSTITUTE AND L	LOCATION					
NCI, NIH,	Bethesda, Mar	yland 20892				
TOTAL MAN-YEAR	RS:	PROFESSIONAL:		OTHER:		
	4	3		1		
CHECK APPROPR	RIATE BOX(ES)					
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(a1)	Minors	` '				
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using diff	erent regions	of the genomi	c clones t	o drive expre	ssion of the c	:hlor-
amphenicol	acetyl trans	ferase gene an	d the prod	uction of tra	nsgenic mice w	hich
express a	human differe	ntiation-assoc	iated kera	tin gene in a	tissue- and d	lifferen-
					rotein, which	
		ied envelope.				

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

650

differentiation-associated keratins with respect to cell division, and requirements for the induction of terminal differentiation products in vitro.

PHS 6040 (Rev 1/84)



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

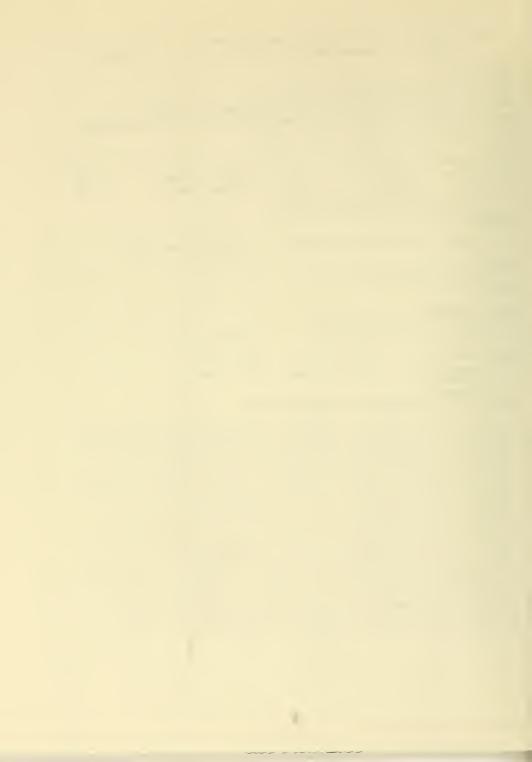
PROJECT NUMBER

Z01CP05051-09 LC

October 1, 1986 to September 30, 1987
TITLE OF PROJECT (80 characters or less. Title must int on ona 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
ABBRANCH Laboratory of Chemoprevention
SECTION
NSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892
TOTAL MAN-YEARS: 6.0 PROFESSIONAL: 3.0 OTHER: 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,

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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

Z01CP05396-04 LC

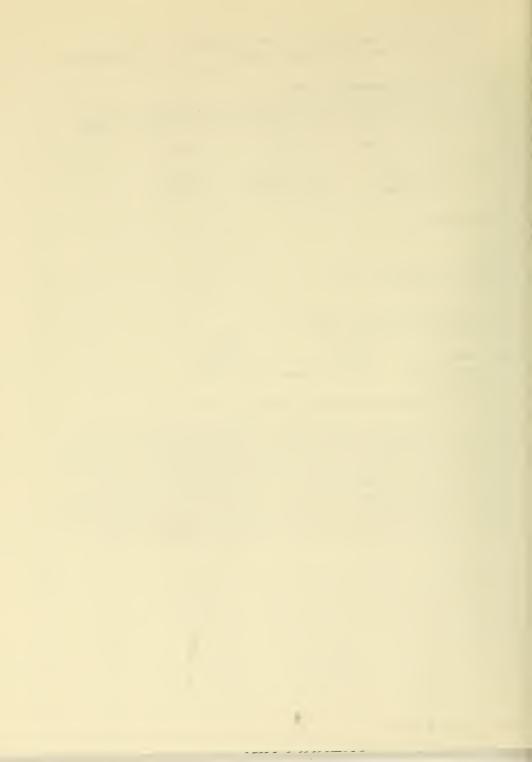
October	1, 1986 to Sept	tember 3	0, 1987					
	ECT (80 characters or less ent of Analogs					eve	lopment of the Rat	
PRINCIPAL INVE	STIGATOR (List other pro-	essional perso	nnel below the Pni	cipal investi	gator) (Nan	ne, trtie,	laboratory, and institute affiliation)
PI:	Shinichi Watar	nabe :	Sr. Staff	Fellow	L	_C I	NCI	
Others:	Eliane Lazar Elisa Vicenzi		Visiting F Guest Rese	archer	Ł	.C I	NCI NCI	
	Linda Durham		Guest Rese	archer	Ĺ	_C I	NCI	
COOPERATING	UNITS (f any)							
None								
Laborator	ry of Chemopre	ention/						
SECTION								
NCI, NIH	LOCATION , Bethesda, Man	yland 2	0892					
TOTAL MAN-YEA	ARS: 3.5	PROFESSION 2.			OTHER:			
CHECK APPROP	an subjects	□ (b) Hu	man tissues	XI	(c) Neit	ther		
_ ` ′	Minors Interviews							
SUMMARY OF W	ORK (Use standard unred	uced type. Do	not exceed the spi	ece 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 <u>E. coli</u> 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.

140-140000



PROJECT NUMBER

Z01CP05398-04 LC

NOTICE OF INTRAMURAL RESEARCH PROJECT 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, leboratory, and institute attillation) PI: Michael B. Sporn Chief LC NCT Others: Lalage M. Wakefield Visiting Associate LC NCI Diane M. Smith Biologist LC NCT Cornelius Knabbe BCSG Fellow NCT MR COOPERATING UNITS (# arry) LAB/BRANCH Laboratory of Chemoprevention INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892 TOTAL MAN-YEARS: 1.8 PROFESSIONAL: OTHER: 1.0 0.8 CHECK APPROPRIATE BOX/ES)

X (c) Neither

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

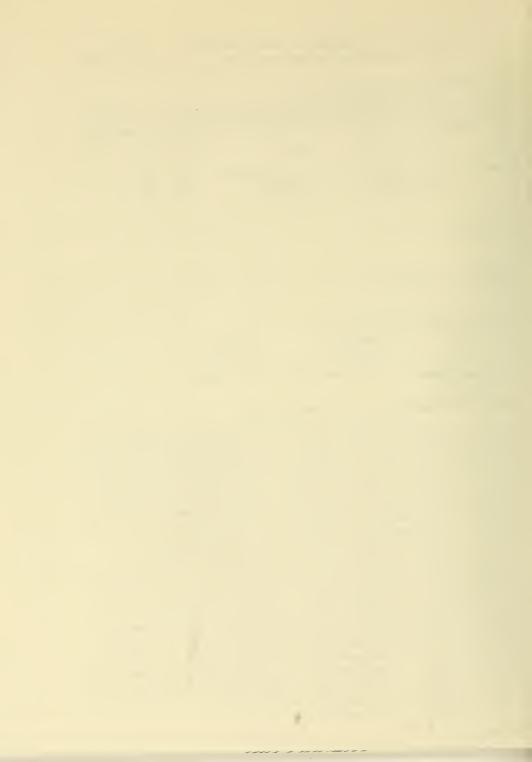
(b) Human tissues

(a) Human subjects

(a1) Minors (a2) Interviews

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.

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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 Sept				
TITLE OF PROJECT (80 characters or less CDNA Cloning and Functi	Title must fit on one line between onal Analysis of T	ransforming Grow	th Factor Beta	
PRINCIPAL INVESTIGATOR (Last other pro	fessional personnel below the Prin	cipal investigator.) (Name, title,	laboratory, and institute affi	Wetion)
PI: Ellen E. Van C	Dbberghen Staff F	ellow	LC NCI	
Others: Carl Baker	Senior	Investigator	LTVB NCI	
COOPERATING UNITS (# arry)				
Monique Dubois-Dalçq, S National Institute of	Section Chief, Labo Neurological and	ratory of Molecu Communicative Di	lar Genetics, sorders and St	roke
ABBRANCH Laboratory of Chemoprey	rention			
ECTION				
NSTITUTE AND LOCATION NCI, NIH, Bethesda, Mar	yland 20892			
OTAL MAN-YEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0.0		-
HECK APPROPRIATE BOX(ES) (a) Human subjects	☐ (b) Human tissues	☑ (c) Neither		
(a1) Minors (a2) Interviews				

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;

Transforming growth factor beta (TGF-beta) is a multifunctional polypeptide,

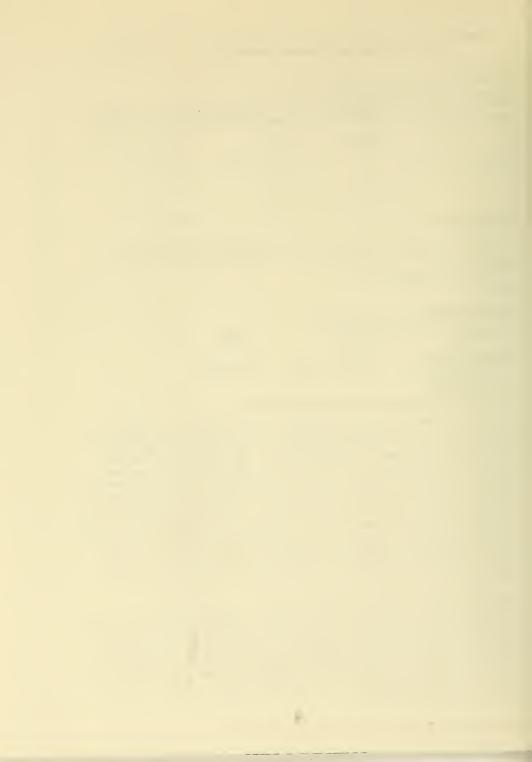
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

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).

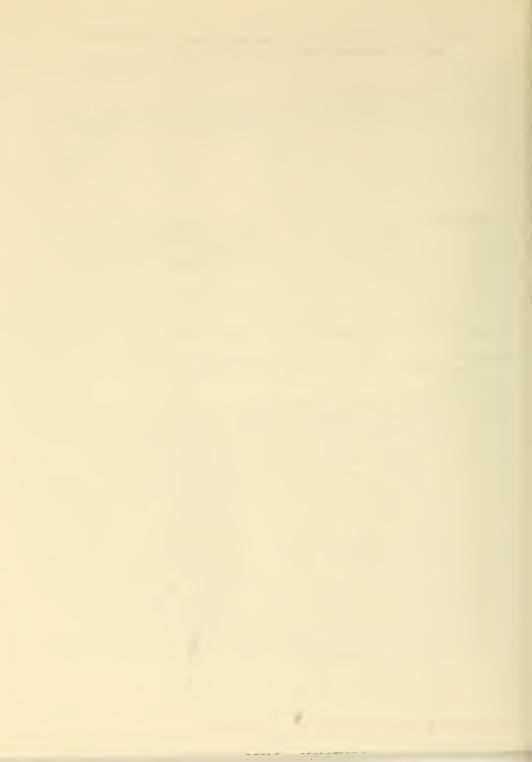


NOTICE OF INTRAMURAL RESEARCH PROJECT

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DRINGIBAL INV	STICATOR (List ather as	dessional personnel below the Principal	tances of	Incerest	in Cancer	Research
PI:	L. K. Keefer	Chief, Chemistr	investigator.) (Na	me, title, laborato	ry, and institute affi LCC	lletion) NC I
1	L. K. KCCICI	chier, chemistr	y Section		LUC	NC I
Others:	YH. Heur	Visiting Fellow			LCC	NCI
1	M. Stershic				LCC	NCI
	A. J. Streete		ate		LCC	NCI
	R. Nims	Chemist			LCC	NC I
	W. Blot	Chief			BB	NCI
	GY. Li	Visiting Fellow			BB	NCI
COOPERATING	UNITS (if any) PRI.	Frederick, MD (J. Hr	abie. L.	Ohannesia	n. D. Will	iams):
SK&F Lab	s., Philadelph	ia, PA (B. Mico); NJ	Med. Sch.	. Newark.	NJ (C. Yar	na): 11.
of Wash.	, Seattle, WA	(S. Nelson); American	Chem. So	c. (J. Ma	lin): Cance	er Res.
Centre,	Moscow, USSR (V. Turusov); Clemson	Univ., C	lemson, So	C (J. Fann	ina)
LAB/BRANCH						
	ry of Comparat	ive Carcinogenesis				
Chemistr	y Section					
NCI. NIH	. Frederick, M	D 21701-1013				
TOTAL MAN-YE	•	PROFESSIONAL:	OTHER:			
3.	3	3.3	OE.r.		0	
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(a) Hum	an subjects	(b) Human tissues		ither		
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	Interviews					
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Mechanis	ms potentially	responsible for form	ation, ac	tivation,	and detoxi	ication
of carcinogenic N-nitroso compounds in the human body are under intense investi-						
gation. Evidence implicating the alpha-nitrosamino radical as the critical intermediate in both activation and inactivation of the potent carcinogen,						
Intermed	late in both a	ctivation and inactiv	ation of	the potent	t carcinoge	en,
		, has been obtained t				
studies.	Certain iron	species have been for	una to co	nvert amir	nes to the	r carcin-
in wive	-mitroso deriv	atives in nonacidic m	edia mode	ing those	e potential	ly found
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liat the	reaction may	be as fast in the lim	it of ver	y low niti	rite concer	itrations

(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.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

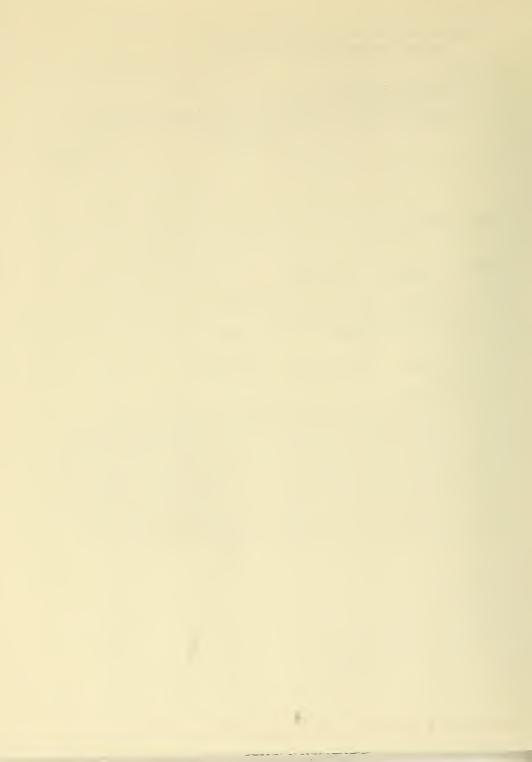
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	. Title must fit on one line between the borders.)		1
The Role of Lipotropes	in Carcinogenesis		
PRINCIPAL INVESTIGATOR (List other pro	fessional personnel below the Principal Investigetor.) (Name, title, laboratory, and institute a	iffiliation)	
PI: L. A. Poirier	Supervisory Research Chemist	LCC	NC I
Others: P. T. Allen	Microbiologist	LCC	NCI
COOPERATING UNITS (if any)			
McArdle Laboratory, Ma	dison, WI (H. Pitot)		
Laboratory of Comparat	ive Carcinogenesis		
SECTION			
	iomethylation Working Group		
NCI, NIH, Frederick, M	aryland 21701-1013		
TOTAL MAN-YEARS:			
1.0	PROFESSIONAL: OTHER:		
CHECK APPROPRIATE BOX(ES)			
(a) Human subjects	☐ (b) Human tissues ☐ (c) Neither		
(a1) Minors			
(a2) Interviews			
	Visual Arra Da and assessed the annual array day 1		
	funed 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.

PHS 6040 (Rev. 1/84)

ALL PARTS



PROJECT NUMBER

0

NOTICE OF INTRAMURAL RESEARCH PROJ	JECT
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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 personnal below the Principal Investigator) (Name, title, laboratory, and institute affiliation) P.I. K.S. Kasprzak Visiting Scientist NCI Others: M.P. Waalkes Senior Staff Fellow LCC NCT J.M. Ward Chief, Tumor Pathol. & Pathogen. Section I.CC NC I 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) LAR/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

(b) Human tissues

(a2) Interviews

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

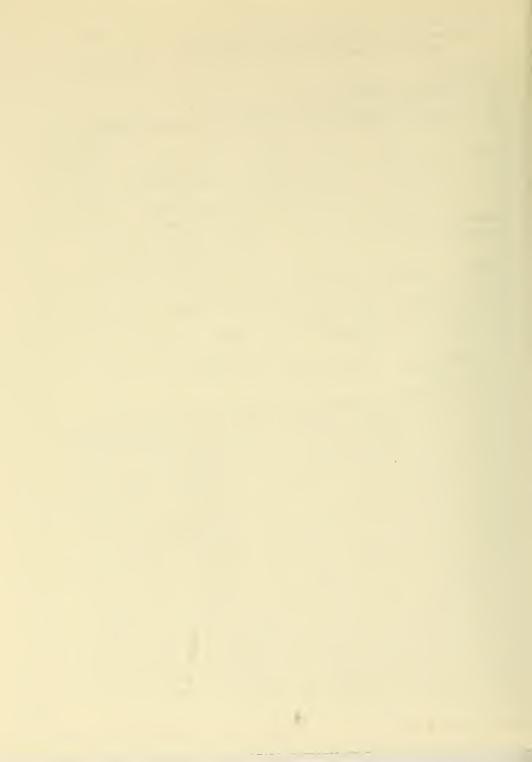
1.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects

(a1) Minors

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.



PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP04680-17 LCC

PERIOD COVERED		
October 1, 1986 to September 30, 1987		
TITLE OF PROJECT (80 cheracters 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.) (Neme, title, leboretory, er	nd institute effiliation)	
PI: L. A. Poirier Supervisory Research Chemist	FCC	NC I
D C Blair		
D. G. Blair Chief, Microbiology Section	LM0	NCI
M. Bhave Visiting Fellow	LCC	NCI
T. Flammang Guest Researcher	LM0	NCI
	-11.5	
COOPERATING UNITS (if any)		
South Charling China (II any)		
None		
Tione .		
AB/BRANCH		
Laboratory of Comparative Carcinogenesis		
ECTION		
Office of the Chief, Biomethylation Working Group		
NSTITUTE AND LOCATION		
NCI, NIH, Frederick, Maryland 21701-1013		
OTAL MAN-YEARS: PROFESSIONAL: OTHER:		
1.0		
HECK APPROPRIATE BOX(ES)		
(a) Human subjects (b) Human tissues (c) Neither		
(a1) Minors		

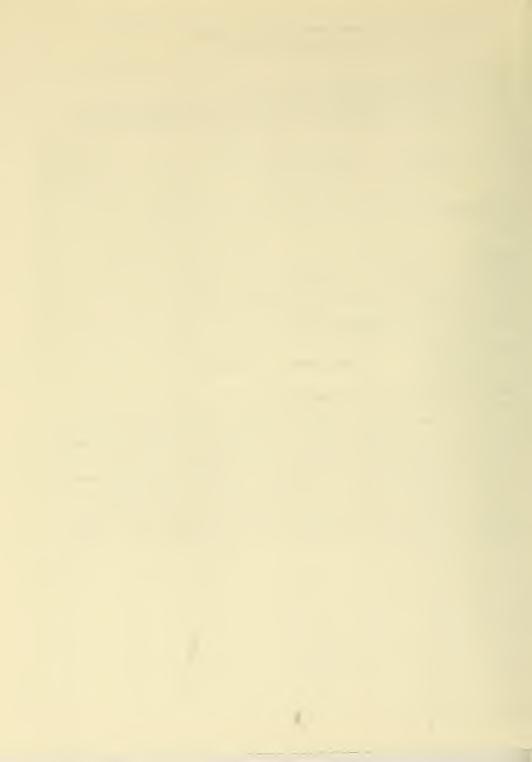
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the spece provided.)

(a2) Interviews

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 methyldeficient rats. This gene is hypomethylated in the liver tumors of rats fed the methyl-deficient diets.

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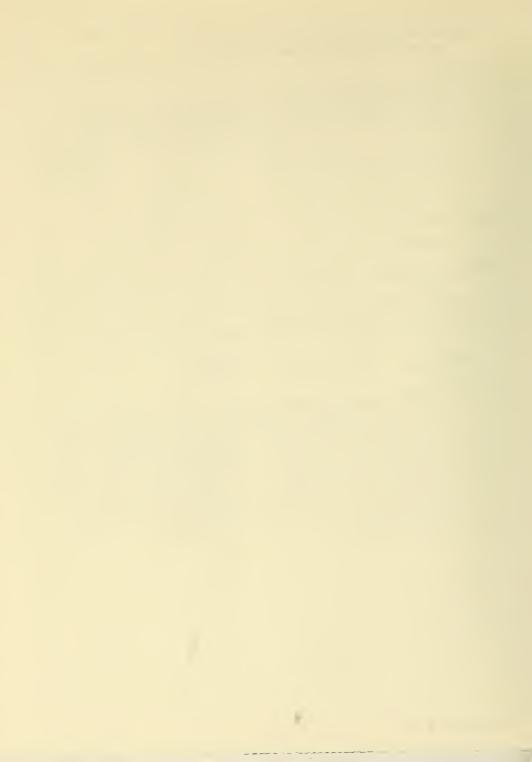
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

			Z01CP04812-19 LCC		
PERIOD COVERED					
October 1, 1986 to Sept					
TITLE OF PROJECT (80 characters or less. T	itle must fit on one line between the border	s.)			
Cell Interactions Durin					
PRINCIPAL INVESTIGATOR (List other profes	ssional personnel below the Principal Invest	gator.) (Neme, title, labora	tory, and institute effiliation)		
PI: U. I. Heine	Chief, Ultrastructur	al Studies Sec	tion LCC NC	Ι	
Others: H. Miki	Visiting Fellow		LCC NC	:1	
K. S. Kasprzak			LCC NC		
COOPERATING UNITS (if any)					
Program Resources, Inc.	, Frederick, MD (E. F.	Munoz)			
LAB/BRANCH					
Laboratory of Comparativ	ve Carcinogenesis				
SECTION					
Ultrastructural Studies	Section				
INSTITUTE AND LOCATION	1 1 01701 1010				
NCI, NIH, Frederick, Mai					
	PROFESSIONAL:	OTHER:	1 0		
2.5	1.5		1.0		
CHECK APPROPRIATE BOX(ES) (a) Human subjects	(h) Human tingunga [X]	(c) Neither			
(a) Human subjects	(b) Human tissues	(c) Maither			
(a2) Interviews					
SUMMARY OF WORK (Use standard unreduc	and hope the not exceed the space organization				
To define the role of ga				t-	
able, promotable, and tu					
JB6 as well as NIH 3T3,					
decanoylphorbol-13-acetate (TPA). Cell-cell communication was measured either					

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.

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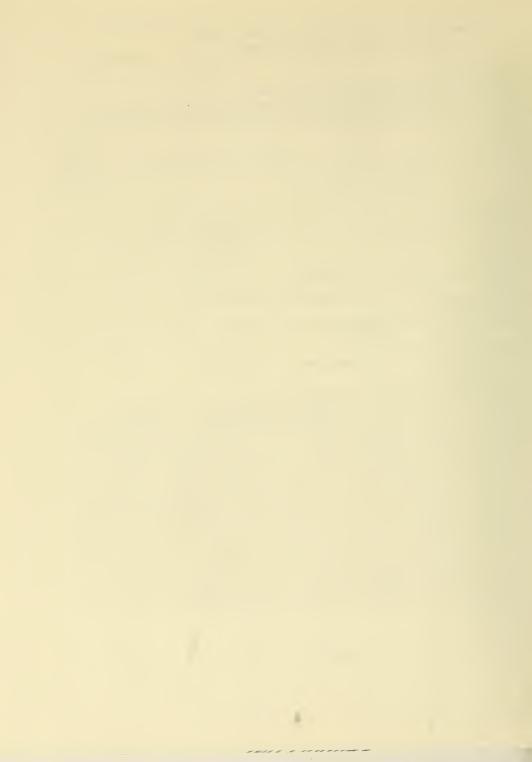
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT
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.)							
	genesis and Tumor Promotion in Nonhuman Primates						
	essional parsonnal below the Principal Investigator.) (Name, title, laboratory, and institute	effilietion)					
PI: A. E. Palmer	Research Veterinarian	LCC	NC I				
Others: J. M. Rice		LCC	NCI				
J. M. Ward	Chief, Tumor Pathol. and Pathogen. Section	LCC	NC I				
L. M. Andersor	n Expert	LCC	NCI				
P. J. Donovan	Chemist	LCC	NC I				
A. O. Perantor	ni Microbiologist	LCC	NCI				
COOPERATING UNITS (if eny)			-				
SEMA, Inc., Rockville,	MD (J. Phillips); Baylor College of Medicine, H	louston	. TX				
(L. J. Lu); Oak Ridge A	ssociated Universities, Oak Ridge, TN (N. Clapp)	,				
LAB/BRANCH							
Laboratory of Comparati	ve Carcinogenesis						
SECTION							
Office of the Chief, Pr	rimate Research Working Group						
INSTITUTE AND LOCATION							
NCI, NIH, Frederick, Ma	ryland 21701						
TOTAL MAN-YEARS:	PROFESSIONAL: OTHER:						
3.2	2.0	.2					
CHECK APPROPRIATE BOX(ES)							
	(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 <u>in vitro</u> cultivability and transplantability to rodents. Selected tumors are <u>subjected</u> 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.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMIIRAL RESEARCH PROJECT

PROJECT NUMBER

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PERIOD COVER						
		tember 30, 1987				
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		gan Specificity in Trans				
PRINCIPAL INV	ESTIGATOR (List other pro	fassional personnel below the Principal Inves	tigator.) (Name, titla, laborat	ory, end in:	stitute affiliation)	
PI:	J. M. Rice	Chief	L	.CC	NCI	
Others:	P. Donovan	Chemist	L	.cc	NCI	
	A. Perantoni	Microbiologist	Ĺ	.CC	NCI	
	T. Enomoto	Visiting Fellow	L	.CC	NCI	
COOPERATING	UNITS (if any)					
Microbio Inc., Fr	logical Associa ederick, MD (B	ates, Inc., Bethesda, MD . Diwan)	(M.L. Wenk); P	rogram	n Resources	,
Laborato	ry of Comparat	ive Carcinogenesis				
SECTION						
		is Section, Developmenta	l Biology Worki	ng Gro	oup	
NCI, NIH		aryland 21701-1013				
TOTAL MAN-YE	ARS:	PROFESSIONAL:	OTHER:			
	2.0	1.0	1.0			
CHECK APPRO	PRIATE BOX(ES)					

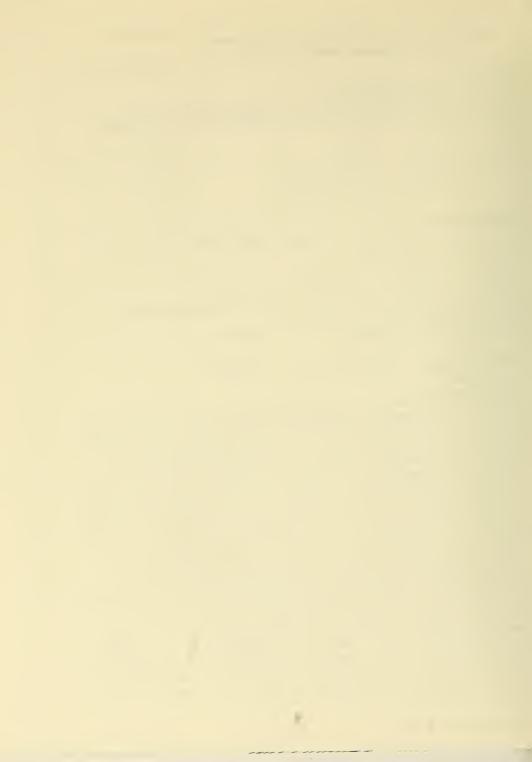
(c) Neither

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

(a) Human subjects (a1) Minors (a2) Interviews

(b) Human tissues

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-thioquanine 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

701CP05288_06_LCC

ERIOD COVERED			
October 1, 1986 to Septe			
TLE OF PROJECT (80 charecters or less. T	itle must fit on one line between the borders.)		
signal fransduction and	the Control of Developmental	Gene Expression	
RINCIPAL INVESTIGATOR (List other profes	sionel personnel below the Principel Investigator.) (Nei	me, title, leboretory, and institute effilietic	on)
D. D. Blumberg	Senior Staff Fellow	LCC	NCI
Others: J. F. Comer	Microbiologist		
R. Das		LCC	NCI
N. Das	Visiting Fellow	LCC	NCI
DOPERATING UNITS (if any)			
· · · · · · · · · · · · · · · · · · ·			
lone			

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:

2.25

1.25

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects

(a1) Minors

(b) Human tissues

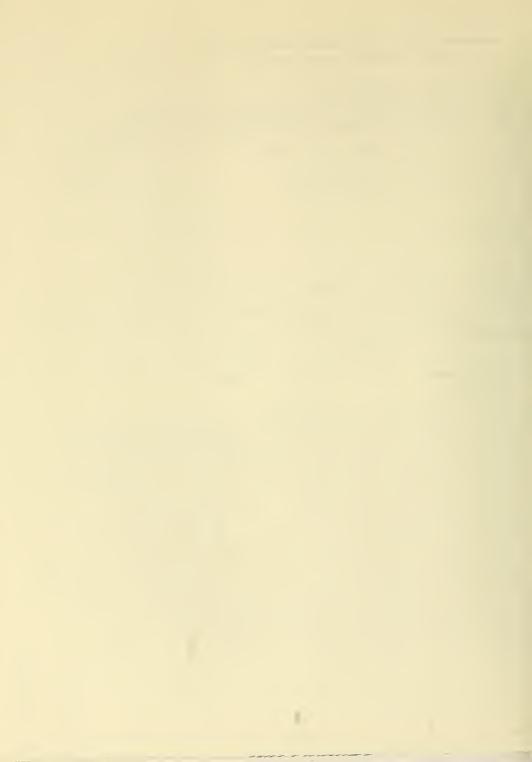
(c) Neither

(a2) Interviews

SUMMARY OF WORK (Use standard 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 (NH₄)₂SO₄ 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++/Calmodulindependent 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. - 157655

PHS 6040 (Rev 1/84)



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

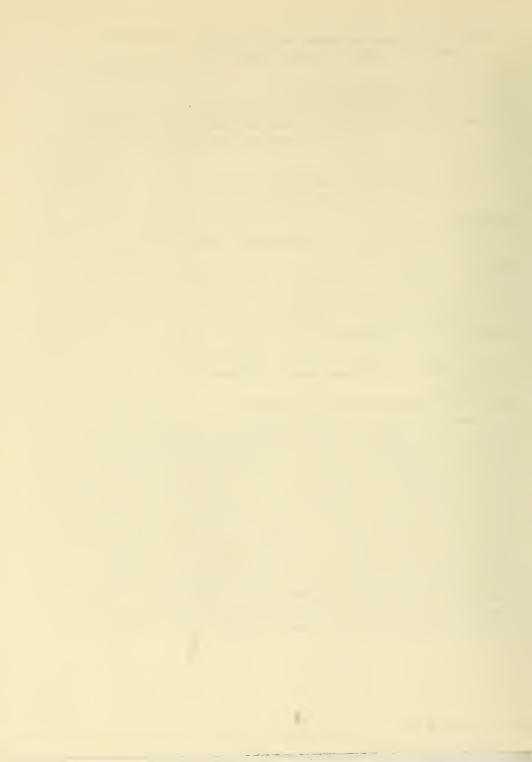
	Z01CP0529	9-06	LCC
Definition Covered October 1, 1986 to September 30, 1987			
TITLE OF PROJECT (80 cherecters or less. Title must ht on one line between the borders.) Interspecies Differences in Transplacental Carcinogenes			n
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, til	le, laboretory, and institute effi	lietion)	
PI: J. M. Ward Chief, Tumor Pathology and Pat	hogenesis 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
		LUU	1401
COOPERATING UNITS (if any)			
Microbiological Associates, Inc., Bethesda, MD (M. L. Winc., Frederick, MD (B. Diwan)	lenk); Program Re	sourc	es,
AB/BRANCH			
Laboratory of Comparative Carcinogenesis			
SECTION			
Tumor Pathology and Pathogenesis Section			
NSTITUTE AND LOCATION			
NCI, NIH, Frederick, Maryland 21701-1013			
TOTAL MAN-YEARS: PROFESSIONAL: OTHER:			
2.2 1.6	0.6		
CHECK APPROPRIATE BOX(ES)			
\sqcup (a) Human subjects \sqcup (b) Human tissues $\boxtimes X$ (c) Neither			
(a1) Minors			

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

(a2) Interviews

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.

entime total



PROJECT NUMBER

Z01CP05301-06 LCC

NOTICE OF	INTRAMURAL	RESEARCH PRO	JECT
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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) Chief, Tumor Pathology and Pathogenesis Section J. M. Ward NCT

Others: S. Rehm Visiting Associate A. Hagiwara Guest Researcher P. Nara Staff Fellow R. Benveniste Medical Officer E. Santos Visiting Associate LCC NCT NC. I LCC On NC I LVC NC I LMM NIAID

COOPERATING UNITS (# 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 . 2.5

OTHER: 2.5

CHECK APPROPRIATE BOX(ES)

(a) Human subjects

(b) Human tissues

(c) Neither

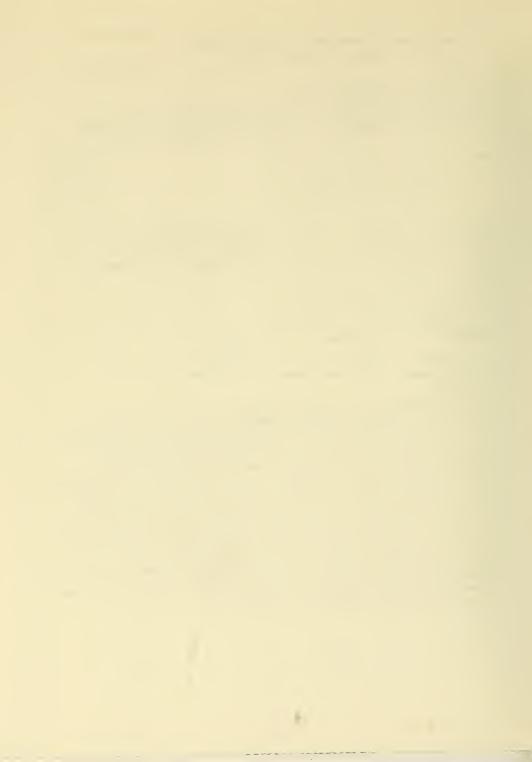
(a1) Minors

(a2) Interviews

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

The pathology and biology of selected experimentally-induced and naturallyoccurring 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).

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NOTICE OF INTRAMURAL RESEARCH PROJECT

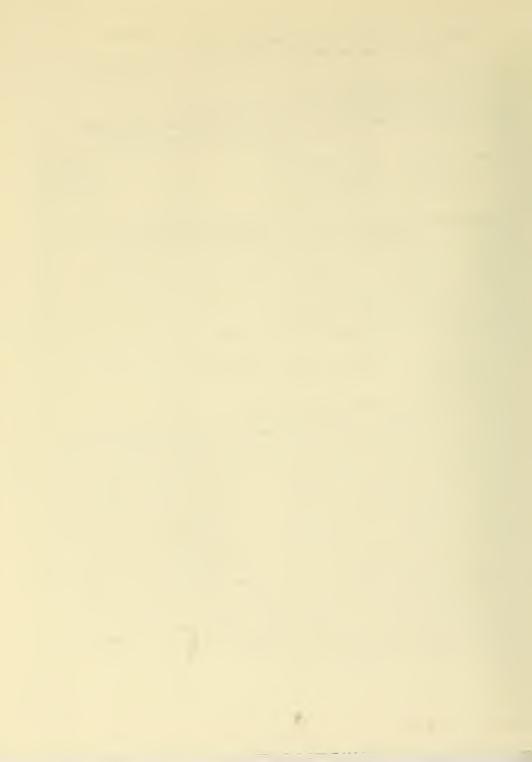
PROJECT NUMBER

Z01CP05303-06 LCC

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PERIOD COVER October		tember 30, 1987					
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PRINCIPAL INVE	ESTIGATOR (List other pro	fessional personnal below the	Principal investi	getor.) (Name, title, lebor	atory, and institute effilia	ation)	
PI:	J. M. Ward	Chief, Tumor F	athology	and Pathogene	sis Section	LCC	NCI
Others:	A. Hagiwara P. Donovan D. Devor R. Cantor	Guest Research Chemist Biologist Staff Fellow	er			LCC LCC LCC	NCI NCI NCI NCI
	ity University	ram Resources, I Medical School, nmark (K. Osterg	Nagoya,	derick, MD (B. Japan (N. Ito	Diwan, J. H); Pathology	ennem	an);
Laborato	ry of Comparat	ive Carcinogenes	is				
SECTION Tumor Pa	thology and Pa	thogenesis Secti	on				
NCI, NIH		aryland 21701-1	013				
TOTAL MAN-YEA	ARS: 2.0	PROFESSIONAL:		OTHER:			
(a1)	PRIATE BOX(ES) nan subjects Minors Interviews	(b) Human tissu	es 🛚	(c) Neither			

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

Z01CP05352-05 LCC

20101033	54-05	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	effiliation)	
PI: L. M. Anderson Expert	LCC	NCI
	200	
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.		
		NCI
A. Hagiwara Guest Researcher	LCC	NC I
A. Perantoni Microbiologist	LCC	NCI
T. Enomoto Visiting Fellow	LCC	NCI
COOPERATING UNITS (if any)		
Program Resources, Inc., Frederick, MD (H. Issaq, R. Kovatch); America	n Haal	t h
Foundation, Valhalla, NY (S. Hecht); and Baylor University, Houston, T	/ /! 1	1
	((L . U	· 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.)

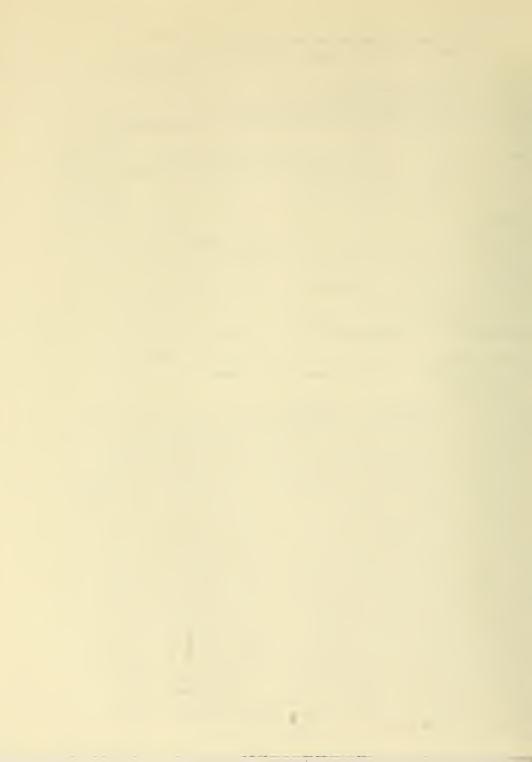
This project addresses carcinogenesis during the perinatal period with regard both to mechanisms underlying susceptibility and to assessment of public health related phenomena. A pharmacogenetic transplacental carcinogenesis experiment has been completed in mice, confirming that both fetal and maternal 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.



PROJECT NUMBER!

Detail Detail 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 (Lust other professional personnel below the Principal Investigator.) (Name. title. Taboratory, and institute artitlational.) 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) LABIBRANCH Laboratory of Comparative Carcinogenesis SECTION Perinatal Carcinogenesis Section INSTITUTE AND LOCATION NCI, NIH, Frederick, Maryland 21701-1013 TOTAL MAN-YEARS: PROFESSIONAL: OTHER: 1.25 CHECK APPROPRIATE BOX(ES) (a) Human subjects (b) Human tissues XX (c) Neither (a1) Minors (a2) Interviews SUMMMANY OF WORK (Isse 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
TITLE OF PROJECT (40 characters or less. Title must lift 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 antilizations.) 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) LABJERANCH Laboratory of Comparative Carcinogenesis Section INSTITUTE AND LOCATION NCI, NIH, Frederick, Maryland 21701-1013 TOTAL MAN-YEARS: PROFESSIONAL: OTHER: 1,25 1.0 0.25 CHECK APPROPRIATE BOXIES) (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
Sensitivity Factors in Special Carcinogenesis Models PRINCIPAL INVESTIGATOR (Ust other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute antituation). 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 S. S. Park Expert LMC NCI H. V. Gelboin Chief LMC NCI COOPERATING UNITS (# 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 CHECK APPROPRIATE BOX(ES) (a) Human subjects (b) Human tissues XX (c) Neither (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
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute artillations) P1: L. M. Anderson Expert L. C. C. 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 D. 0.25 CHECK APPROPRIATE BOX(ES) (a) Human subjects (b) Human tissues XX (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
PI: L. M. Anderson Expert 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 COOPERATING UNITS (M 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 MANL-YEARS: 1.25 CHECK APPROPRIATE BOXIES) (a) Human subjects (b) Human tissues XX (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
J. M. Ward A. Hagiwara Guest Researcher S. S. Park Expert H. V. Gelboin Chief COOPERATING UNITS (H 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 DIAL MANYEARS: 1.25 PROFESSIONAL: 1.25 TOTAL MANYEARS: (a) Human subjects (a) Human subjects (a) Human subjects (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
COOPERATING UNITS (# 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 XX (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
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 MANYEARS: PROFESSIONAL: OTHER: 1.25 1.0 0.25 CHECK APPROPRIATE BOX(ES) (a) Human subjects (b) Human tissues XX (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
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: 1.25 PROFESSIONAL: OTHER: 1.25 CHECK APPROPRIATE BOX(ES) (a) Human subjects (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
Laboratory of Comparative Carcinogenesis SECTION Perinatal Carcinogenesis Section INSTITUTE AND LOCATION NCI, NIH, Frederick, Maryland 21701-1013 TOTAL MAN-YEARS: 1.25 CHECK APPROPRIATE BOXIES) (a) Human subjects (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
SECTION Perinatal Carcinogenesis Section INSTITUTE AND LOCATION NCI, NIH, Frederick, Maryland 21701-1013 TOTAL MAN-YEARS: 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.) Determination of the factors which influence susceptibility to chemical carcinogens and of means of modulating this susceptibility are important goals. Such
Perinatal Carcinogenesis Section INSTITUTE AND LOCATION NCI, NIH, Frederick, Maryland 21701-1013 TOTAL MANYEARS: 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
INSTITUTE AND LOCATION NCI, NIH, Frederick, Maryland 21701-1013 TOTAL MANYEARS: 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
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
TOTAL MANYEARS: 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 spece provided.) Determination of the factors which influence susceptibility to chemical carcinogens and of means of modulating this susceptibility are important goals. Such
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.) Determination of the factors which influence susceptibility to chemical carcinogens and of means of modulating this susceptibility are important goals. Such
Determination of the factors which influence susceptibility to chemical carcinogens and of means of modulating this susceptibility are important goals. Such
gens and of means of modulating this susceptibility are important goals. Such
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 circulation levels of NDMA and increased by led to an increase in large types.
lating 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, tetradecanoyl-
phorbol acetate (TPA). (3) An immunohistochemical study with a specific mono-
clonal antibody to isozymes of cytochrome P450 induced by polycyclic aromatic
hydrocarbons (PAH) has revealed that this procedure can be used for semiquantita-
tive determination of metabolic phenotype of liver, and that in extrahepatic
tissues, staining is especially intense in, and perhaps limited to, the endo- thelium 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 directacting 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.



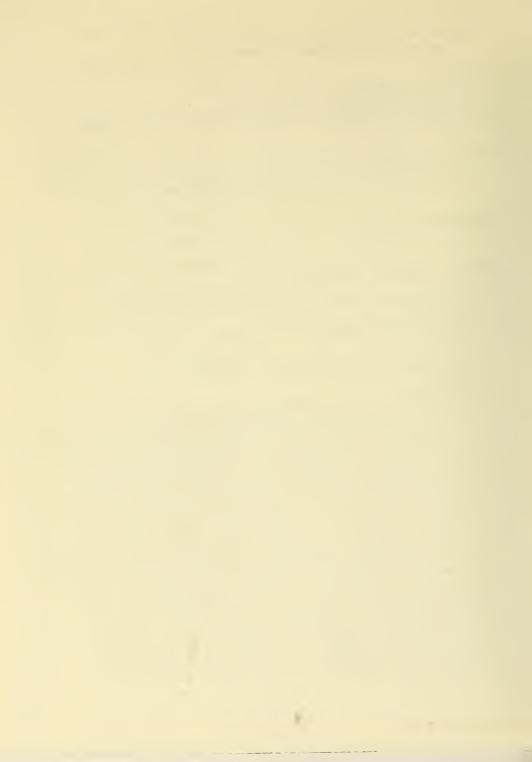
PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05399-04 LCC

				2010F03393-04 LCC
	1, 1986 to Sept			
Oncogene	Expression in	. Title must fit on one line between the li Chemically Induced Tui	mors	
PRINCIPAL INV	ESTIGATOR (List other pro	fessional personnel below the Principal	nvestigator.) (Neme, title, labor	atory, and institute effiliation)
PI:	J. M. Rice	Chief		LCC NCI
Others:	A. O. Peranton	i Microbiologist		LCC NCI
	M. Watatani	Visiting Fellow		LCC NCI
}	C. D. Reed	Senior Health Serv	ices Officer	LCC NCI
	J. M. Ward	Chief, Tumor Patho		
COOPERATING	UNITS (if any)			
Microbio	logical Associa	tes, Inc., Bethesda,	MD (M. L. Wenk)	
Laborato	ry of Comparati	ve Carcinogenesis		
SECTION				
Perinata		s Section, Developmen	al Biology Worki	ng Group
NCI, NIH		ryland 21701-1013		
TOTAL MAN-YE	ARS:	PROFESSIONAL:	OTHER:	
	3.0	2.5	0.5	;
(a) Hur	man subjects) Minors) Interviews	☐ (b) Human tissues	☐ (c) Neither	
		duced type. Do not exceed the space pr	ovided 1	
The expre	ession of activ	ated cellular oncogend oncogene expression	es in chemically to progression fr	om the normal to the
		e studied using 3T3 t		
niques ar	nd monoclonal a	ntibodies directed aga	ainst the specifi	c oncogene products.
Itive type	es of tumors ha	ve 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 $T \rightarrow \bar{A}$ 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.



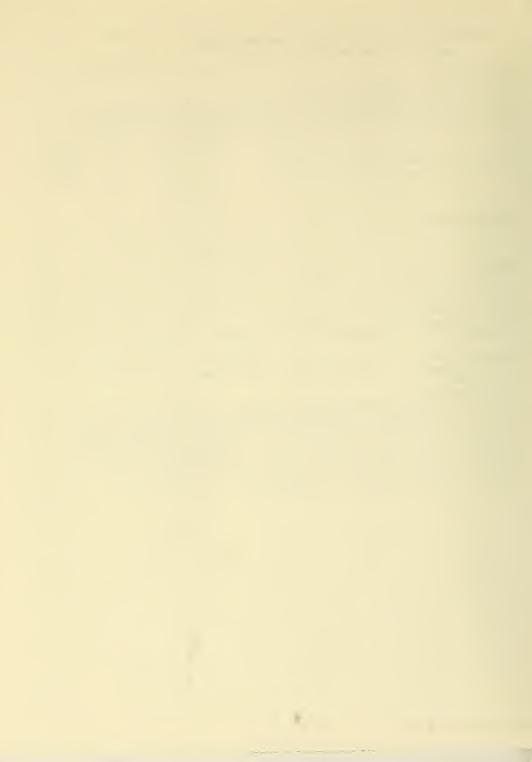
PROJECT NUMBER

Z01CP05465-03 LCC

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October																
TITLE OF PROJE																
The Regu	lato	ry	Role of	Reti	noids	and	Growth	Fac	ctors	in	Tissu	e Di	ffere	entiat	ion	
PRINCIPAL INVE	STIGA	TOR	(List other pro	fessionel .	personne	l below th	e Principal	Invest	gator.) (N	eme, ti	tle, labor	etory, ar	nd institu	te effiliation	7)	
PI:	U.	Ι.	Heine	Ch	ief,	Ultra	struct	ural	Stud	dies	Sect	ion		LCC	NC	I
Others:			Roberts Sporn		searc ief	h Chei	mist							LC LC	NC NC	_
COOPERATING	UNITS	(ıf an	v)													
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Program F	Reso	urc	es, Inc.	, FCF	RF, F	reder	ick, M	D (E	. F.	Muno	oz)					
LAB/BRANCH																
Laborator	ry o	f C	Comparati	ive Ca	arcin	ogenes	sis									
SECTION																
Ultrastru	uctu	ral	Studies	Sect	ion											
INSTITUTE AND																
NCI, NIH,	, Fr	ede	rick, Ma	rylar	nd 2	170 1- 3	1013									
TOTAL MAN-YEA				PROFES	SSIONAL				OTHER:							
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☐ (a) Hum			ects	□ (b)	Hum	an tissi	ues	X	(c) Ne	either						
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☐ (a2)																
SUMMARY OF W														_		
The invol	vem	ent	or tumo	or gro	wth :	ractor	r-beta	(10	F-β)	in e	embry	ona l	deve	lopmer	nt	
of the mo	ouse	wa	s invest	igate	a in	emory	yos of	10	to 18	day	s of	gest	tatio	n, usi	ing	
antibodie	:2 L	aisi	eu ayain	156 SV	าแกลา	LIC DE	epride	s or	tne	161-	 B mor 	nomer	^ T.O	locali	70	

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.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

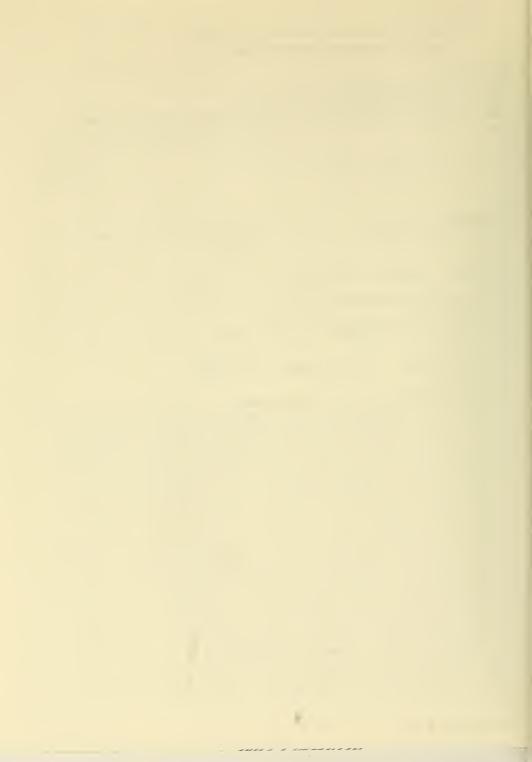
PROJECT NUMBER

Z01CP05487-02 LCC

October 1, 1986 to September 30, 1987									
TITLE OF PROJECT (80 cherecters 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 effiliations)	etion)								
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 eny)									
Stanford Research Institute, Palo Alto, CA (W. Bradford); Program Resour	res								
Inc., Frederick, MD (A. W. Andrews, L. Channesian)	003,								
, , , , , , , , , , , , , , , , , , , ,									
LAB/BRANCH									
Laboratory of Comparative Carcinggenesis									
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 standard unreduced type. Do not exceed the space provided.)									
Fecapentaenes from human feces have been found to be direct-acting mutag	ione :	and							
are therefore prime candidates as human carcinogens, especially for the									
bowel. A variety of in vitro studies by other investigators have demons									
potent mutagenic effects of fecapentaene-12 (FP-12) in bacterial cells.									
cell transforming activity in vitro is low and mutagenic activity for ma									
cells is weak. Thus, animal experiments have become necessary to charac									
the in vivo toxic and carcinogenic effects. We first studied the purity									
stability of FP-12 to determine the most effective handling procedures d		,							
animal exposure. The chemical was moderately stable under argon but qui									
decomposed after exposure to air. Vitamin E has shown promise for stabi	lizir	na							
fecapentaene solutions for use in carcinogenesis studies. Several types	of	19							
animal experiments were performed. Skin painting studies in SENCAR mice	rave	halad							
neither initiating activity nor complete carcinogenesis to the skin by r									
exposure. Intrarectal and subcutaneous administration to mice and rats		.cu							
not provided convincing evidence of the carcinogenesis of FP-12, althoug		+							
studies are still in progress. In a preliminary but small intrarectal m	IOUS C	, ,							
study 1/15 mice had a small colonic carcinoma and 3 had foci of atvoice	1 001	onic							
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									

PERIOD COVERED

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

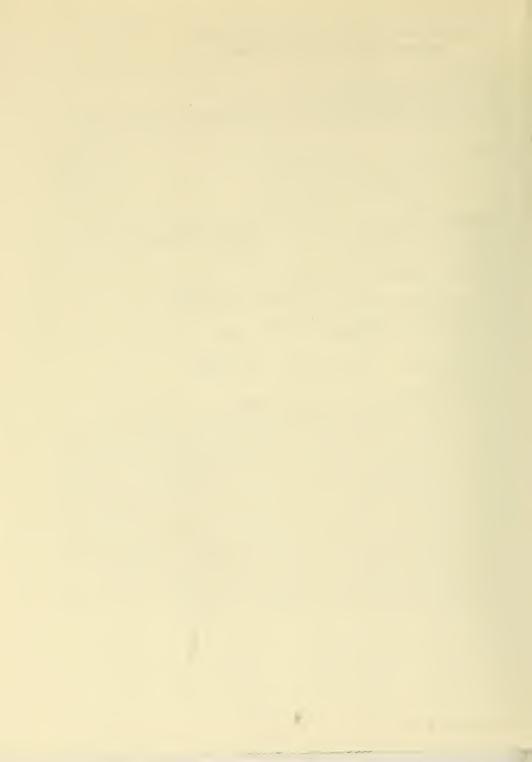
701CP05488-02 LCC

October 1, 1986 to September 30, 1987								
TITLE OF PROJECT (80 characters or less Title must lit on one line between the borders.) Mechanisms of Inorganic Carcinogenesis: Cadmium								
PRINCIPAL INVESTIGATOR (List other pri	PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute effiliation)							
PI: M. P. Waalkes	s Senior Staff Fellow	LCC NCI						
Others: M. Bhave		LCC NCI						
M. Miller	Senior Staff Fellow	LCC NCI						
A. O. Peranto	oni Microbiologist	LCC NCI						
K. S. Kasprza		LCC NCI						
T. Koizumi	Visiting Fellow	LCC NCI						
S. Rehm	Visiting Scientist	LCC NCI						
COOPERATING UNITS (if eny)	Training Serentise	200 1101						
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)								
Laboratory of Comparat	cive Carcinogenesis							
Office of the Chief, 1	Inorganic Carcinogenesis Working Group							
NCI, NIH, Frederick, N	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 stenderd unreduced type. Do not exceed the space provided.)

present.

The mechanisms of cadmium carcinogenesis are under active investigation. In rats, subcutaneous injection of cadmium induced injection-site tumors in a doserelated 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.



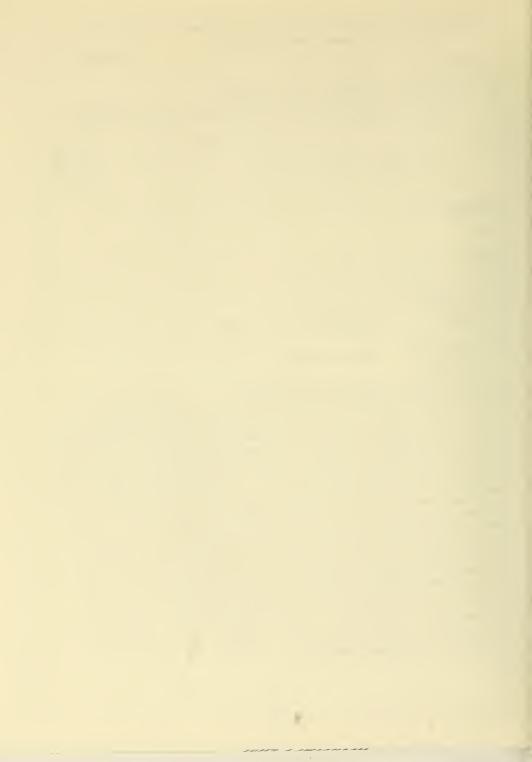
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

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PERIOD COVERED		
October 1, 1986 to September 3		
TITLE OF PROJECT (80 cherecters or less. Title must fit of		
Effects of Chemical Carcinogen		
PRINCIPAL INVESTIGATOR (List other professional person		
PI: M. S. Miller Seni	or Staff Fellow	LCC NCI
Others: J. M. Rice Chie	f, Perinatal Carcinogenesis Se	ction LCC NCI
L. M. Anderson Expe	rt	LCC NCI
M. P. Waalkes Seni	or Staff Fellow	LCC NCI
J. S. Rhim Rese	arch Microbiologist	LCMB NCI
	•	
COOPERATING UNITS (if any)		
None		
LAB/BRANCH		
Laboratory of Comparative Carc	inogenesis	
SECTION		
Perinatal Carcinogenesis Section	on	
INSTITUTE AND LOCATION		
NCI, NIH, Frederick, Maryland	21701-1013	
TOTAL MAN-YEARS. PROFESSION	NAL: OTHER:	
1.25	1.0	
CHECK APPROPRIATE BOX(ES)		
☐ (a) Human subjects 🏻 🏋 (b) Hu	man tissues	
(a1) Minors		
(a2) Interviews		
CURRENCY OF WORK (Up. 10-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-		

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-9,10epoxide (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 steroidinducible 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.



PROJECT NUMBER

	NOTICE OF INT									
	•				Z01CP0498	6-10 LEC				
PERIOD COVER	ED									
	1, 1986 to Sep									
	ECT (80 charecters or less			rs.)						
Molecular Basis of Steroid Hormone Action										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, leboratory, and institute effillation)										
PI:	Michael G. Co	rdingley	Visiting A	ssociate	LEC	NCI				
Others:	Gordon L. Hag	er	Head, Horm	one Action &						
			Oncogene	sis Section	LEC	NCI				
	Anna Riegel		Visiting F	ellow	LEC	NCI				
	Ronald G. Wol	ford	Microbiolo	gist	LEC	NCI				
	Diana S. Bera	rd	Microbiolo	gist	LEC	NCI				
COOPERATING	UNITS (if any)									
None										
LAB/BRANCH	ry of Experime	ntal Carcinon	anacic							
SECTION	ity of Experime	- Caremog	C11C3 13							
	Action and Onc	ogenesis Sect	ion							
INSTITUTE AND		ogenes is see a	1011							
	l, Bethesda, Ma	ryland 20892								
TOTAL MAN-YEA	ARS:	PROFESSIONAL:		OTHER:						
	2	1.5		0.5						
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(c) Neither

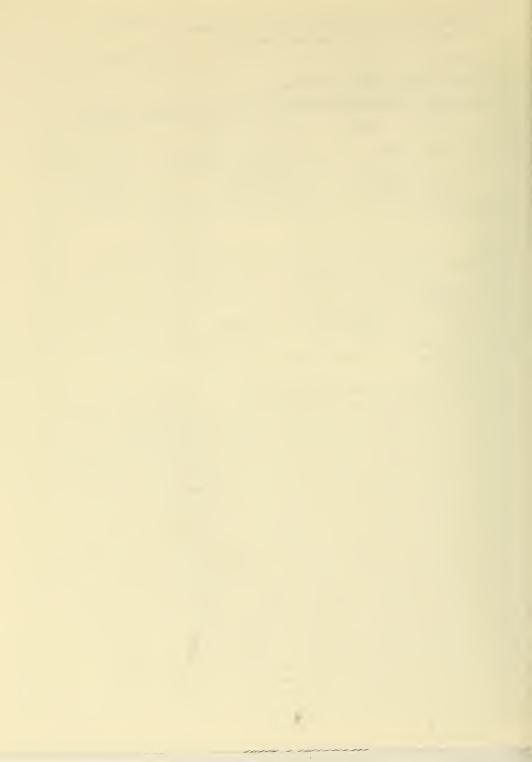
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(b) Human tissues

(a) Human subjects

(a1) Minors (a2) Interviews

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 steroidactivated 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.



PROJECT NUMBER

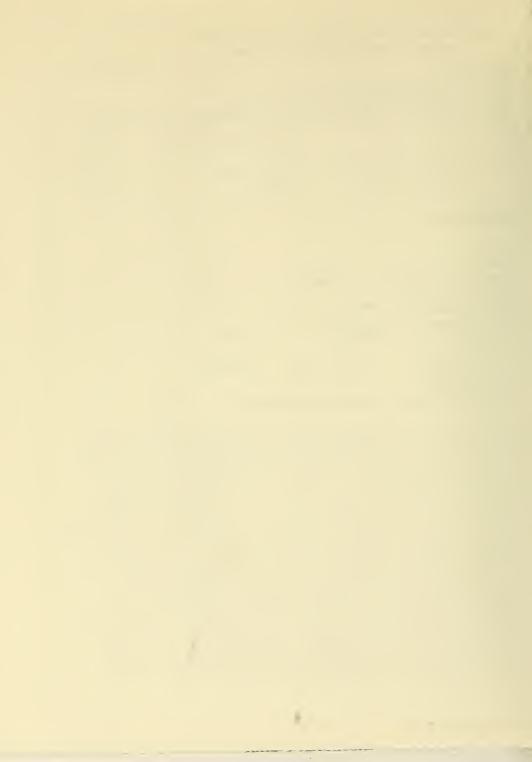
NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05262-06 LEC

PERIOD COVER	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, laboretory, and institute affiliation)									
PI:	Ritva P. Evart	s	Veterinary	Medica1	Officer	LEC NCI			
Others:	Snorri S. Thor Peter Nagy Elizabeth R. M		Chief Visiting F Biologist	ellow		TEC NCI FEC NCI			
COOPERATING	UNITS (if any)				_				
None									
LAB/BRANCH									
	y of Experimen	tal Carcinoge	nesis						
SECTION									
	Carcinogenesis	Section							
INSTITUTE AND	LOCATION								
NCI, NIH,	Bethesda, Mar	yland 20892							
TOTAL MAN-YE	ARS:	PROFESSIONAL:		OTHER:					
3.0		2.0		111					
_	PRIATE BOX(ES) nan subjects	(b) Human ti	iceuse [X]	(c) Neithe	•				
(a) Hull		(b) Human t	155465	(0) 14011110					
	Interviews								
LJ (az) Interviews SUMMARY OF WORK (Use standard unreduced type. Do not exceed the spece 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-6phosphatase 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-Stransferase 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.



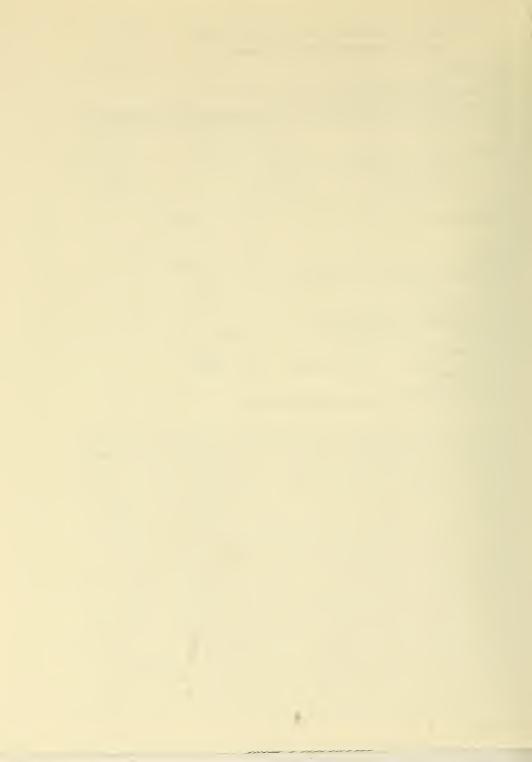
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT.

701CP05263-06 LEC

PERIOD COVERED									
October I, 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 INVE	ESTIGATOR (List other pro	fessional personnel bel	ow the Principal Investi	igator.) (Name, title, labore	tory, and institute effiliation)				
PI:	Mark J. Miller	•	Senior Staf	f Fellow	LEC NCI				
Others:	Arthur David C	llson	Computer Pr	ogrammer	LEC NCI				
	Snorri S. Thor	rgeirsson	Chief		LEC NCI				
	Peter J. Wirth	1	Expert		LEC NCI				
	Lori Hampton		Biologist		LEC NCI				
COOPERATING	UNITS (if any)								
None									
LAB/BRANCH									
	ry of Experimen	ital Carcinog	enesis						
SECTION									
INSTITUTE AND	LOCATION								
NCI, NIH,	, Bethesda, Mar	yland 20892							
TOTAL MAN-YEA	ARS:	PROFESSIONAL:		OTHER:					
1.9		1.2		0.7					
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(a) Hum	nan subjects	(b) Human	tissues 🛛	(c) Neither					
☐ (a1)	Minors								
☐ (a2)	Interviews								
SUMMARY OF W	NIMMARY OF WORK (I be standard uppdured type Oc not exceed the space opputed.)								

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.



October 1, 1986 to September 30, 1987

PROJECT NUMBER

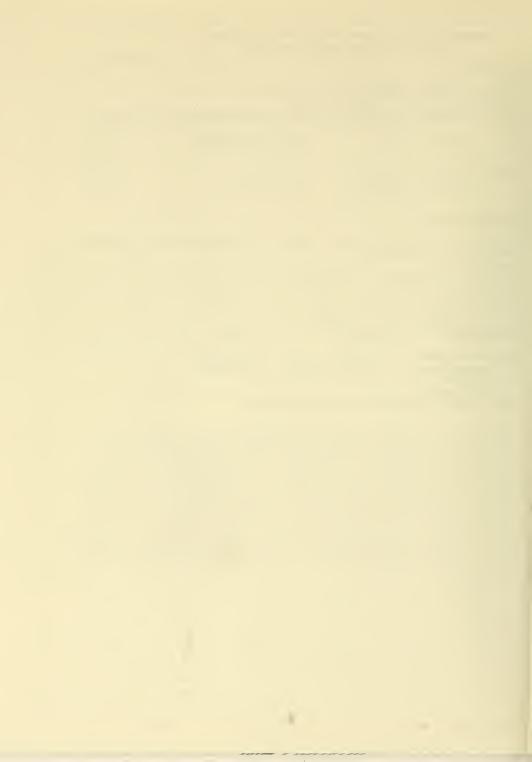
Z01CP05283-05 LEC

TITLE OF PROJ	ECT (80 charecters or less.	Title must fit on one li	ne between the bord	ers.)			
Condition	nal Expression	of Mammalian	Genes				
PRINCIPAL INVI	ESTIGATOR (List other prof	fessional personnel bel	ow the Principal Inve	stigator.) (Name, title, lat	oratory, and institute	affiliation)	
PI:	Gordon L. Hage	r		one Action & sis Section		LEC NCI	
	Diana S. Berar Michael G. Cor		Microbiolo Visiting A			LEC NCI	
COOPERATING UNITS (If any) Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Manitoba, Canada (Arnold H. Greenberg)							
	ry of Experimen	tal Carcinog	enesis				
	Action and Onco	genesis Sect	ion				
NCI, NIH	LOCATION , Bethesda, Mar	yland 20892					
TOTAL MAN-YE		PROFESSIONAL: 0.2		OTHER:			
☐ (a) Hum ☐ (a1)		(b) Human	tiss ues] (c) Neither			
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.

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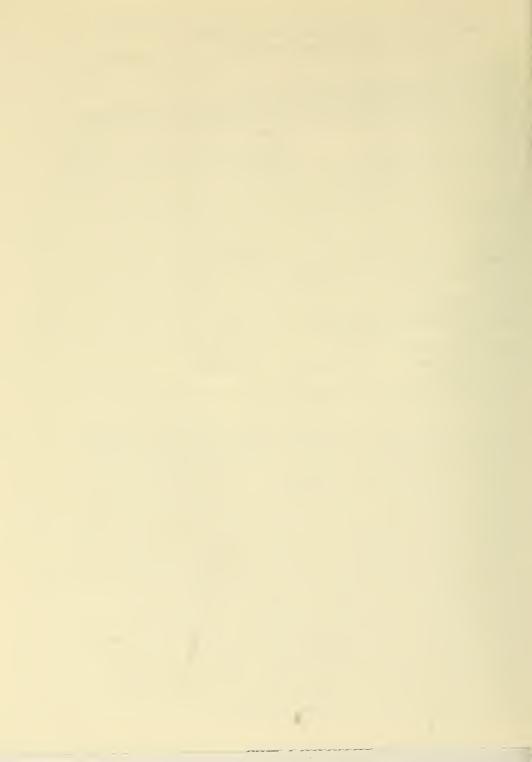
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05313-05 LEC

PERIOD COVER		ember 30 1987						
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							
				gator.) (Name, title, leboratory, and i	actitute offiliation)			
PHINCIPAL INVE	STIGATOR (LIST Offiair pro-	lessional personnel below the	rincipal investig	pator.) (warrie, title, reporatory, and r	· ·			
PI:	Peter J. Wirth	1	Expert		LEC NCI			
Others:	Ritva P. Evart Lori L. Hampto Snorri S. Thom	on	Veterina Biologis Chief	ry Medical Officer t	LEC NCI LEC NCI			
COOPERATING	UNITS (if any)							
Universi	ty of Toronto,	Canada (Dr. M. 1	Waheed Ro	omi)				
LAB/BRANCH								
Laborato	ry of Experimer	ntal Carcinogene:	sis					
SECTION								
INSTITUTE AND								
NCI, NIH	, Bethesda, Mar	ryland 20892						
TOTAL MAN-YEA	ARS:	PROFESSIONAL:		OTHER:				
1.3		0.9		0.4				
(a1)	an subjects Minors Interviews	(b) Human tissue		(c) Neither				
SUMMARY OF V	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 twoto 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.



PROJECT NUMBER

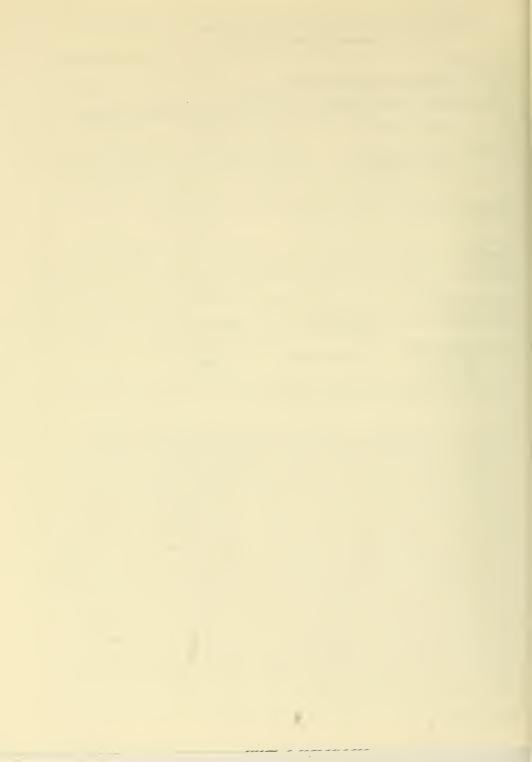
NOTICE OF INTRAMURAL RESEARCH PROJECT

701CP05317-04 LEC

PERIOD COVERED								
October 1, 1986 to September 30, 1987								
TITLE OF PROJE	CT (80 cherecters or less	Title must fit on one	ine between the b	orders.)				
Opal Supr	pressor tRNA i	n Human and	other Gen	omes				
	STIGATOR (List other pro-				title, laboretory, and i	nstitute affilietion)		
PI:	Dolph L. Hatf	ie Id	Research	Biologist	LEC	NCI		
0.1								
Others:	Byeong Jae Le		Visiting		LEC			
	Malini Rajago		Visiting		LEC	NCI		
	O. Wesley McB	ride	Section 1	Head	NCI			
COOPERATING L	JNITS (if eny)							
None								
LAB/BRANCH								
Laborator	ry of Experime	ntal Carcino	genesis					
SECTION								
INSTITUTE AND I	LOCATION							
NCI, NIH.	, Bethesda, Ma	ryland 2089	2					
TOTAL MAN-YEA		PROFESSIONAL:		OTHER:				
.2.5		2.5		0				
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(a) Huma	an subjects	(b) Human	tissues	(c) Neithe	er			
(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.



MENT OF HEALTH AND HUMAN SERVICES - FUBLIC HEALTH SERVIC

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP5373-04 LEC

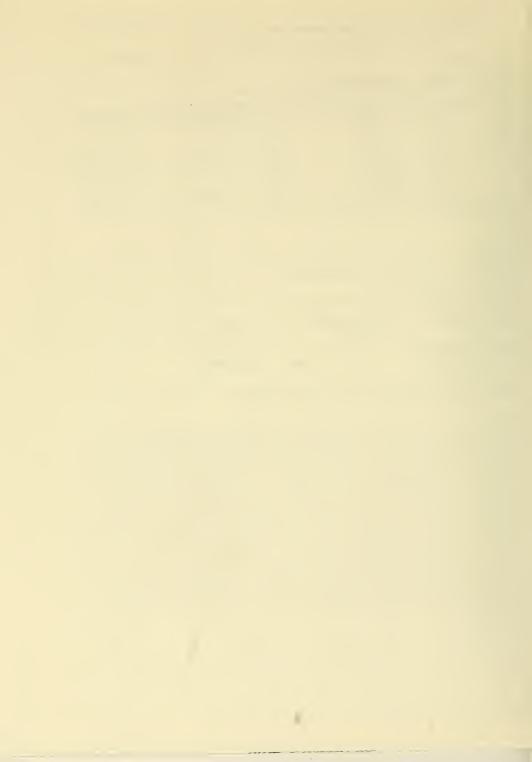
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		cterization of					
		dessional personnel below th			• • • • • • • • • • • • • • • • • • • •		
PI:	Anthony C. Hug	gget t	Visiting	g Associate	LEC	NCI	
Others:	Henry C. Krut:		Expert		LEC		
	Mrunal S. Chaj			Staff Fellow	LEC		
	Peter J. Wirth		Expert		LEC	NCI	
	James B. McMal		Expert		DDRG		
	Anita B. Rober			Investigator	LCP	NCI	
	Snorri S. Tho	rgeirsson	Chief		LEC	NCI	
COOPERATING	UNITS (if any)						
None							
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		(b) Human tiss	ues 🗵	(c) Neither			
☐ (a1)							
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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 liverderived 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 liverderived 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.

PHS 6040 (Rev 1/84)

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

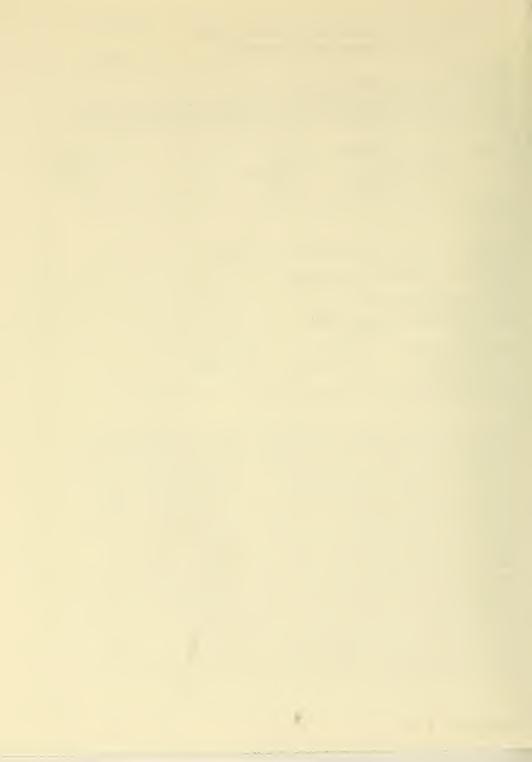
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

NOTICE OF INTRAMURAL RESEARCH PROJECT.

701CP05374-04 LFC

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	1, 1986 to Sep							
TITLE OF PROJE	CT (80 characters or less	s. Title must fit on one i	ne between the borde	75.)				
				eins Relevant t				
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute effillation)								
PI:	Peter P. Roll	ler	Head, Biopo	lymer Chemistry	Section LEG	. NCI		
Others:	Snorri S. Tho	orgeirsson	Chief		LEC	NCI		
	Chien-Hua Niu	ı T	Expert		LEC	NCI		
	Anthony C. Hu	Jagett	Visiting As	sociate		NCI		
	Preston H. Gr		Chemist			NCI		
						1101		
COOPERATING L	JNITS (if any)							
Kossuth I	University, De	ebrecen, Hung	ary (Dr. Z.	Dinya)				
AB/BRANCH								
Laborator	ry of Experime	ental Carcino	genesis					
SECTION								
	er Chemistry S	Section						
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NCI, NIH, Bethesda, Maryland 20892								
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☐ (a2) l	Interviews							

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 0-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.



NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

		Z01CP053/9-04 LEC
PERIOD COVERED		
October 1, 1986 to September 30, 1987		
TITLE OF PROJECT (80 cherecters or less. Title must fit on one line between	the borders.)	
Analysis of Polypeptide Changes During Co	ellular Differentiati	ion and Transformation
PRINCIPAL INVESTIGATOR (List other professional personnel below the Prin	cipal Investigator.) (Name, title, laboral	tory, and institute effiliation)
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, Bi	iologist, American
Red Cross; John P. Kupferschmid, Clinica	Associate, IR SU, N	HLBI
LAB/BRANCH		
Laboratory of Experimental Carcinogenesis	>	
SECTION		
INSTITUTE AND LOCATION		
NCI, NIH, Bethesda, Maryland 20892	OTHER.	

0.5

(c) Neither

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

0.7

prophing?

(b) Human tissues

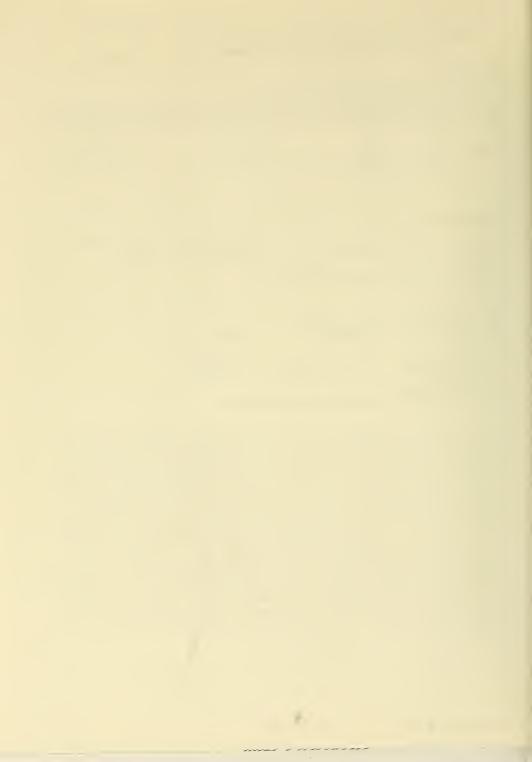
This project was initiated to analyze, both qualitatively and quantitatively, changes in total cellular protein patterns during cellular senescence using the technique of quantitative two-dimensional polyacrylamide gel electrophoresis. A human endothelial cell culture system has been developed which 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 12-0-tetradecanoylphorbol-13-acetate (TPA), recombinant gammainterferon (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.

PHS 6040 (Rev 1/84)

1.2

CHECK APPROPRIATE BOX(ES) (a) Human subjects

> (a1) Minors (a2) Interviews

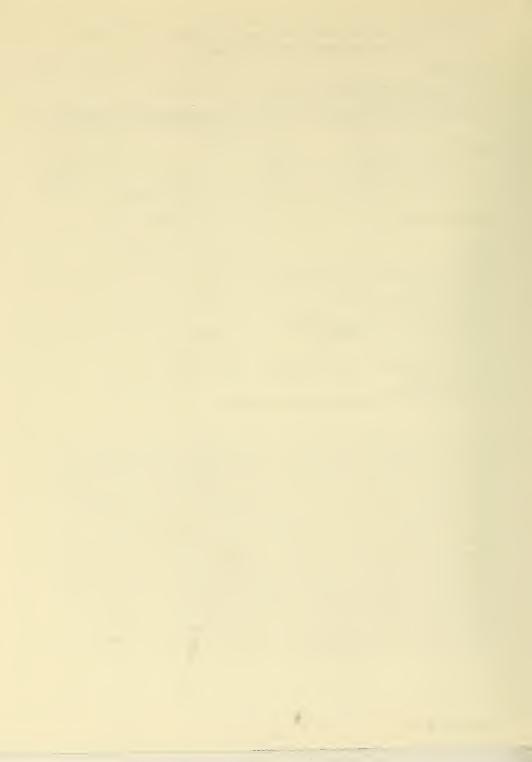


DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05447-03 LEC

PERIOD COVE								
October 1, 1986 to September 30, 1987								
	JECT (80 cherecters or less.				•			
Isolation	and Character	ization of	Proteins	from	Two-Dimensio	nal Polyacr	ylami	de Gels
PRINCIPAL INV	ESTIGATOR (List other pro-	fessional personne	ol below the Pnnci	oal Investig	getor.) (Name, title, labo	oratory, and institute	effilietion)
PI:	Anthony C. Hugg	gett	Visiting	Assoc	iate		LEC	NCI
Others:	Peter J. Wirth		Expert				LEC	NCI
	Preston Grantha	am	Chemist				LEC	
	Snorri S. Thora	geirsson	Chief				LEC	
	Peter P. Roller		Head, Bio	mv Load	er Chemistry	Section	LEC	
						00001011		1101
COOPERATING	UNITS (if eny)							
None								
LAB/BRANCH								
	y of Experiment	tal Carcin	ogenesis					
SECTION								
	er Chemistry Sec	ction						
INSTITUTE AND								
	, Bethesda, Mary					_		
TOTAL MAN-YE	EARS:	PROFESSIONAL	4		OTHER:			
1.8		0.8			1.0			
	PRIATE BOX(ES)			_				
		(b) Hum	an tissues		(c) Neither	•		
☐ (a1)	Minors							
☐ (a2)	Interviews							
SUMMARY OF WORK (Use stendard 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.

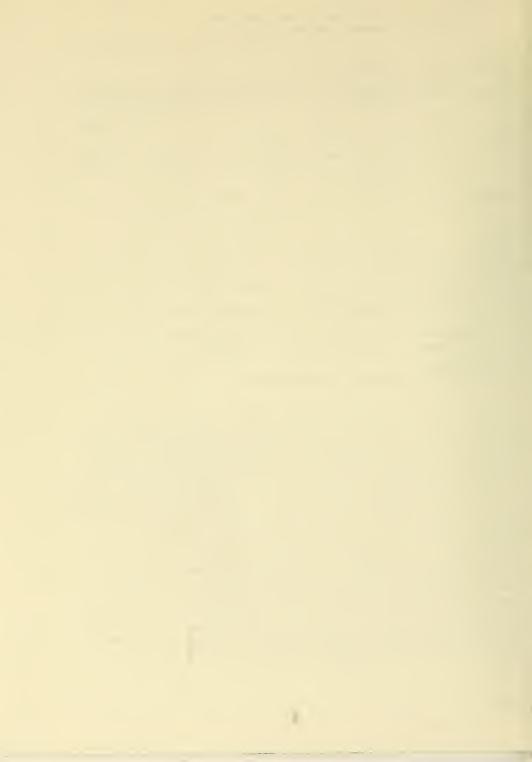


PROJECT NUMBER

Z01CP05448-03 LEC

October 1, 1986 to September 30, 1987										
TITLE OF PROJECT (80 charecters or less. Title must fit on one line between the borders.)										
	e Triphosphate									
PRINCIPAL INVE	STIGATOR (List other pro	fessional parsi	onnel below the Pi	rıncıpal İnvesti	gator.) (I	Neme, title	, laboratory	, and institu	ite effiliatio	n)
PI:	Chien-Hua Niu		Expert						LEC	NCI
Others:	Kyouhoon Han		Visiting						LEC	NCI
	Peter P. Roll	er	Head, Bio	polymer	Chem	nistry	Secti	on	LEC	NCI
COOPERATING L	JNITS (if eny)									
None										
LAB/BRANCH										
Laborator	ry of Experime	ntal Car	cinogenes	is						
SECTION	J 51 211 1110		•e gees							
Biopolyme	er Chemistry S	ection								
INSTITUTE AND										
NCI. NIH.	, Bethesda, Ma	rvland	20892							
TOTAL MAN-YEA		PROFESSIO			OTHER	:	· · · · · · · · · · · · · · · · · · ·			
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(a) Huma		☐ (b) H	ıman tissues	X	(c) N	leither				
☐ (a1)		(-,			(-,					
	Interviews									
SUMMARY OF WORK (Use standard unreduced type, Do not exceed the space provided.)										
John M. S. Waller and Sandada Sandada Sandada Sandad Sanda										

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 quanine 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

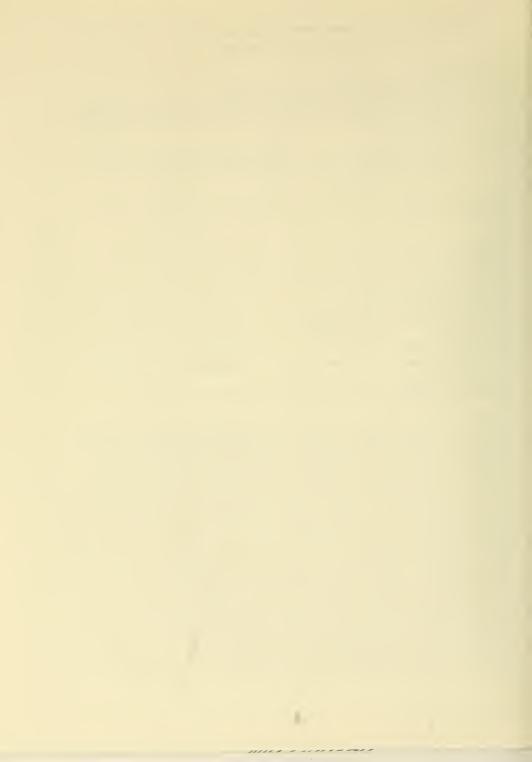
NOTICE OF INTRAMURAL RESEARCH PROJECT

701CD0E440 02 LEC

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PERIOD COVER									
	l, 1986 to Sep								
	CT (80 cherecters or less								
Conformational Studies of Growth Factors and Transforming Related Peptides									
	PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, leboratory, and institute affiliation)								
PI:	Chien-Hua Niu		Expert						
011	V hara Har		111.11.1.						
Others:	Kyouhoon Han		Visiting			C+:			
	Peter P. Roll		Head, Bio	polymer C	nemistr	y Section			
	Snorri S. Tho	rgeirsson	Chief						
20000001000									
COOPERATING	UNIIS (IF BRY)								
None									
LAB/BRANCH									
Laboratory of Experimental Carcinogenesis									
SECTION									
Biopolymer Chemistry Section									
INSTITUTE AND LOCATION									
NCI, NIH, Bethesda, Maryland 20892									
TOTAL MAN-YEA	ARS:	PROFESSIONAL:		OTHER:					
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CHECK APPROP		_							
	an subjects	(b) Human t	issues	(c) Neithe	er				
_ ` `	Minors								
☐ (a2)	Interviews								

SUMMARY OF WORK (Use stendard 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 Glumin 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).



PROJECT NUMBER

701CP05450-03 LEC

0.4

(c) Neither

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 NCT COOPERATING UNITS (if any) Universite de Paris XI, Dept de Chimie Biologique Lab Hormones. 94270 Bicetre, France (Dr. Helene Richard-Foy) Laboratory of Experimental Carcinogenesis Hormone Action and Oncogenesis Section INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892 TOTAL MAN-YEARS: PROFESSIONAL . OTHER: 1.4

(a2) Interviews SUMMARY OF WORK (Use standard unreduced type, Do not exceed the space provided.)

(b) Human tissues

東京とかりまたか

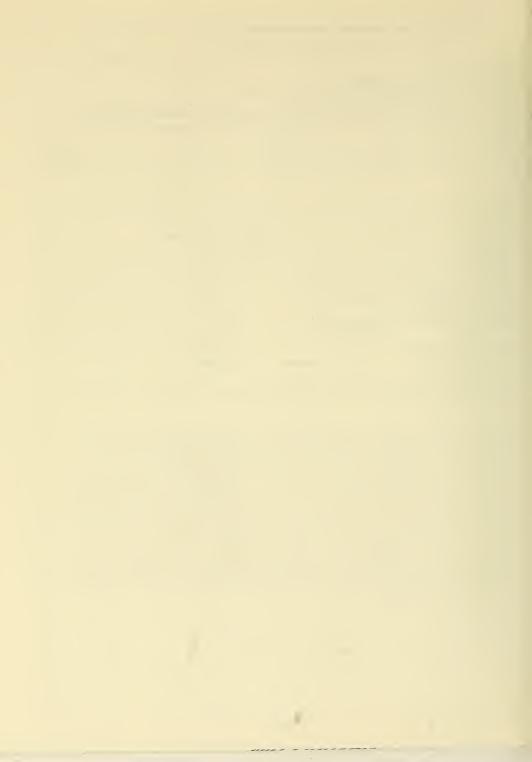
The genetic information in mammalian cells exits is organized into a highly condensed nucleoprotein structure whose basic repeating subunit is the nucleosome. The major function of this structure is usually thought to be packaging the very large amounts of DNA into a minimal volume. Recent evidence indicates 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.

PHS 6040 (Rev. 1/84)

1.8

CHECK APPROPRIATE BOX(ES) (a) Human subjects

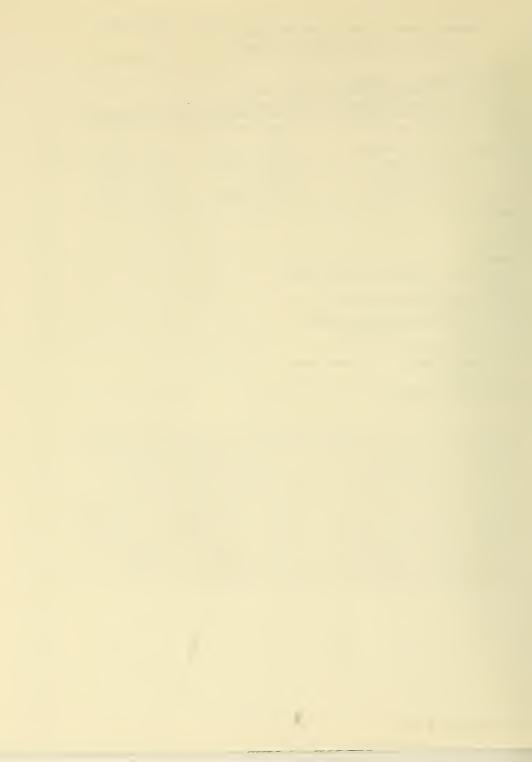
(a1) Minors



PROJECT NUMBER DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT 701CP05452~03 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-vun Chuna Senior Staff Fellow LEC NCI Others: Snorri S. Thorgeirsson Chief LFC. NCT Miriam Falzon Visiting Fellow NCT LEC Shu-hua Yu Visiting Fellow LEC NCT Nancy Sanderson Chemist LEC NCI Dennis R. Roop Microbiologist LCCTP NCI COOPERATING UNITS (if env) None LAB/BRANCH Laboratory of Experimental Carcinogenesis Chemical Carcinogenesis Section INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892 TOTAL MAN-YEARS: PROFESSIONAL OTHER: 2.1 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.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

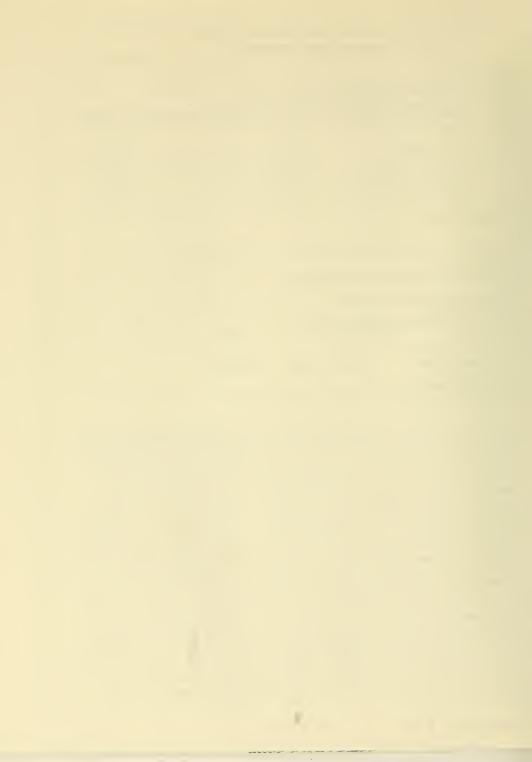
NOTICE OF INTRAMURAL RESEARCH PROJECT

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PERIOD COVERED		
October 1, 1986 to September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between	en the borders.)	
Genetic Determinants in Chemical Hepato	carcinogenesis	
PRINCIPAL INVESTIGATOR (List other professional personnel below the P	nncipal Investigator.) (Neme, title, labora	tory, and institute affiliation)
PI: Snorri S. Thorgeirsson Chie	f	LEC NCI
Others: Peter Nagy Visi	ting Fellow	LEC NCI
	rinary Medical Officer	
Susan H. Garfield Chem		LEC NCI
Michael G. Cordingley Visi	ting Associate	LEC NCI
Michael M. Gottesman Sect		LMB NCI
COOPERATING UNITS (# eny)		
laboratory of Immunopathalani MIAID (D	. II C M TTT\	
Laboratory of Immunopathology, NIAID (D	r. H. C. Morse, 111)	
LAB/BRANCH		
Laboratory of Experimental Carcinogenes	is	
SECTION		
Chemical Carcinogenesis Section		
INSTITUTE AND LOCATION		
NCI, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: PROFESSIONAL:	OTHER:	•
1.7	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

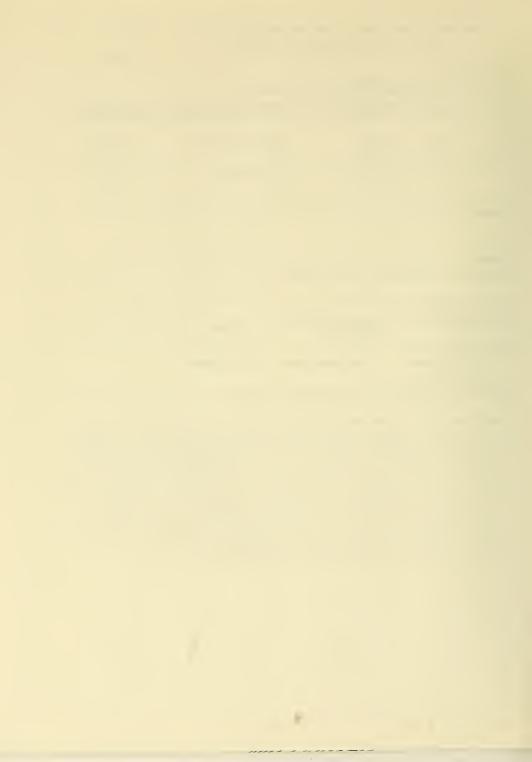
Z01CP05495-02 LFC

PERIOD COVER	ED					
October	1, 1986 to Sep	tember 30, 1987	7			
		. Title must fit on one line t				
Amino Ac	id at the Suppi	ression Site in	Rabbit Be	eta-Globin I	Readthrough P	rotein
PRINCIPAL INVE	STIGATOR (List other pro	fessional personnel below	the Principal Invast	igator.) (Name, titla,	leboratory, and institute	effiliation)
PI:	Dolph L. Hatf	ield	Research	Biologist	LEC	NCI
				,		
Others:	Snorri S. Thou	rgeirsson	Chief		LEC	NCI
	Michael Bustin		Research	Chemist	LMC	NCI
					2110	
COOPERATING	UNITS (if any)					
None						
LAB/BRANCH			***************************************			
Laborator	rv of Experimen	ntal Carcinoger	nesis			
SECTION	J					
INSTITUTE AND	LOCATION					
NCI NIH	, Bethesda, Ma	aryland 20892				
TOTAL MAN-YEA		PROFESSIONAL:		OTHER:		
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_ ` ′	Interviews					
		tuned have On and a second	***************************************	41		
SUMMARY OF W	IOHK (USB Standard unred	luced type. Do not axceed	tne space provided	J.)		

Rabbit beta-globin readthrough protein is the only naturally occurring readthrough 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

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characterizing the amino acid at the suppression site.



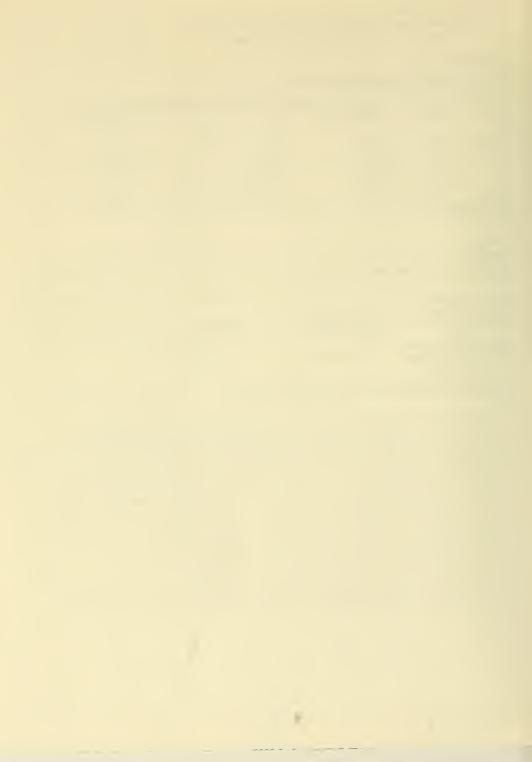
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

	NOTICE OF INT	Z01CP05500-01 LEC					
PERIOD COVERI	PERIOD COVERED						
October 1, 1986 to September 30, 1987							
TITLE OF PROJE	CT (80 characters or less	. Title must fit on one line	between the bords	•			
Polypept	ide Modulation	in MCF-7 Cell	s by Estro	gen and Growth	1 Factors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboration of the professional personnel below the Principal Investigator.)				**			
PI:	Snorri S. Tho	rgeirsson	Chief		LEC NCI		
Others:	Peter J. Worl	and.	Viciting	Follow.	LEC NOT		
Others.	Peter J. Wirt		Visiting Expert .		LEC NCI		
	Lori L. Hampt	•	Biologist				
	Diane A. Bron		Biologist		LEC NCI		
	Robert B. Dic			vestigator	MB NCI.		
	Marc E. Lippm		Section C		MB NCI		
COOPERATING		a11	Section C	mei	MB NCI		
OCC. Elettina	J, G (iii diy)						
None							
LAB/BRANCH							
Laborato	ry of Experime	ntal Carcinoge	nesis				
SECTION							
INSTITUTE AND							
	, Bethesda, Ma			-			
TOTAL MAN-YEA	ARS:	PROFESSIONAL:		OTHER:			
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(a) Huin		(b) Human us	Sues M	(c) Neither			
/	Interviews						
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SUMMARY OF W	ORK (Use standard unrec	исеа туре. Оо пот ехсева	the space provide	a.)			
Ti	6						
The purp	ose of this pro	oject was to ut	tilize the	established h	uman mammary tumor		
cell lin	es (MCF-7, MCF	-/(gpt) [produc	ed by trai	nsfection with	the Eco-gpt		
selectab	le gene marker], MCF-7(<u>ras</u>) [produced b	y transfectio	n with Eco-gpt and		

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.

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PROJECT NUMBER

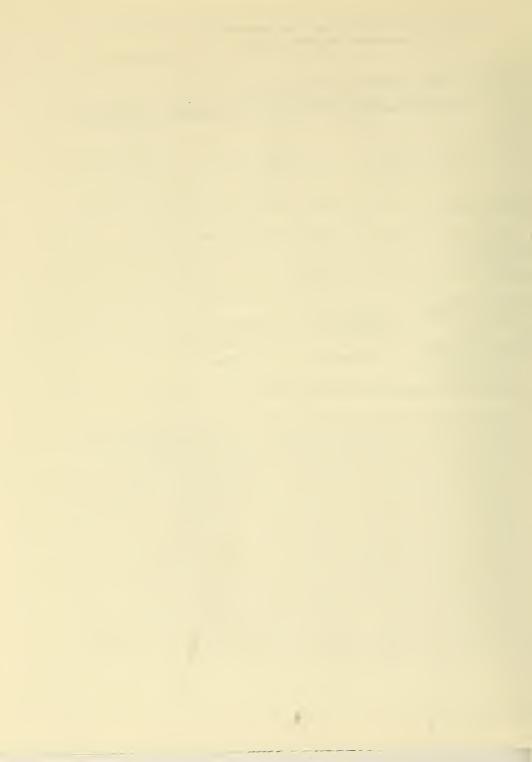
NOTICE OF INTRAMURAL RESEARCH PROJECT

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	Cellular	Polypeptides	Associated v	vith Metasta	sis of Rat M	Mammary Tumor Ce	11s
PF	RINCIPAL INVEST	IGATOR (List other pro	fessional personnel belo	w the Principal Inves	ngator.) (Name, title, la	boratory, and institute affilieti	on)
	PI:	Snorri S. Th	orgeirsson	Chief		LEC NCI	
	Others:	Peter J. Wor Peter J. Wir		Visitin Expert	g Fellow	LEC NCI LEC NCI	
		Lori L. Hamp		Biologi	st	LEC NCI	
CC	OPERATING UNI	ITS (if any)					
	Departmen (Dr. Unta	t of Patholo e Kim)	gy, Roswell M	Memorial Par	k Institute,	Buffalo, New Yo	ork
LAI	B/BRANCH			**********			
	Laborator	y of Experim	ental Carcino	genesis			
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(a1) Minors (a2) Interviews

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 nonmetastatic 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 nonmetastatic 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.



PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05502-01 LFC 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 & LEC NCI Oncogenesis Section Others: Anna Tate Riegel Visiting Fellow Microbiologist LEC NCI Diana S. Berard LFC NCI COOPERATING UNITS (if any) None LAB/BRANCH Laboratory of Experimental Carcinogenesis Hormone Action and Oncogenesis Section INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892 TOTAL MAN-YEARS: PROFESSIONAL: OTHER:

0.1

(c) Neither

(a1) Minors (a2) Interviews SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

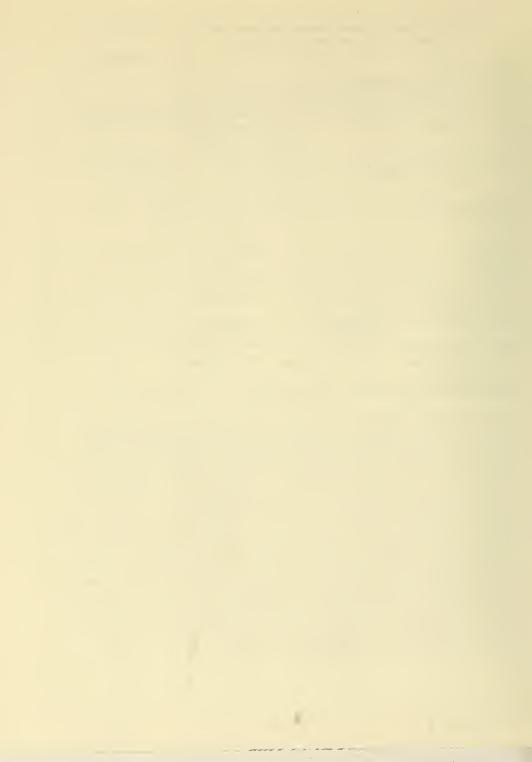
0.9

(b) Human tissues

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 ZO1CP04986-10 LEC) that two tightbinding 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 crosslinking 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 ZO1CP05450-03 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.

1.0

CHECK APPROPRIATE BOX(ES) (a) Human subjects



NOTICE OF INTRAMURAL RESEARCH PROJECT

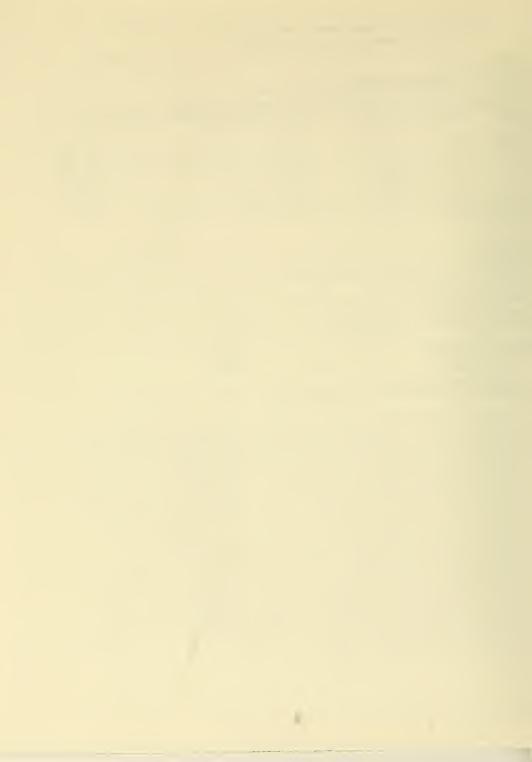
701CP05503_01 LEC

PROJECT NUMBER

							_
PERIOD COVERED							
October 1, 1986 to Sep	otember 30, 19	87					
TITLE OF PROJECT (80 characters or less	s. Title must fit on one line	e between the borde	rs.)				
Biological Effects of	a Rat Liver-D	erived Grow	th Inhil	bitor			
PRINCIPAL INVESTIGATOR (List other pro	ofessional personnel below	w the Principal Inves	ngetor.) (Name	e, title, laborato	ry, and institute effi	lietion)	
PI: Mrunal S. Cha	apekar	Senior Sta	ff Fello	OW		LÉC	NCI
0							
Others: Snorri S. Tho		Chief				LEC	NCI
Peter P. Roll		Head, Biop	olymer (Chemistr	y Section	LEC	NCI
Peter J. Wirt		Expert				LEC	NCI
Anthony C. Hu		Visiting A	ssociate	9		LEC	NCI
James B. McMa		Expert				DDRG	NCI
Robert I. Gla	zer	Pharmacolo	qist			LBC	NCI
COOPERATING UNITS (if any)							
••							
None							
LAB/BRANCH							
Laboratory of Experime	ntal Carcinoge	enesis					
SECTION							
Biopolymer Chemistry S	ection						
INSTITUTE AND LOCATION							
NCI, NIH, Bethesda, Ma	ryland 20892						
TOTAL MAN-YEARS:	PROFESSIONAL:		OTHER:				
1.2	1.2		0				
CHECK APPROPRIATE BOX(ES)							
	(b) Human tis	sues 🛛	(c) Neith	er			
(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.



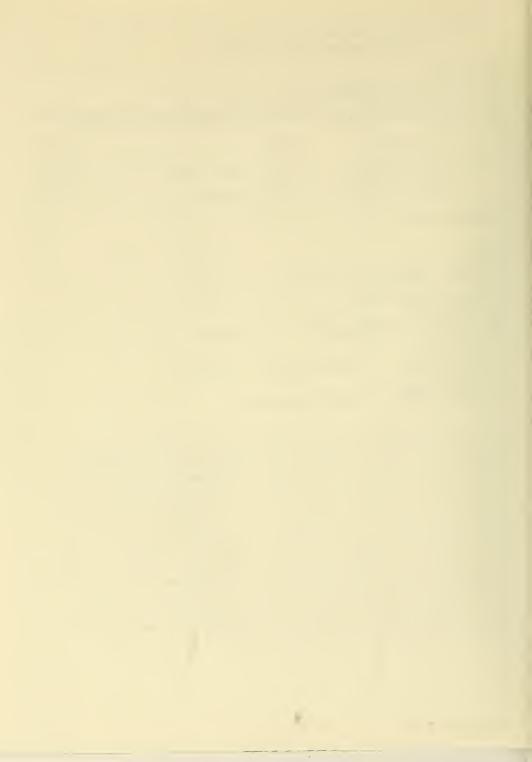
PROJECT NUMBER

		NOTICE OF II	NTRAMURAL	RESEARCH I	PROJECT			
							Z01CP05504	-01 LEC
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				iai balow tha Filicip	er investigator.)	i (Neme, title, labora	tory, and institute affili	etion)
P:		Chien-Hua Niu		Expert				LEC NCI
01	thers:	Timothy Benja Peter P. Roll Snorri S. Tho	er rgeirsson	Chief		Chemistry	Section	LEC NCI LEC NCI
		Anthony C. Hu	ggett	Visiting	Associa	te		LEC NCI
-	ODERATION	0.13.070.07					_	
	OFERATIN	G UNITS (if any)						
No								
	BIBRANCH							
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		er Chemistry Se	action					
INS	TITUTE AN	D LOCATION	ección					
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SUA	MARY OF	WORK (Use standard unre	educed type. Do not	exceed the space	amuded)			
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of	normal	and neoplasti	c rat liver	rs using tw	n-dimens	ional gal	plasma memb	ranes
121	DAGE	have veves led	1.00	3 dailing tw	o-a mens	ional gel e	rectrophore	515

(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.

PHS 6040 (Rev 1/84)

#15 v 6 16/12

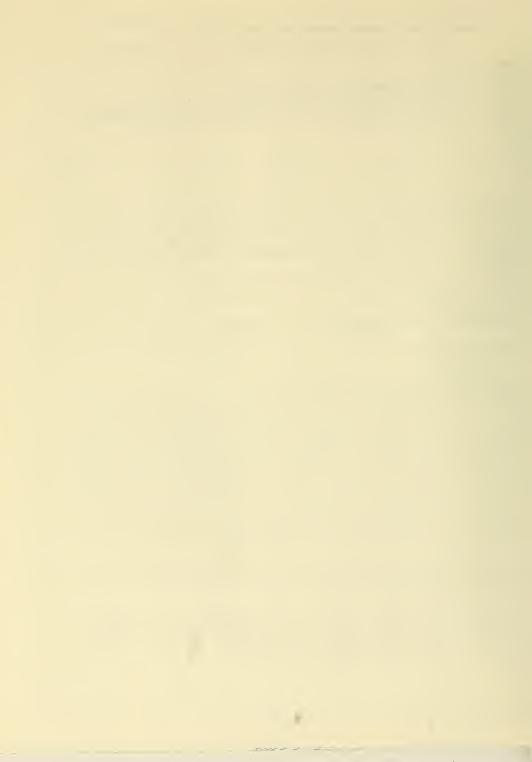


DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP04491-11 LEP

October 1, 1986 through	September 30, 198	7	
TITLE OF PROJECT (80 characters or less		n the borders.) s in Neoplastic Transformation	
		ncipal Investigator) (Name, title, leboratory, and institute affiliation)	
PI: U. Saffiotti	Chief	LEP NCI	
COOPERATING UNITS (MARY) 10 Negr	ri Pharmacol. Res.	Insititute, Milan, Italy (F. Bertolero);
Commission of European (Commun. Research Co	enter, Ispra, Italy, (E. Sabbioni): Lab	٠.
Dogliotti): Inst. of His	Istituto Superiore	di Sanita', Rome, Italy (M. Bignami, E logy, Univ. of Padua, Italy (S. Garbisa	
LABUBRANCH Laboratory of Experiment		rugy, mirv. or Fanua, Itary (S. Garbisa	-
SECTION	al rathology		
Office of the Chief			
NCI, NIH, Bethesda, Mary			
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER: 0.4	
CHECK APPROPRIATE BOX(ES)		(a) Market	
(a) Human subjects (a1) Minors	(b) Human tissues	☑ (c) Neither	
(a2) Interviews			
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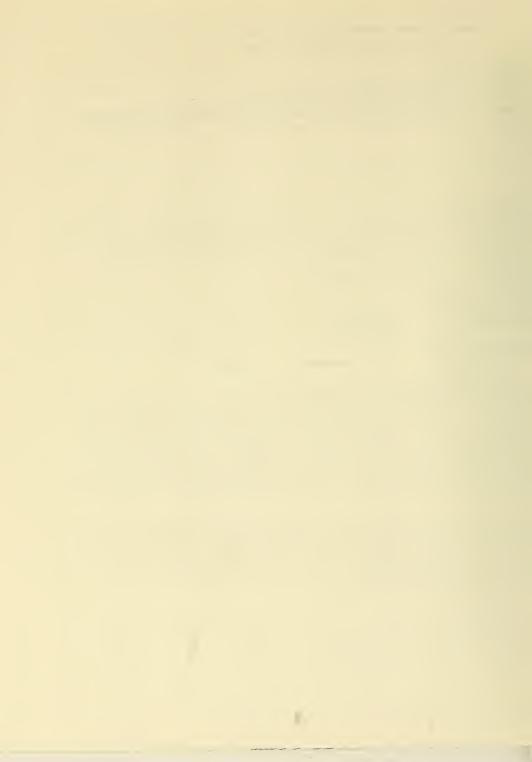
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

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 become the beginning
	Bioenergetic Pathways in Chemically-Transformed Epithelial Cells
	PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute effiliation) PI: A. F. Kanlan Posparch Chamist
	PI: A. E. Kaplan Research Chemist LEP NCI
	COOPERATING UNITS (# 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)
	LABUBRANCH
ł	Laboratory of Experimental Pathology
ľ	SECTION Office of the Chief
ł	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
ł	(a2) Interviews
ı	SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)
ı	Studies of routes of energy losses from neoplastic cells continued using a model
ш	consisting of a control rat hepatocyte line and its N_nitross N_mothlugge +
н	Turnied Counterpart. Studies of energy loss focused on the onzume lastic date
ľ	drogenase (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
	Doints between pH 5.98 and 9.44 in control cells but only two in tumorigenic cells.
ŀ	The latter correlate with markedly increased rate of synthesis and excretion of
ŀ	lactic acid.
ł	
ľ	The availability of new monoclonal antibodies against tubulin, cytokeratin and
١	turn resulted in more clearly defined differences between the control and tumori
15	genic rat nepatocyte lines. In the latter, cytokeratin is markedly diminished in
112	Tuurestelle, but the addredation nattern is not altonod, tubulia and anti-
0	altered in the patterns of their individual aggregates and also show a slight overall decrease in fluorescence.
ľ	The state of the s
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PHS 6040 (Rev. 1/84)



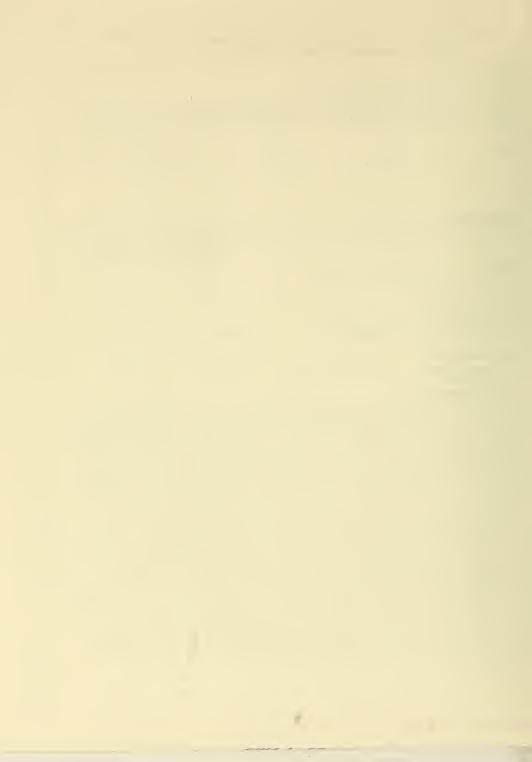
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

CH PROJECT 701 CP05265 O6 LED

					701CPU3203-00 LEP	
PERIOD COVERED						
October 1, 19	986 to Sept	ember 30, 1987				
TITLE OF PROJECT (8	O charecters or less	. Title must fit on one line	between the border	rs.)		
Effects of Ch	nemical Car	cinogens on Tr	ansforming	DNA Sequences	and Expression	
PRINCIPAL INVESTIGA	TOR (List other pro	fessional personnel below	the Pnncipel Invest		tory, and institute effillation)	
PI:	U. Saffiot	ti	Chief	LE	EP NCI	
0.1						
Others:	M. I. Lerm		Expert		IB NC I	
	S. F. Stin	son	Biologist	LE	EP NCI	
			-			
COOPERATING UNITS	(if any)		C+ . 12 N			
Laboratory of	central N	ervous System	Studies, N	ational Institu	ute of Neurological	
and Communica	itive Disor	ders and Strok	e, NIH (1).	Y. Goldgaber,	D. C. Gajdusek).	
LAB/BRANCH						
	Experimen	tal Pathology				
SECTION						
Office of the						
INSTITUTE AND LOCA						
NCI, NIH, Bet	hesda, Mar					
TOTAL MAN-YEARS:	1.5	PROFESSIONAL:		OTHER:		
	1.5	0.4		1.1		
CHECK APPROPRIATE				/ > > 1.1 /s4		
(a) Human s		(b) Human tis	sues 🗀	(c) Neither		
(a1) Mind						
(a2) Inter						
SUMMARY OF WORK	Use standard unrec	fuced type. Do not exceed	the space provide	d.)		
drive the de	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 abo	relopment o	i neopiasia wn	ose mailign	ant potential r	results from changes	
caused by che	mical carc	mogens. (A).	IJNAase 1-	nypersensitive	(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 protooncogene. 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 Nown'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.



PROJECT NUMBER

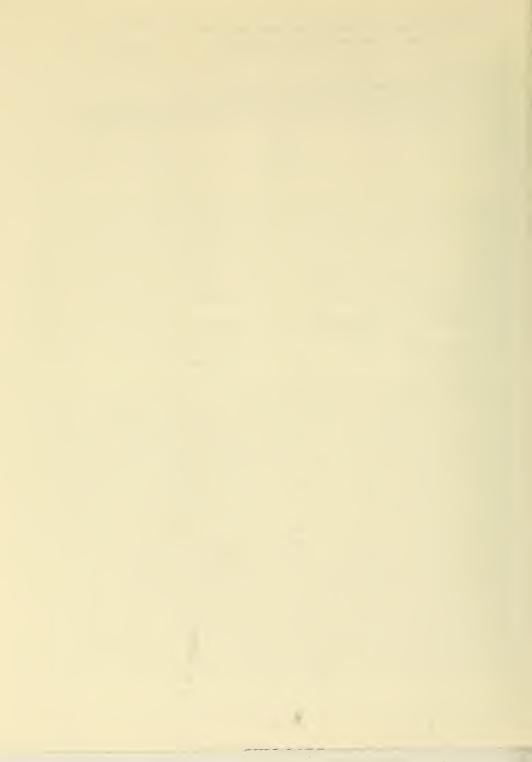
NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05274-06 LEP

October 1, 1986 through	September 30, 1987	
	Title must fit on one line between the border	
	is by Chemical and Physi	
	essional personnel below the Principal Invest	igator.) (Name, title, laboratory, and institute affiliation)
PI: U. Saffiotti	Chief	LEP NCI
Others: S. F. Stinson	Biologist	LEP NCI
COOPERATING UNITS (if eny)		
Department of Pathology, MD (E. M. McDowell, K. P		, School of Medicine, Baltimore,
LAB/BRANCH		
Laboratory of Experiment	al Pathology	
SECTION	30	····
Respiratory Carcinogenes	is Section	
INSTITUTE AND LOCATION		
NCI, NIH, Bethesda, Mary	land 20892	
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.2	1.2
CHECK APPROPRIATE BOX(ES)	(h) 11	(a) Alajahan
	(b) Human tissues	(c) Neither
(a1) Minors (a2) Interviews		
	uced type. Do not exceed the space provided	
		from different respiratory tract
		I treatments with chemical, physical
		ultifactorial study were analyzed.
Fourteen groups of hamst	ers were treated with th	ne following variables: single
		sourea (MNU) at 5 weeks of age: 15
weekly instillations of	benzo[a]pyrene (BP) adso	orbed on ferric oxide (Fe-0) in
		on was either only at the larynx or
		the tracheal epithelium induced
		three major determinants of the
		and tracheal wounding, which was a
		nly in the trachea but also in the
		ly, concurrent intraperitoneal
		idence and severity and decreased
		ratracheal administration of
		n with BP, than 5 days after. cratracheal administration of

of different forms of silica (quartz, cristobalite, tridymite).

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

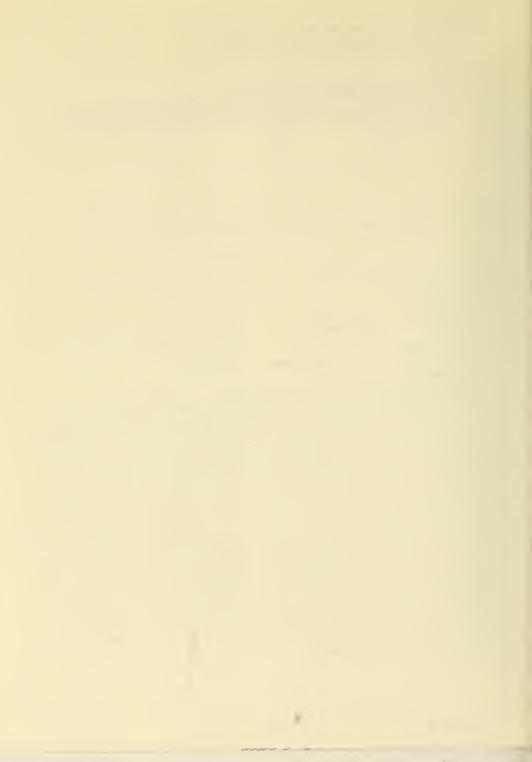


DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05276-06 LEP

I control of the cont			
PERIOD COVERED			
October 1, 1986 through			
TITLE OF PROJECT (80 cherecters or less			
Growth Control in Epithe			
PRINCIPAL INVESTIGATOR (List other pro			
PI: M. E. Kaighn	Expert	LEP	NC I
Others: U. Saffiotti	Chief	LEP	NC I
COOPERATING UNITS (if any) Mario Negri Pharmacol. F	Res. Institute, Milan,	Italy (F. Bertolero)	
Laboratory of Experiment	al Pathology		
SECTION Tissue Culture Section			
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Mary	land 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL:	OTHER: 1.0	
CHECK APPROPRIATE BOX(ES) (a) Human subjects (a1) Minors (a2) Interviews	☐ (b) Human tissues	☑ (c) Neither	
SUMMARY OF WORK (Use standard unred	luced type. Do not exceed the space pro	ovided)	
Mouse keratinocytes were			on" without a
crisis, in a newly devel	oped serum-free mediu	m. IFP/MK2, consisting	of low calcium
MEM with non-essential a	mino acids supplement	ed with eight factors.	Three lines
have been isolated to da	te (MK1, MKDC4, and M	K/2057C). The MK1 line	has now under-
gone more than 400 doubl	ings. Giemsa banding	has revealed significa	int karvotynic

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/MK? medium with 1.0 ng/ml TGF- eta_{ullet} 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

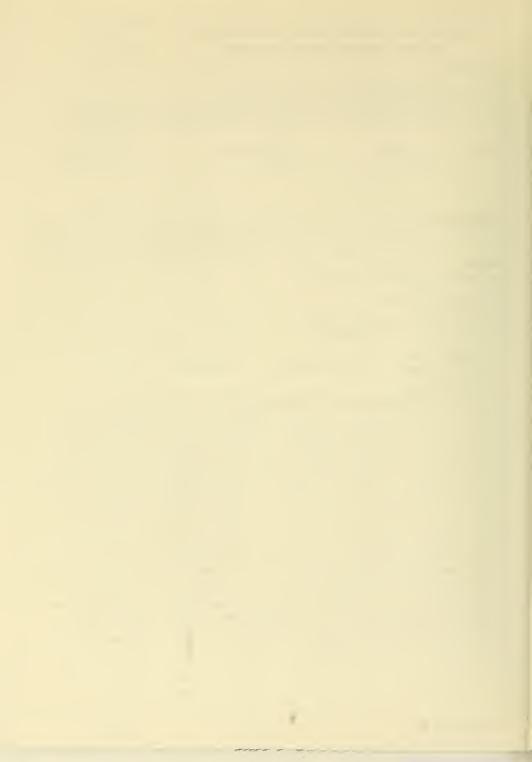
NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05494-02 LEP

October 1, 1986 to Septe	ember 30, 1987		
Cellular and Molecular S	. Title must fit on one line between the border Studies in Normal and Nec	plastic Human Prostati	
	fassional personnel below the Principal Investi		,
PI: M. E. Kaighn	Expert		LEP NCI
Others: J. F. Lechner R. Reddel	Microbiolog Expert		LHC NCI
N. Acduci	Experc	'	LITO NOT
COOPERATING UNITS (if any)			
Departments of Urology a	and Surgery, Northwestern	University Medical So	chool, Chicago
IL (J. Kozlowski); Labor	ratory of Oral Medicine,	National Institute of	Dental Research
NIH (M. I. Lerman); Univ	versity of Texas Medical	School, Houston, TX ([). Sirbasku)
LAB/BRANCH			
Laboratory of Experiment	al Pathology		
SECTION Tissue Culture Section			
INSTITUTE AND LOCATION			
NCI, NIH, Bethesda, Mary	land 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:	
1.5	0.5	1.0	
CHECK APPROPRIATE BOX(ES) (a) Human subjects (a1) Minors (a2) Interviews	☑ (b) Human tissues ☐	(c) Neither	
SUMMARY OF WORK (Use standard unred	luced type. Do not exceed the spece provided	ho mala of known ander	
genomic DNA from prostat epithelial cells. The "cell line, NP-2s, has be 797, 1978) was recovered (P4-8F) consisting of PF pp. 195-225, 1980), with 50 nM; calcium, 0.5 mM; bovine pituitary extract amine, $0.5 \mu M$; and chole end of their lifespan we RSV-LTR promoter and the	ject is to investigate the concerning of a non- immortalization" of a no- en accomplished. This I from liquid nitrogen and MR4 (Lechner et al., Met mout trace element concerning epidermal growth factor, p. 0.5%; bovine serum alburatoxin, 0.1 nM. Overning transfected with plase gene encoding the SV40 (187). The treated cells	forming normal human promal human prostatic edine (Lechner et al., ed cultured in serum-frhods in Cell Biology, trate, supplemented wi 0.5 ng/ml; insulin, 5 umin, 250 µg/ml; phospight cultures of cells mid p-RSV-T consisting large T-antigen (Brash	prostatic epithelial MCI, 60: ree medium Vol. 21B, ith selenite, 5.0 µg/ml; phoethanol-s near the g of the net al.

layered 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.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

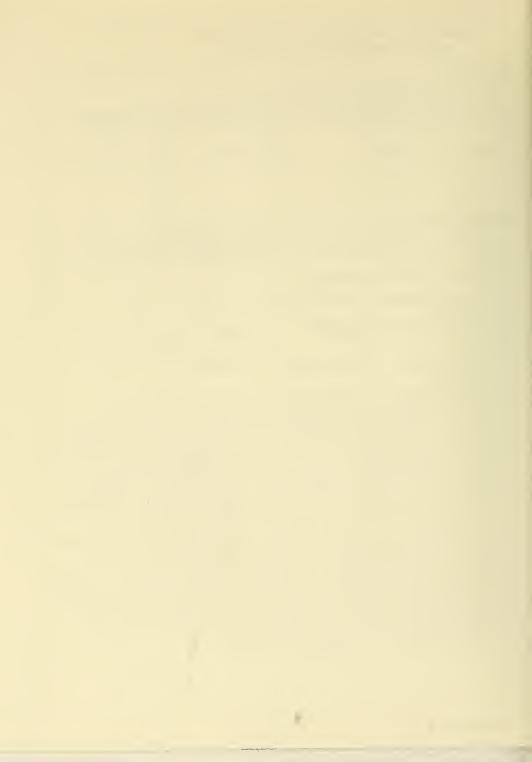
NOTICE OF INTRAMURAL RESEARCH PROJECT

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October 1, 1986 to Se					
TITLE OF PROJECT (80 characters or les.					
Repair of Carcinogen		3 -			
PRINCIPAL INVESTIGATOR (List other pre			tigetor.) (Neme, title, leb		
P.I. Curtis C. F	larris	Chief		LHC	NCI
Others: Simon Plumr	70.0	Vi	. Fallow	LUC	NCT
others: Stillon Pluill	ner	VISITING	Fellow	LHC	NCI
COOPERATING UNITS (If any)					
	logy Honshoy Mo	diasl Con	ton Uonchou	DA /A F	Dogg\.
Department of Physical					
Department of Patholo MD (B.F. Trump); Karo					
LAB/BRANCH	JIIIISKA IIISLILULI	e, SLUCKI	orm, sweden (K.C. Grain	s troiii)
Laboratory of Human (`arcinogenecic				
SECTION	arcinogenesis				
Carcinogen Macromoleo	cular Interaction	n Section			
INSTITUTE AND LOCATION	diai inceraceroi	3666100			
NCI NIH, Bethesda, Maryland 20892					
TOTAL MAN-YEARS:	PROFESSIONAL:		OTHER:		
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(a) Human subjects	(b) Human tissu	es 🗆	(c) Neither		
(a1) Minors	. ,		(-)		
(a2) Interviews					
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)					
Normal adult human ti	ssues and cultur	red brond	hial epitheli	al cells a	and

fibroblasts exhibit O6-alkylquanine-DNA alkyltransferase activity in vitro by catalyzing the repair of the promutagenic alkylation lesion 06-methylquanine 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 06-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.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

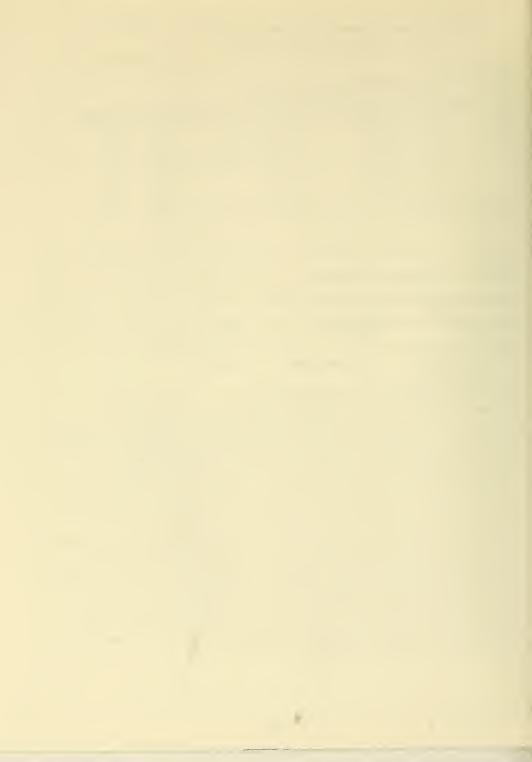
NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05293-06 LHC

	October 1, 1986 to Sept						
	TITLE OF PROJECT (80 characters or less			·s.)			
ŀ	ras Oncogene Transfecti						
	PRINCIPAL INVESTIGATOR (List other pro						
	P.I. George H. Yoa		Senior Sta		LHC	NCI	
ı	Others: John F. Lechr		Section Ch		LHC	NCI	
ı	Ainsley Westo		Visiting F		LHC	NCI	
ı	James C. Will			aining Fellow		NCI	
	Paul Amstad		Visiting'F	ellow	LHC	NCI	
-	Curtis C. Har		Chief		LHC	NCI	
	Lance Liotta		Chief		LP	NCI	
	D. C. Rao		<u>Visiting.F</u>	ellow	LP	NCI	
l	COOPERATING UNITS (if any)						
١							
ļ							
l	LAB/BRANCH						
ŀ	Laboratory of Human Car	cinogenesis					
İ							
ŀ	Carcinogen Macromolecul	lar Interaction	Section				
ı		1					
ŀ	NCI, NIH, Bethesda, Mar	TPROFESSIONAL:		OTHER:			
ı					^		
ŀ	2.0 CHECK APPROPRIATE BOX(ES)	L	1.0	1.	J		
ı	(a) Human subjects	(b) Human tiss	2012	(c) Neither			
ı	(a) Human subjects	E (b) Haman tist	54 05	(0) 110111101			
ı	(a2) Interviews						
ŀ	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						
ı							
l	testing the effect of v	/-на- <u>ras</u> expres	sion on cn	romosomai sta	bility.	10 determin	ne
١	the effect of v-Ha-ras						as
ı	transfected by protopla						
1	later to observe effects on chromosomal structure. Increased numbers of						
١	chromosome breaks and gaps were observed in v-Ha-ras-transfected NHBE cells.						
Ì	Multistage progression of v-Ha- <u>ras</u> -transfected NHBE cells was studied by						

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-ISA, 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-ISA), and xenogeneic transfer of TBE-series tumor tissues between mice. The TBE-ISA 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.

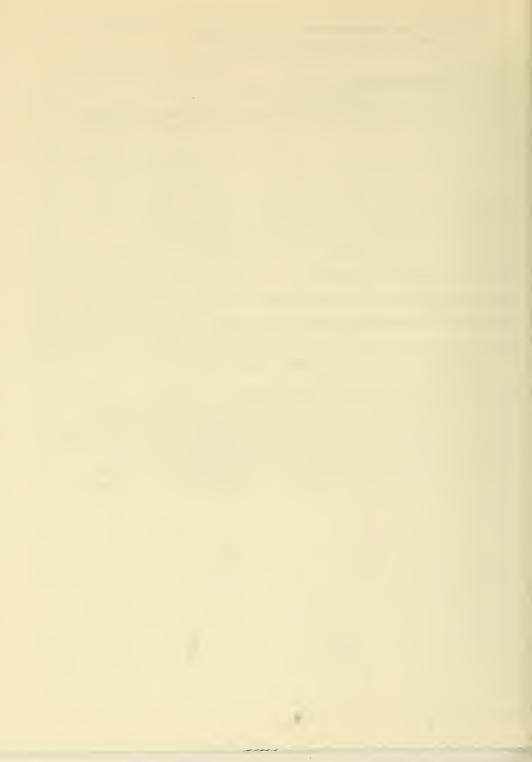
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	ND HUMAN SERVICES - PUBLIC HEAR	
		Z01CP05324-05 LHC
PERIOD COVERED	tamban 20 1007	
October 1, 1986 to Sept TITLE OF PROJECT (80 characters or less	Title must fit on one line between the borde	rs.)
Genetic Studies of Tumo	or Suppression	
		tigator) (Name, title, laboratory, end institute affiliation)
P.I.: Curtis C. Ha	rris Chief	LHC NCI
COOPERATING UNITS (if any)		
University of Californ	ia at Irvine, Irvine, CA	(E. Stanbridge)
University of Maryland	, Baltimore, MD (E. Gab	rielson)
LAB/BRANCH		
Laboratory of Human Can	cinogenesis	
SECTION		
Carcinogen Macromolecuinstitute and Location	lar Interaction Section	
NCI. NIH. Bethesda. Man	cyland 20802	
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	_ (5) //5///2// =	(6) 115.11.15.
(a2) Interviews		
SUMMARY OF WORK (Use standard unred	uced type. Do not exceed the space provide	
SUMMARY OF WORK (Use standard unred Genetic changes related	d to carcinogenesis are	being studied using hybrids of
SUMMARY OF WORK (Use standard unred Genetic changes related human lung carcinoma ce	d to carcinogenesis are ells with normal human b	being studied using hybrids of ronchial epithelial cells. Initial
SUMMARY OF WORK (Use standard unred Genetic changes related human lung carcinoma co studies suggest that a	d to carcinogenesis are ells with normal human b limited population doub	being studied using hybrids of ronchial epithelial cells. Initial ling potential (mortality) is the
SUMMARY OF WORK (Use standard unred Genetic changes related human lung carcinoma co studies suggest that a dominant genetic trait	d to carcinogenesis are ells with normal human b limited population doub in hybrid cells. Other	being studied using hybrids of ronchial epithelial cells. Initial ling potential (mortality) is the hybrid cell lines have been
Genetic changes related human lung carcinoma co studies suggest that a dominant genetic trait isolated and are being	d to carcinogenesis are ells with normal human b limited population doub in hybrid cells. Other characterized for doubl	being studied using hybrids of ronchial epithelial cells. Initial ling potential (mortality) is the
Genetic changes related human lung carcinoma constitution studies suggest that a dominant genetic trait isolated and are being tumorigenicity in athyr	d to carcinogenesis are ells with normal human b limited population doub in hybrid cells. Other characterized for doubl nic nude mice. The effe	being studied using hybrids of ronchial epithelial cells. Initial ling potential (mortality) is the hybrid cell lines have been ing potential, karyotype and
Genetic changes related human lung carcinoma constitution studies suggest that a dominant genetic trait isolated and are being tumorigenicity in athyr	d to carcinogenesis are ells with normal human b limited population doub in hybrid cells. Other characterized for doubl nic nude mice. The effe	being studied using hybrids of ronchial epithelial cells. Initial ling potential (mortality) is the hybrid cell lines have been ing potential, karyotype and cts of individual chromosomes are
Genetic changes related human lung carcinoma constitution studies suggest that a dominant genetic trait isolated and are being tumorigenicity in athyr	d to carcinogenesis are ells with normal human b limited population doub in hybrid cells. Other characterized for doubl nic nude mice. The effe	being studied using hybrids of ronchial epithelial cells. Initial ling potential (mortality) is the hybrid cell lines have been ing potential, karyotype and cts of individual chromosomes are
Genetic changes related human lung carcinoma constitution studies suggest that a dominant genetic trait isolated and are being tumorigenicity in athyr	d to carcinogenesis are ells with normal human b limited population doub in hybrid cells. Other characterized for doubl nic nude mice. The effe	being studied using hybrids of ronchial epithelial cells. Initial ling potential (mortality) is the hybrid cell lines have been ing potential, karyotype and cts of individual chromosomes are
Genetic changes related human lung carcinoma constitution studies suggest that a dominant genetic trait isolated and are being tumorigenicity in athyr	d to carcinogenesis are ells with normal human b limited population doub in hybrid cells. Other characterized for doubl nic nude mice. The effe	being studied using hybrids of ronchial epithelial cells. Initial ling potential (mortality) is the hybrid cell lines have been ing potential, karyotype and cts of individual chromosomes are
Genetic changes related human lung carcinoma constitution studies suggest that a dominant genetic trait isolated and are being tumorigenicity in athyr	d to carcinogenesis are ells with normal human b limited population doub in hybrid cells. Other characterized for doubl nic nude mice. The effe	being studied using hybrids of ronchial epithelial cells. Initial ling potential (mortality) is the hybrid cell lines have been ing potential, karyotype and cts of individual chromosomes are
Genetic changes related human lung carcinoma constitution studies suggest that a dominant genetic trait isolated and are being tumorigenicity in athyr	d to carcinogenesis are ells with normal human b limited population doub in hybrid cells. Other characterized for doubl nic nude mice. The effe	being studied using hybrids of ronchial epithelial cells. Initial ling potential (mortality) is the hybrid cell lines have been ing potential, karyotype and cts of individual chromosomes are
Genetic changes related human lung carcinoma constitution studies suggest that a dominant genetic trait isolated and are being tumorigenicity in athyr	d to carcinogenesis are ells with normal human b limited population doub in hybrid cells. Other characterized for doubl nic nude mice. The effe	being studied using hybrids of ronchial epithelial cells. Initial ling potential (mortality) is the hybrid cell lines have been ing potential, karyotype and cts of individual chromosomes are
Genetic changes related human lung carcinoma constitution studies suggest that a dominant genetic trait isolated and are being tumorigenicity in athyr	d to carcinogenesis are ells with normal human b limited population doub in hybrid cells. Other characterized for doubl nic nude mice. The effe	being studied using hybrids of ronchial epithelial cells. Initial ling potential (mortality) is the hybrid cell lines have been ing potential, karyotype and cts of individual chromosomes are
Genetic changes related human lung carcinoma constitution studies suggest that a dominant genetic trait isolated and are being tumorigenicity in athyr	d to carcinogenesis are ells with normal human b limited population doub in hybrid cells. Other characterized for doubl nic nude mice. The effe	being studied using hybrids of ronchial epithelial cells. Initial ling potential (mortality) is the hybrid cell lines have been ing potential, karyotype and cts of individual chromosomes are
Genetic changes related human lung carcinoma constitution studies suggest that a dominant genetic trait isolated and are being tumorigenicity in athyr	d to carcinogenesis are ells with normal human b limited population doub in hybrid cells. Other characterized for doubl nic nude mice. The effe	being studied using hybrids of ronchial epithelial cells. Initial ling potential (mortality) is the hybrid cell lines have been ing potential, karyotype and cts of individual chromosomes are

PHS 6040 (Rev. 1/84)

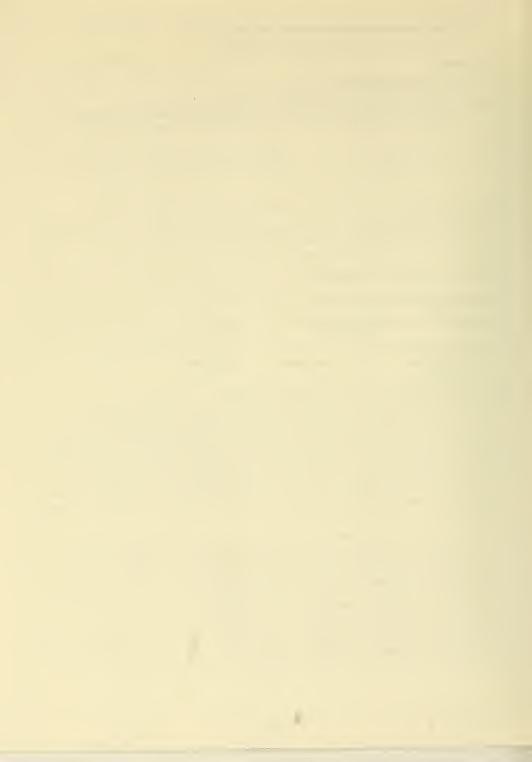
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PROJECT NUMBER DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT. Z01CP05325-05 LHC PERIOD COVERED October 1, 1986 to September 30, 1987 TITLE OF PROJECT (80 characters or less. Title must fit on 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 NCT Others: Curtis C. Harris Chief LHC NCT LHC NCI Tohru Masui Visiting Associate 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 Fpidemiology Section
INSTITUTE AND LOCATION NCI, NIH, Rethesda, Maryland 20892 OTAL MAN-YEARS: | PROFESSIONAL: TOTAL MAN-YEARS: OTHER: CHECK APPROPRIATE BOX(ES) (a) Human subjects (c) Neither (b) Human tissues (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

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.



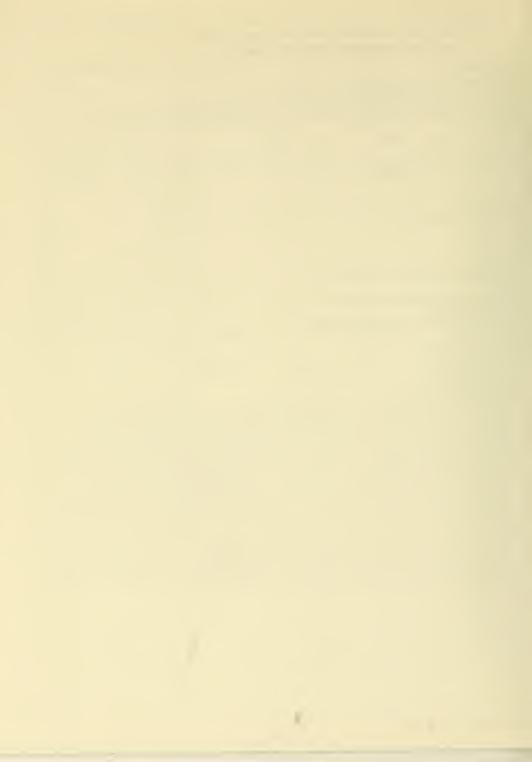
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	ECT (80 charecters or less							
HLA Ant	igens: Struct	ure, Funct	ion and	Diseas	se Associ	ation		
PRINCIPAL INVE	STIGATOR (List other pro	fessional personnel	below the Pr	incipal Inves	tigator.) (Name	, title, laboratory,	end institute a	ffiliation)
P.I.:	Dean L. Mar	n	Section	Chief			LHC	NCI
Others:	William Bla	ttner	Chief,	Family	Studies	Section	EEB	NCI
	James Geode		Expert				EEB	NCI
				· .				
COOPERATING (UNITS (if any)							
LAB/BRANCH								
Laborat	ory of Human C	arcinogene	sis					
SECTION								
Biochem	ical Epidemiol	ogy Section	n					
INSTITUTE AND		- 34						
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TOTAL MAN-YEA	RS:	PROFESSIONAL:			OTHER.			
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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

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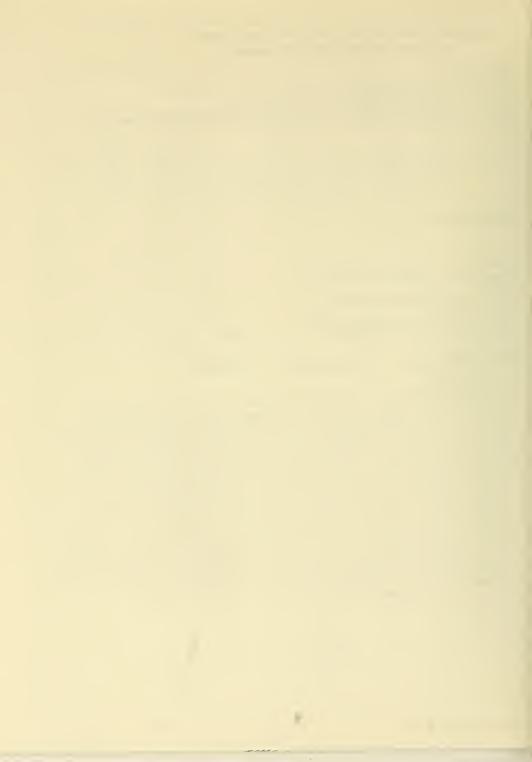
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October 1, 1986 to Sep	tember 30, 1987		
TITLE OF PROJECT (80 characters or les	s. Title must fit on one line between the borders.)		
Immunologic Studies of	Human T-Cell Lymphoma Virus		
PRINCIPAL INVESTIGATOR (List other po	rofessional personnal below the Principal Investigator.) (Name, title,	leboratory, and institute aff	iliation)
P.I.: Dean L. Mann	n Section Chief	LHC NCI	
Others: Mikulas Popo	ovic Medical Officer	LTCB NCI	
Robert Gallo) ` Chief	LTCB NCI	
William Blat	tner Chief, Family Studies Section	EEB NCI	
Jeffrey Clar	k Senior Staff Fellow	EEB NCI	
	· ·		
COOPERATING UNITS (if any)			
LAB/BRANCH			
Laboratory of Human Ca	rcinogenesis		
SECTION			
Biochemical Epidemiolo	gy Section		
INSTITUTE AND LOCATION			
NCI, NIH, Bethesda, Ma	ryland 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.

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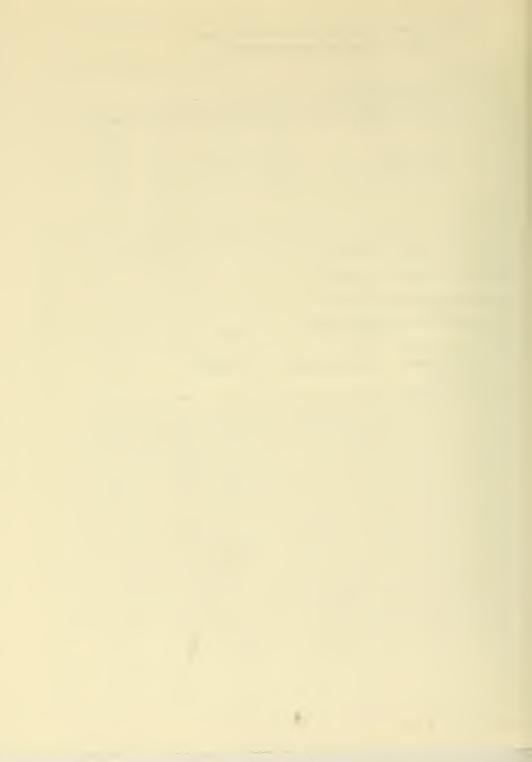


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TITLE OF PROJECT (80 cherecters or less								
Model Systems for Study								
PRINCIPAL INVESTIGATOR (List other pro								
P.I.: John F. Lechn	er	Section (Chief	LHC	NCI			
Others: Angela Somers		Visiting	Fellow	LHC	NCI			
Brenda Gerwin		Research		LHC	NCI			
Helen Reddel		Guest Res		LHC	NCI			
Curtis C. Har	ris	Chief	ocar onar	LHC	NCI			
047 073 07 1141	. , ,	on re-		25				
COOPERATING UNITS (if any)								
Duke University, Depart	ment of Pharm	acology, [Ourham, NC	(G. Rosen); Baltimore			
V.A. Hospital, Baltimor	e, MD (E. Gab	rielson);	Institute	of Occupa	tional Health,			
Helsinki, Finland (K. L	<u>innainmaa)</u>							
LAB/BRANCH								
Laboratory of Human Car	<u>cinogenesis</u>							
SECTION	Continu							
In Vitro Carcinogenesis	Section							
NCI, NIH, Bethesda, Mar	wland 20002							
TOTAL MAN-YEARS:	PROFESSIONAL:		OTHER:					
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(a2) Interviews								
SUMMARY OF WORK (Use standard unred	uced type. Do not excee	d the space provid	led.)					
Methods to culture huma	n pleural mes	othelial ((NHM) cell	s have bee	n improved.			
Pure cultures are initi	ated by pelle	ting the r	nesothelia	1 cells fr	om pleural			
effusion fluid and inoc	ulating the r	esuspende	d cells in	to dishes	containing LHC			
basal nutrient medium s	upplemented w	ith serum	(3%), hyd	rocortison	e (0.5			
micromoles), insulin (5	micrograms/m	1) epiderr	nal growth	factor (E	GF) (5 ng/ml),			
twansfermin /10 microsy	2mc/ml) + 22c	o olomonte	and 2%	chomically	-raduced			

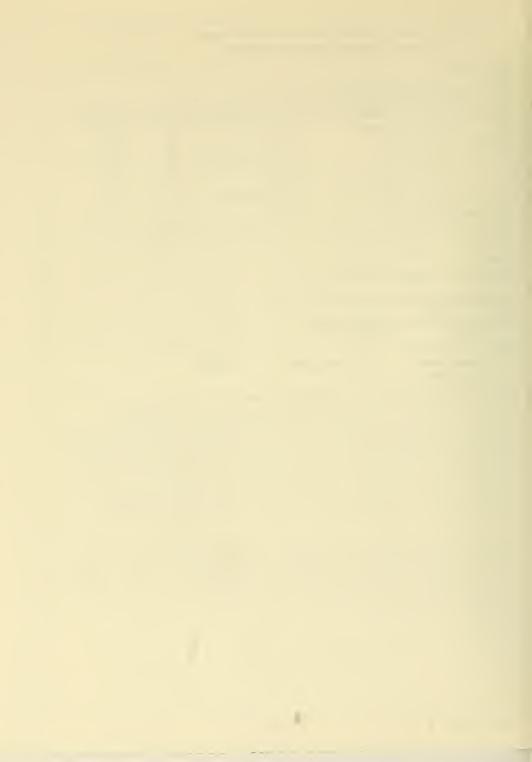
(factor-free) serum (FFS). Using this pseudo-defined protocol, mesothelial cell cultures have been established from more than 200 donors. The cells can be subcultured at clonal density with a colony-forming efficiency of more than 10% and high density cultures can be subcultured four to six times before 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.

PHS 6040 (Rev. 1/84)



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	
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ctober 1, 1986 to September 30, 1987 TLE OF PROJECT (80 characters or lass. Title must lit on one line between the borders.)	
olecular Analysis of Gene Regulation and Proliferative Control in Human Cel	l c
RINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, leboratory, and institute affiliation)	13
.I.: Brenda I. Gerwin Research Chemist LHC NCI	
thers: 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 Sporm Chief LC NCI	
Michael Sporn Chief LC NCI DOPERATING UNITS (# any)	
azelton Labs, Rockville, MD (M. Moore); Institute of Occupational Health,	
elsinki, Finland (K. Linnainmaa)	
B/BRANCH	
aboratory of Human Carcinogenesis	
ECTION	
arcinogen Macromolecular Interaction Section STITUTE AND LOCATION	
CI. NIH. Bethesda. Maryland 20892	
OTHER:	
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(a) Human subjects (b) Human tissues (c) Neither	
(a1) Minors (a2) Interviews	
IMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)	
hese experiments have shown that human mesothelial cells, as compared to	
ibroblasts, are more sensitive to induction of structural chromosomal	
berrations by exposure to asbestos fibers. In addition, it has been shown	that.
alignant mesothelioma cell lines produce PDGF A-chain and B-chain mRNA at m	uch
righer levels than do normal cells. PDGF-like activity is detected in medium	m
onditioned by tumor cells, but not by normal cells. TGF-beta mRNA is expre	ssed
t similar levels in normal cells and tumor cells, but TGF-beta protein is	
ecreted in greater amounts by normal cells. Normal cells respond to mitoge	nic
timuli from PDGF and possess PDGF receptors. These findings suggest the	
ossibility of an autocrine mechanism for the generation of mesothelioma.	
wo-dimensional gel analysis of normal human bronchial epithelial cells afte	n TDA
r TGF-beta treatment has indicated several protein alterations which might	ITA
orrelate with squamous differentiation. The magnitude of the alterations i	s not
reat, implying that this technique may not display the most critical change	s.
t is of interest that Northern blot analysis indicates that a 2-hour treatm	ent
f human bronchial epithelial cells with TPA, but not TGF-beta, can induce a	n
ncrease in IL-1 beta.	

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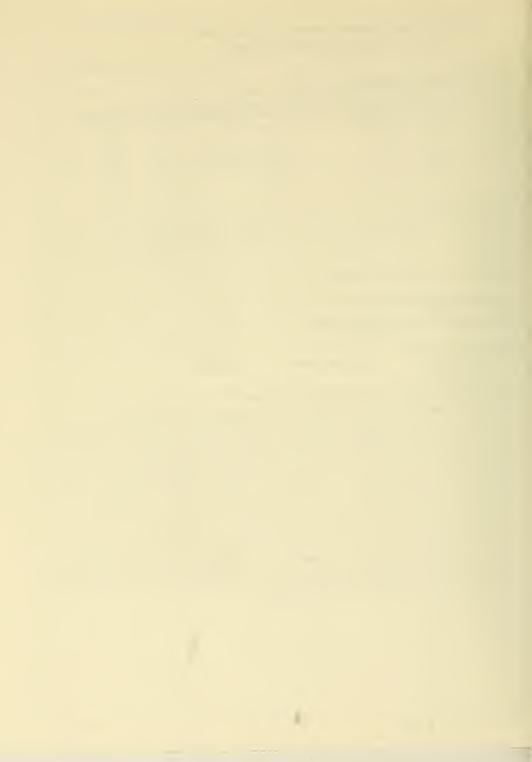
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October 1, 1986 to Sep			ers.)					
Control of Growth and			•	Epithelial	Cells			
PRINCIPAL INVESTIGATOR (List other pro								
P.I.: John F. Lech	ner	Section C	hief	LHC	NCI			
Others: Tohru Masui		Visiting	Associate	LHC	NCI			
Yang Ke		Visiting	Fellow	LHC	NCI			
Curtis C. Ha	rris	Chief		LHC	NCI			
COOPERATING UNITS (if eny)								
Univ. of MD School of	Medicine, Balt	., MD (B.	F. Trump);	Georgetown	Univ. School			
of Medicine, Washingto	n, DC (H. Yeag	er); VA Ho	spital, Was	shington, D	OC (P. Schafer)			
LAB/BRANCH								
Laboratory of Human Ca	rcinogenesis							
SECTION	Cinogenesis		~					
In Vitro Carcinogenesi	s Section							
INSTITUTE AND LOCATION								
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SUMMARY OF WORK (Use standard unre-								
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								
several times; will undergo 35 population doublings; and have expected epithelial cell characteristics of keratin, desmosomes and cell surface antigens. NHBE								
cells inoculated at cl								
of 28 hr; the majority	of the cells	are small	and migrato	ory and hav	re few			
tonofilaments. Adding								
growth rate of NHBE ce	Ils in a dose-	dependent	tashion. I	n contrast	, human lung			
carcinomas either repl		r fail to	grow at all	when inoc	ulated 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.

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PROJECT NUMBER

Z01CP05426-03 LHC

NOTICE OF INTRAMURAL RESEARCH PROJECT

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) George E. Mark, III P. I .: LHC NCT Andrea Pfeifer Visiting Fellow Others: NCT Paul Amstad Visiting Fellow LHC Section Chief 1 HC NCI Dean L. Mann LHC NEI Curtis C. Harris Chief NCT Snorri S. Thoraeirsson LEC Chief .

COOPERATING UNITS (if any)

Dept. of Genetics, Harvard Medical School (N. Perrimon); Dept. of Radiation Medicine, Georgetown Univ. School of Medicine (U. Kasid).

1 A	R/R	RAN	СН

Laboratory of Human Carcinogenesis

SECTION

Carcinogen Macromolecular Interaction Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: PROFESSIONAL: O.THER: 0.7 0.5 0.2

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
(a1) Minors

(b) Human tissues

(c) Neither

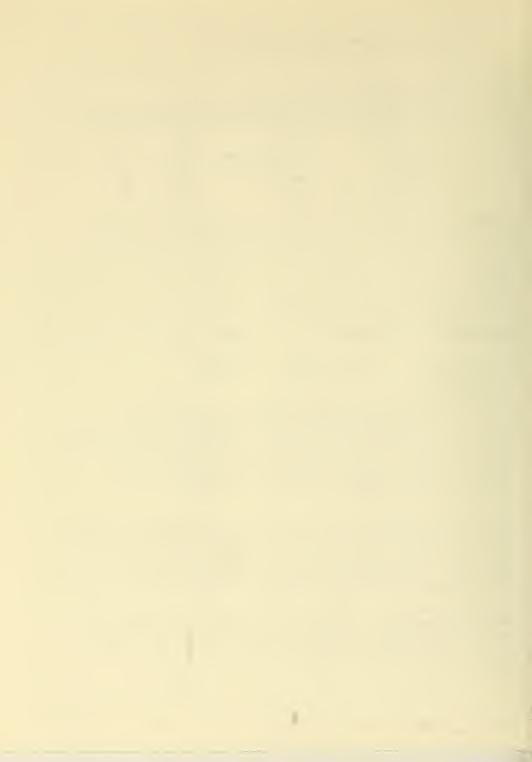
(a2) Interviews

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

The <u>raf</u> 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 <u>raf</u> protein and show approximately 50% sequence relatedness. <u>Raf</u>, when non-activated, may be seen as a diffuse cytoplasmic protein. In neuroepitheliomas, where we believe <u>raf</u> 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 G4I8 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.



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		fassional personnel below t	The second second	tigator) (Name,			
P.I.:	Curtis C. Hai	rris	Chief		LHC	NCI	
Others:	Paul Amstad		Visiting		LHC	NCI	
	Andrea Pfeife		Visiting	Fellow	LHC	NCI	
	George E. Mai	rk, III	Expert		LHC	NCI	
	Roger Reddel		Expert		LHC	NCI	
COOPERATING UN	IITS (if eny)						
		iversity of Upp r (A. Klein-Sza		ital (C.	Betsholtz)		
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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 $1\overline{2}$). 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.

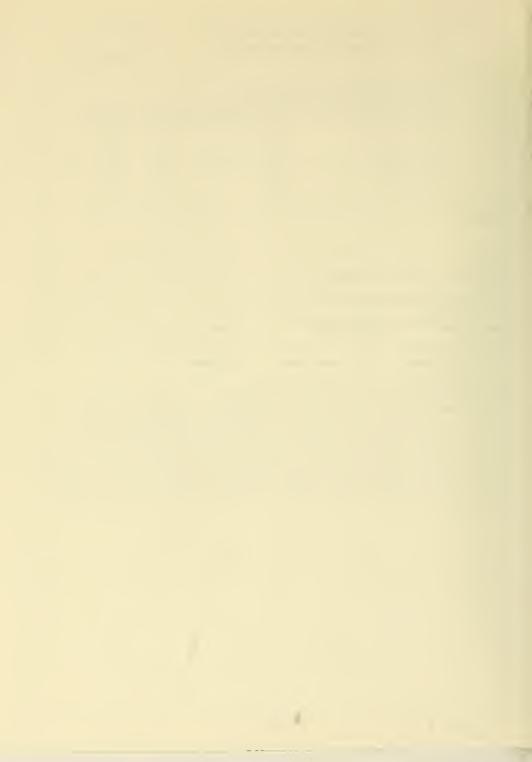
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	tion of myc On				lial	Cells
						lory, and institute affiliation)
P.I.:	Brenda Gerwi		Research		LHC	
Others:	Curtis C. Ha	rris	Chief		LHC	NCI
	George Yoaku	m	Sr. Staff	Fellow	LHC	NCI
	Paul Amstad		Visiting	Fellow	LHC	NCI
	George Mark		Expert		LHC	NCI
COOPERATING	UNITS (if any)					
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	ry of Human Ca	rcinogenesis				
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					fect	ed with a variety of
						v-myc on the same
nlasmid	and the transī	ocated c-myc	frame of th	e CA46 Burk	itt'	s Tymphoma (BL) cell
line Th	he transfected	cells were t	hen selecte	d for resis	tanc	ce to inducers of
						OS) or TPA. The CA46
transloca	ated c-mvc den	e was the mos	t effective	oncodene i	n in	iducing resistance to
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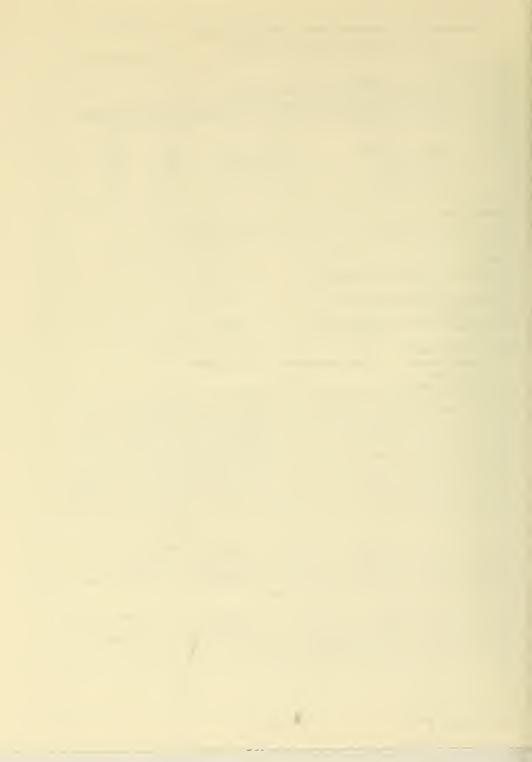
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TITLE OF PROJECT (80 characters or less									
The Biological Activity									
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, leboratory, and institute effiliation)									
P.I.: Curtis C. Har	ris	Chief		LHC	NCI				
Others: Simon M. Plum	mer		Fellow	LHC	NCI				
Jin-Su Choi			Fellow	LHC	NCI				
Dean L. Mann				LHC	NCI				
Vincent Wilso	n	Sr. Staff	Fellow	LHC	NCI				
		•							
COOPERATING UNITS (if any)									
Dept. Toxicology, Karol	inska Institu	te, Sweden	(R. Grafst	rom)					
Microbiological Associa	ites, Inc., Be	thesda, MD	(R. Currer	i, L.L. Yar	ng)				
LAB/BRANCH									
Laboratory of Human Car	cinogenesis								
SECTION									
Biochemical Epidemiolog	y Section								
INSTITUTE AND LOCATION									
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(a) Human subjects	(b) Human tis	ssues L	(c) Neither						
(a1) Minors									
(a2) Interviews									
SUMMARY OF WORK (Use standard unred									
Fecapentaene-12 (fec-12	!), a candidate	e carcinoge	en in the p	athogenesi	s 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

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.

finding that this compound induces transformation in murine Balb 3T3 cells.

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.



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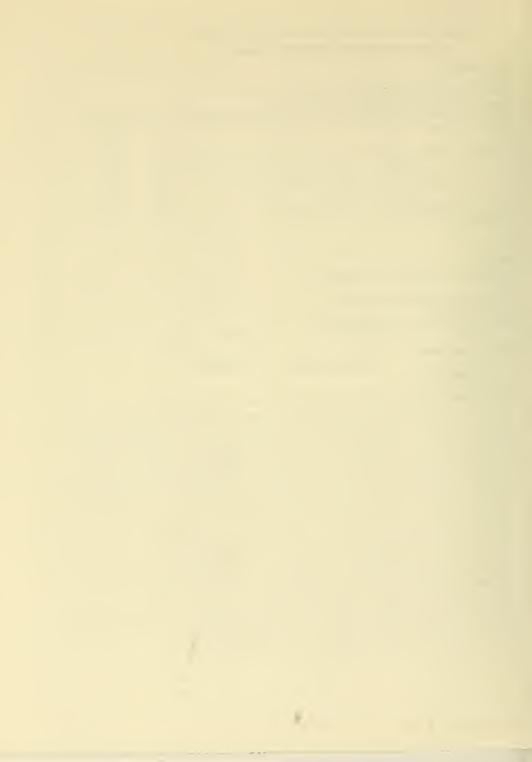
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TITLE OF PROJEC	CT (80 cheracters or lass	. Title must fit on one line	between the border	s.)					
Immunolog	y of AIDS and	AIDS-Related	Diseases						
PRINCIPAL INVES	PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute effiliation)								
P.I.:	Dean L. Mann	Section	Chief		LHC	NCI			
Others:	William Blat	tner Chief, I	Family Stud	ies Section	EEB	NCI			
	J. J. Goeder		, and the second		EEB	NCI			
	R. J. Bigger	Medical	Officer		EEB	NCI			
	Stanley H. W	eiss Medical	Staff Fell	ow	EEB	NCI			
	R. C. Gallo				LTCB	NCI			
		vic Medical	Officer		LTCB	NCI			
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Biochemic	al Epidemiolo	gy Section							
INSTITUTE AND L	OCATION								
NCI, NIH,	Bethesda, Ma	ryland 20892							
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(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 antibodypositive 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.



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PROJECT NUMBER

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Analysis o	of Hydrocarbor	n-Macromolecul	ar Adducts	in Humans	and Cancer	Risk		
		fessional personnel below	the Principal Invest	igator.) (Neme, title,	laboratory, and inst	itute affilietion)		
P.I.:	Ainsley West	on	Visiting A	ssociate	LHC	NCI		
Others:	Curtis C. Har	rris	Chief		LHC	NCI		
	Glennwood E.	Trivers	Res. Biolo	gist	LHC	NCI		
	Simon M. Plun		Visiting F	Fellow	LHC	NCI		
	Vincent Wilso	on	Sr. Staff	Fellow	LHC	NCI		
	Dean L. Mann		Section Ch	nief	LHC	NCI		
	David Manches	ster	Expert			NCI		
COOPERATING U	NITS (if any)							
University	of Oulu, Fir	ıland (K. Vaha	kangas), Lo	uisiana St	ate Univers	ity, Baton		
		n), M.Ř.C., Ca						
		nore, MD (B. F						
LAB/BRANCH								
Laboratory	of Human Car	rcinogenesis						
SECTION								
Biochemica	al Epidemiolog	y Section						
INSTITUTE AND L	OCATION							
	Bethesda, MD	20892						
TOTAL MAN-YEAR	IS:	PROFESSIONAL:		OTHER:				
2.0			2.0		0.0			
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(a) Huma		(b) Human tis	sues \square	(c) Neither				
☐ (a1) M								
☐ (a2) I	nterviews							
SUMMARY OF WO	RK (Use standerd unred	luced type. Do not exceed	the space provided	1.)				

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 enzymelinked 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.



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. T.: Douglas E. Brash Sr. Staff Fellow

LHC

0.5

NCI

COOPERATING UNITS (if any)

Department of Dermatology, Massachusetts General Hospital, Boston, MA (H. Baden)

LAB/BRANCH

1.0

Laboratory of Human Carcinogenesis SECTION

Carcinogen Macromolecular Interaction Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892 TOTAL MAN-YEARS:

PROFESSIONAL:

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues

property.

(c) Neither

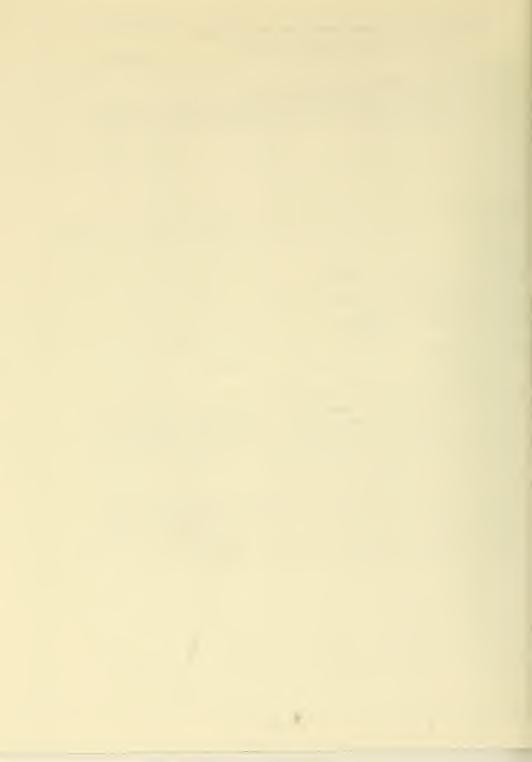
OTHER:

(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 restrictiondigested, 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.



PROJECT NUMBER

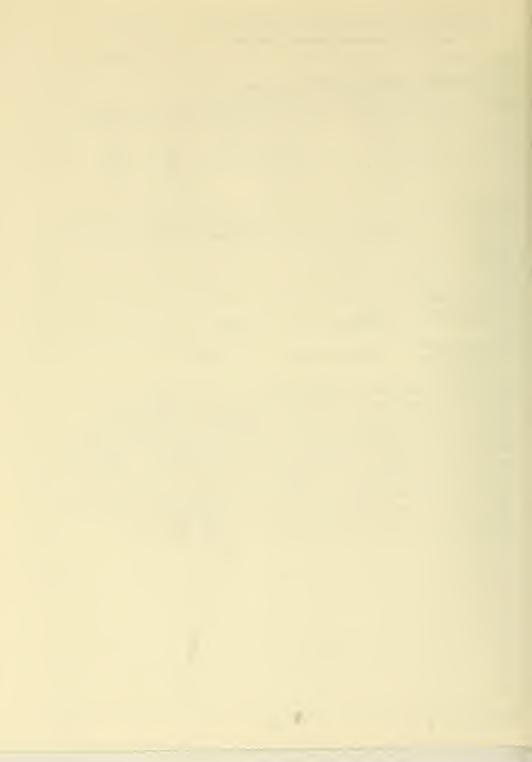
NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05479-02 LHC

October 1, 1986 to September 30, 1987 TITLE OF PROJECT (80 characters or less. Title must int 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 attiliation) 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). ABURANCH Laboratory of Human Carcinogenesis
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 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). ABURDANCH Laboratory of Human Carcinogenesis
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigetor) (Name, little, 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 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). ABURDANCH Laboratory of Human Carcinogenesis
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 Curtis C. Harris Chief LHC NCI Cooperating Units (# 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). ABURDANCH Laboratory of Human Carcinogenesis
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 Curtis C. Harris Chief LHC NCI Cooperating Units (# 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). AB/BRANCH Laboratory of Human Carcinogenesis
Philip Smith Hall-Shields Fellow LHC NCI Dean Mann Section Chief LHC NCI Curtis C. Harris Chief LHC NCI Chief LHC NCI COOPERATING UNITS (# 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). ABUBRANCH Laboratory of Human Carcinogenesis
Philip Smith Hall-Shields Fellow LHC NCI Dean Mann Section Chief LHC NCI Curtis C. Harris Chief LHC NCI Chief LHC NCI COOPERATING UNITS (# 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). ABUBRANCH Laboratory of Human Carcinogenesis
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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). ABURRANCH Laboratory of Human Carcinogenesis
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). ABURDANCH Laboratory of Human Carcinogenesis
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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). ABUBRANCH Laboratory of Human Carcinogenesis
Boston, MA (J.M. Essigman); Department of Toxicology, Karolinska Institute, Stockholm, Sweden (R.C. Grafstrom). ABUBRANCH Laboratory of Human Carcinogenesis
Stockholm, Sweden (R.C. Grafstrom). ABUBRANCH Laboratory of Human Carcinogenesis
AB/BRANCH Laboratory of Human Carcinogenesis
Laboratory of Human Carcinogenesis
Biochemical Epidemiology Section
NCI, NIH, Bethesda, MD 20892
OTAL MAN-YEARS: PROFESSIONAL: OTHER:
2.0 1.0
CHECK APPROPRIATE BOX(ES)
」 (a) Human subjects
(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
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

nee 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 06-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.

pr. Lempings



PROJECT NUMBER

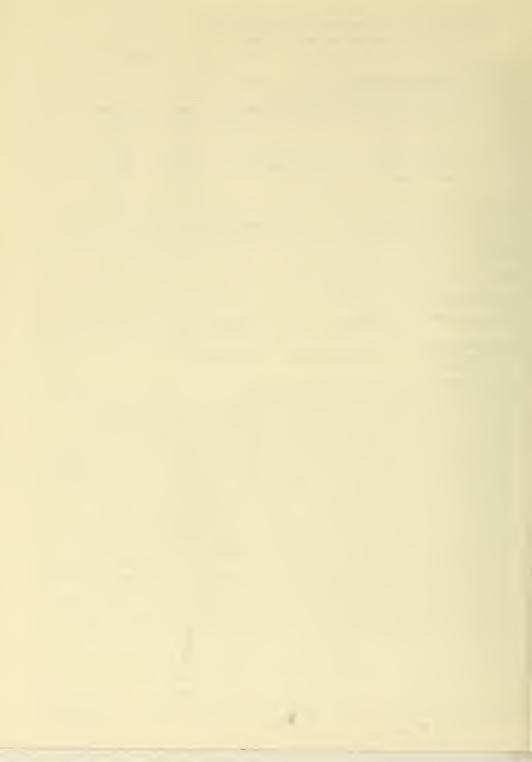
NOTICE OF INTRAMURAL RESEARCH PROJECT

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TITLE OF PROJEC	CT (80 cheracters or less	. Title must fit o	on one line between th	e borders.)				
	olymorphisms							
PRINCIPAL INVES	TIGATOR (List other pro	etessionel perso	nnel below the Princip	al Investigator) (Name, title, le	boratory, and	institute effillation)	
P.I.:	Ainsley West	on	Visiting As	sociate		LHC	NCI	
Othouse	Cumtia C IIa	22 _	Ch : - £				W0.7	
Others:	Curtis C. Ha		Chief			LHC	NCI	
	James C. Wil	rey				LHC	NCI	
	Brenda I. Ge		Research Ch			LHC	NCI	
	Dean L. Mann		Section Chi	ef		LHC	NCI	
COOPERATING U								
New Engla	nd Medical Ce	nter, Bo	ston, MA $(T.$	Krontiri	is); Chi	ldren's	Hospital o	f LA,
Los Angel	es, CA (W. Be	nedict);	National In	stitute o	of Occup	ational	Health, Os	lo,
Norway (A	. Haugen)							
LAB/BRANCH								
	y of Human Ca	rcinagen	esis					
SECTION								
	<u>al Epidemiolo</u>	gy Secti	on					
INSTITUTE AND L								
NCI, NIH,	Bethesda, MD							
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(a) Huma		区 (b) Hu	ıman tissues	☐ (c) N	Veither			
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☐ (a2) I	nterviews							
SUMMARY OF WO	ORK (Use standard unred	duced type Do	not exceed the space	provided.)				

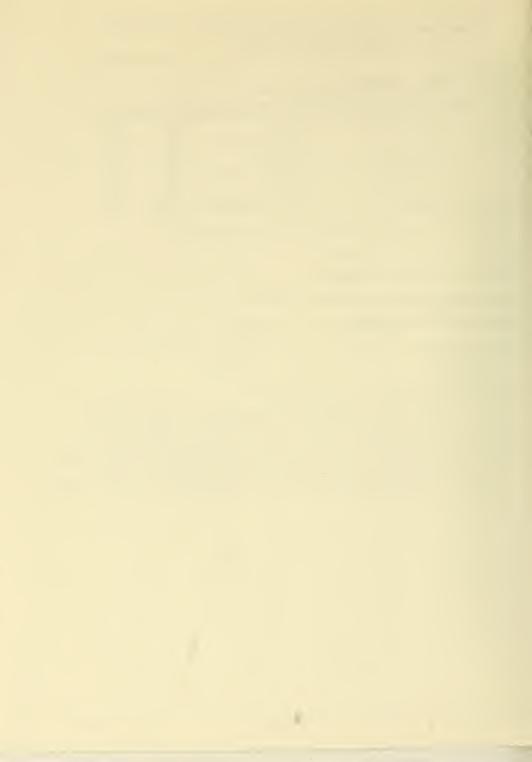
There is a clear association between smoking and lung cancer, but it is still not known why some individuals, who are heavily exposed to large concentrations of chemical carcinogens, do not develop tumors, whereas others do. These observations provide circumstantial evidence for the involvement of a 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.

INC. WENDER



PROJECT NUMBER

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SV40 Large T-Antigen PRINCIPAL INVESTIGATOR (List other pro	fessionel personnel below the	e Principal Invas	tigator.) (Name, title, la	boretory, and institu	ute affiliation)	
P.I.: R. R. Redde	1	Expert		LHC	NCI	
Others: Y. Ke		Visiting	Fellow	LHC	NCI	
M. McMenami		Biologis		LHC	NCI	
J. Quintero		Biologis		LHC	NCI	
B. I. Gerwi			Chemist	LHC	NCI	
C. C. Harri J. Rhim	S	Chief		LHC	NCI	
T. McLemore			robiologist f Fellow	L CMB L ETM	NCI	
COOPERATING UNITS (if any)		-JIJLGI	1.16110W	LEHT	1401	
Fox Chase Cancer Cente	er, Philadelphia	a. PA (A.	Klein-Szant	.0)		
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LAB/BRANCH						
Laboratory of Human Co	arcinogenesis		· · · · · ·			
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NCI, NIH, Bethesda, M TOTAL MAN-YEARS:	D_20892					
TOTAL MÁN-YEARS:	PROFESSIONAL:		OTHER:			
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CHECK APPROPRIATE BOX(ES) (a) Human subjects	(b) Human tissu	10c	(c) Neither			
(a) Human subjects	(b) Human ussu	162 —	(c) Neither			
(a2) Interviews						
SUMMARY OF WORK (Use standard unred	uced type. Do not exceed th	e space provide	d.)			
This is a project to o	obtain immortal:	ized nont	umoriaenic c	oll lines	of human	
mesothelial and bronch	hial epithelial	origin.	Five lines	have been a	or numan establishe	-d
from normal human bron	nchial epithelia	al (NHBE)	cells, one	by infection	on with	
adenovirus12-SV40 hybi	rid virus, two b	by infect	ion with wil	d type SV40	O virus. a	nd
two by transfection v	ia strontium pho	osphate c	oprecipitati	on with a i	plasmid, p	RSV-
T, containing origin-	ninus SV40 early	/ region	sequences.	One line ha	as been	
established from norma	al mesothelial o	cells by	transfection	with the p	plasmid, p	rRSV-
T. These cell lines a carcinogenesis.	are being used t	to study	aspects of m	uitistage		
car emogenes is.						

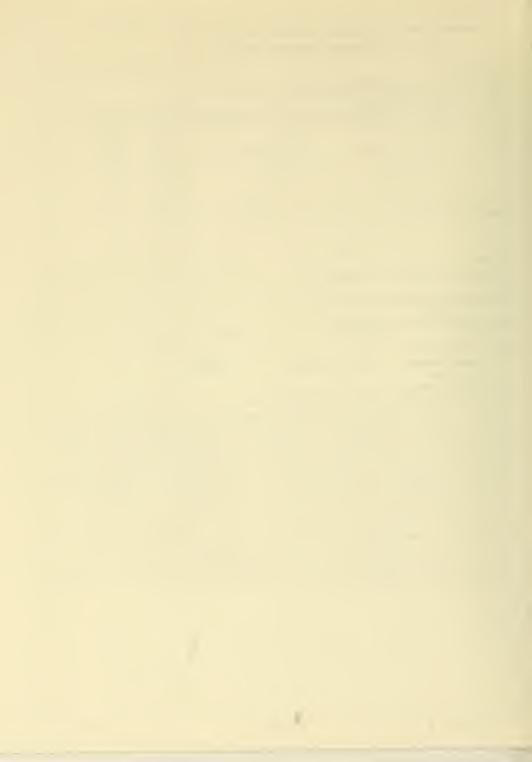


PROJECT NUMBER

DEPARTMENT OF HEALTH				1				
NOTICE OF IN	TRAMURAL RESEAR	CH PROJE	CT					
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October 1, I986 to Sep	tember 30, 1987							
TITLE OF PROJECT (80 characters or les	s. Title must fit on one line bet	ween the border	s.)					
Oxidant-Induced DNA Da	mage							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Neme, title, laboratory, and institute affiliation)								
P.I.: Curtis C. Ha	rris Chie	ef		LHC	NCI			
Others: Philip C. Sm	ith Hal	1 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	I.	0		0.0				
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(a) Human subjects	(b) Human tissue	es 🗆	(c) Neither					
(a1) Minors								
(a2) Interviews								
SUMMARY OF WORK (Use standard unre-	duced 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 8-0Hdeoxyguanosine (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.

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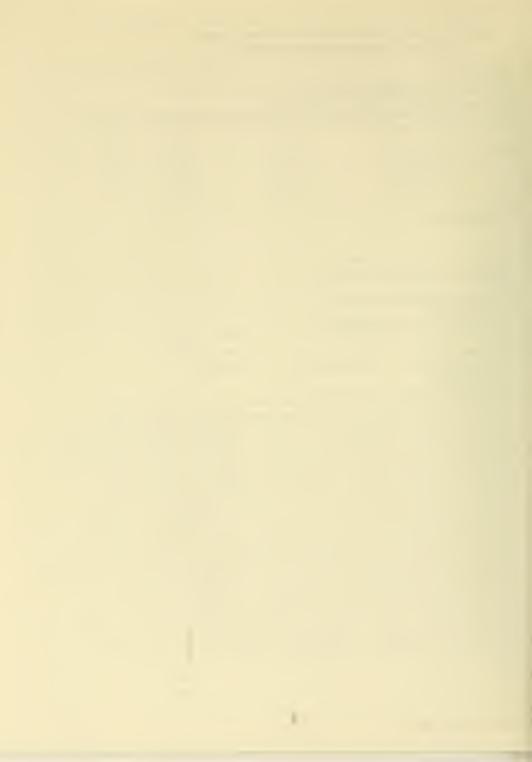


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PERIOD COVERE	D				[20.	10F03307-03 LHC	
October 1	, 1986 - Sept	ember 30, 1987					
TITLE OF PROJE	CT (80 cheracters or less	. Title must fit on one line	between the bo	rders.)			_
Cell Surf	ace Antigens	on Human Lung (Carcinoma	ı S			
PRINCIPAL INVES	STIGATOR (List other pro	fessionel personnel below	the Principal In	vestigator.) (Name, title,	laboratory, i	and institute affiliation)	_
P.I.:	Dean L. Mann		Section	Chief	LHC	NCI	
0+1	Carrier March						
Others:	George Mark		Expert		LHC	NCI	
	Roger Reddel		Expert		LHC	NCI	
	John Lechner		Section	Chief	LHC	NCI	
	Curtis Harri	S	Chief		LHC	NCI	
COOPERATING U	NITS (if any)						
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LAB/BRANCH							
Laborator	y of Human Car	rcinogenesis					
SECTION							
Biochemica	al Epidemiolog	y Section					
NCI, NIH,	Bethesda, Ma						
TOTAL MAN-YEAR	is:	PROFESSIONAL:		OTHER:			
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		لص (ك) Human tiss	ues l	☐ (c) Neither			

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

(a2) Interviews

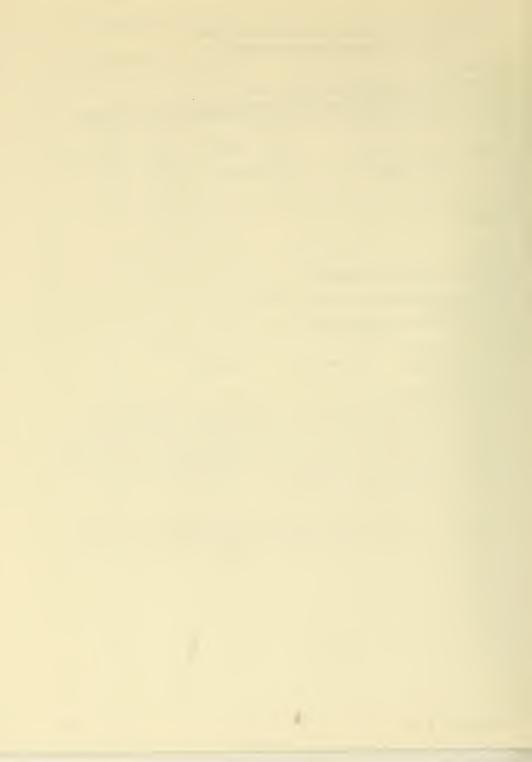
The expression of a variety of cell surface antigens were studied on normal bronchial epithelial cells, small cell lung carcinomas, mesothelial cells, mesotheliomas, as well as bronchial epithelial cells, fibroblasts and mesothelial cells transfected with T antigen. The monoclonal antibodies used to 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 \overline{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.



PROJECT NUMBER

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PERIOD COVERED	1005 1 0		_						
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								
Isolation	of Growth a	ind Differentiat	tion Genes	by Subtrac	tion Libr	raries			
P.I.:	PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I.: George E. Mark, III Expert IHC NCT								
P.1.;	George E. N	lark, III	Expert		LHC	NCI			
Others:	Andrea Pfei	for	Visiting	Follow	LHC	NCI			
o chers.	Curtis C. H		Chief	reriow	LHC	NCI			
John Lechner Section Chief LHC NCI									
Section Uniet LHC NCI									
·									
COOPERATING UNI	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 APPROPRIA						•			
☐ (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 sub-									
tracted 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.									
1	- 6.6 6 11 1								
As a spin-	orr of this	subtraction pr	ocedure it	has been	realized	that genomic			
subtractio	on can also	be accomplished	using a s	light modi	fication	of the basic			
protocols.	inis woul	d enable the di	rect ident	ification	of "reces	sive" genes su	ch		
as the one	es involved	in retinoblasto	ma, Wilm's	tumor, an	d muscula	r dystrophy.			

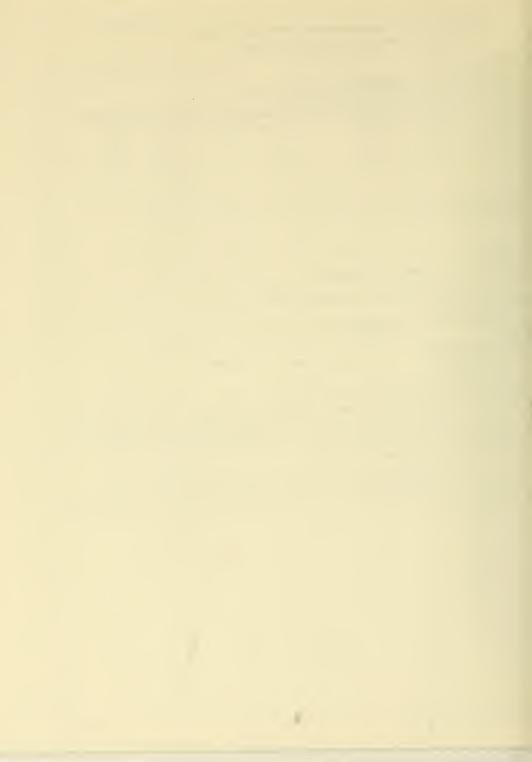
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PROJECT NUMBER

NOTICE OF	INTRAMURAL	RESEARCH	PROJECT
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PERIOD COVERED			Z01CP05509-03 LHC
October 1, 1986 to Sentember	r 30 1987		
TITLE OF PROJECT (80 characters or less. Title mus	t fit on one line between the borders.)		
Detection of Mutations in F	Proto-Oncogonos		
PHINCIPAL INVESTIGATOR (List other professional p	personnel below the Principal Investiga	tor.) (Name, title, leboret	ory, and institute affiliation)
P.I.: Douglas E. Brash	Sr. Staff	Fellow LH	HC NCI
Others: Curtis C. Harris	Chief	1.6	IC NOT
	OHIE	LF	IC NCI
COOPERATING UNITS (if any)			
LAB/BRANCH			
Laboratory of Human Carcino	Jenes 1 s		
Carcinogen Macromolecular I	iteraction Section		
INSTITUTE AND LOCATION			
NCI, NIH, Bethesda, MD 2089			
TOTAL MAN-YEARS PROFESS	01	HER:	
CHECK APPROPRIATE BOX(ES)	0.25	0.0	o
(a) Human subjects (b)	Human tissues 🖾 (c) Neither	
(a1) Minors	- Tarries - 100000	Neither	
(a2) Interviews			
SUMMARY OF WORK (Use stendard unreduced type.	Do not exceed the space provided)		
We have isolated DNA from 6	human lung tumor cel	l lines as we	ell as several tumor
digested, electrophoresed, a single-base mismatches in the coden 12 region and coden 61			
codon 12 region and codon 61	region.	nd N-ras prot	o-oncogenes at the
Thus far, two previously unc	haracterized lung tu	mor lines app	ear to carry
A line previously shown to h the normal allele.	ave a K1-12- <u>ras</u> muta	tion has been	found to have lost
one norman arrene.			
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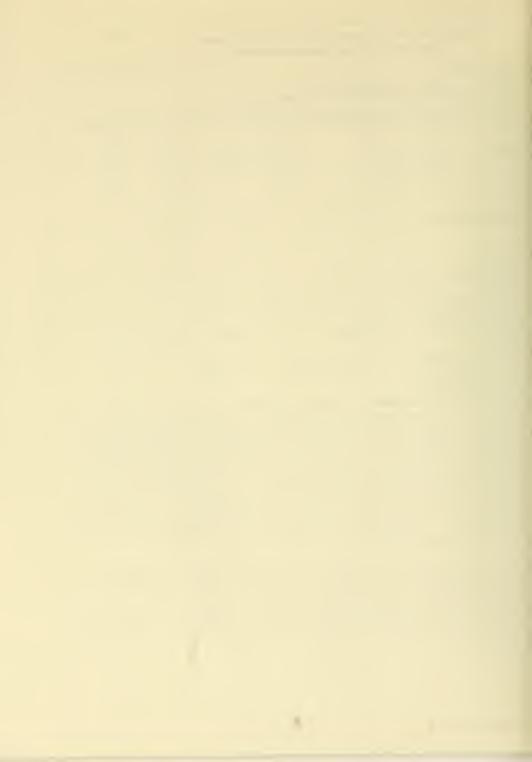


PROJECT NUMBER DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT. Z01CP05510-03 LHC PERIOD COVERED October 1, 1986 to September 30, 1987 TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Activities of Promoters/Enhancers in Human Cells PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, leboratory, and institute effiliation) Roger R. Reddel P.I.: Expert I HC NCI Others: Douglas E. Brash Sr. Staff Fellow NCI LHC Brenda Gerwin Research Chemist LHC NCT 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: 0.5 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 chloramphenical acetyl transferase (CAT) assay system. In the immortalized lines, SV40-enhanced adenovirus MLP, transactivated HTLV-I

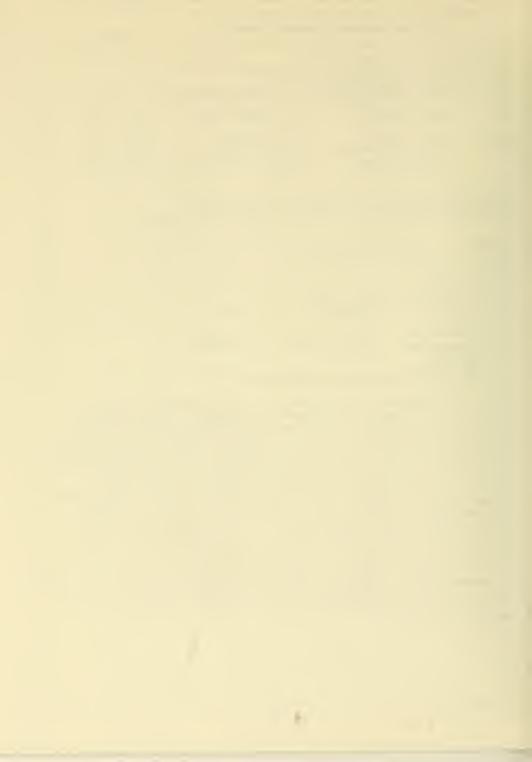
In the immortalized lines, SV40-enhanced adenovirus MLP, transactivated HTLV-I LTR and transactivated HTV LTR were highly active. Under the conditions of the assay, the metallothionien promoter was measurably active, but not inducible by cadmium, and MMTV was inducible but showed weak activity. These 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.

who we need

PHS 6040 (Rev. 1/84)



PROJECT NUMBER OFPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT Z01CP04496-10 LMC PERIOD COVERED October 1, 1986 to September 30, 1987 TITLE OF PROJECT (80 charecters or less. Title must lit on one line between the borders.) Chromosomal Proteins and Chromatin Function PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Neme, title, laboratory, and institute affiliation) PI. Michael Bustin Acting Section Chief 1 MC NC I Others: David Landsman Visiting Associate 1 MC NC.T Thyagarajan Srikantha Visiting Fellow LMC NCI Nirmolini Soares Lab. Tech. (Microbiol.) 1 MC NC T COOPERATING UNITS (# 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: 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 retropseudogene family with 50 copies per genome. Each family transcribes a singlesize 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. ent outhers

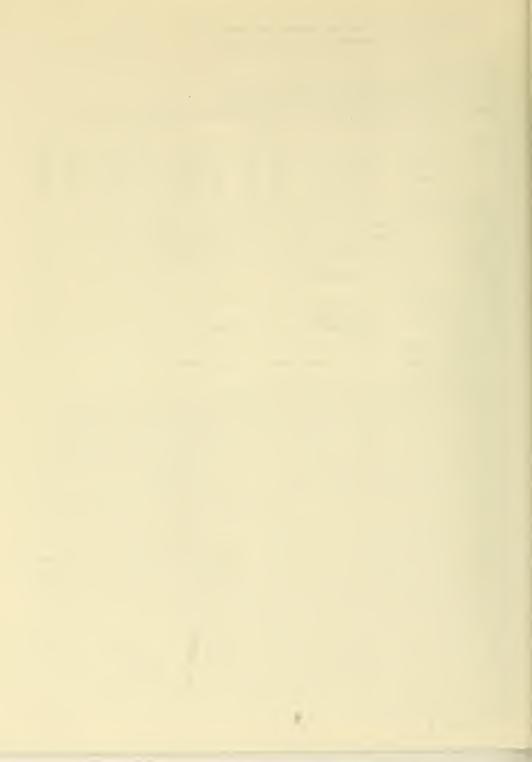


NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP04517-11 LMC

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tosum (XP) and with the dysplastic nevus syndrome (DNS) of hereditary cutaneous melanoma are being studied. We have developed new assays utilizing plasmids as											
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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. 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.



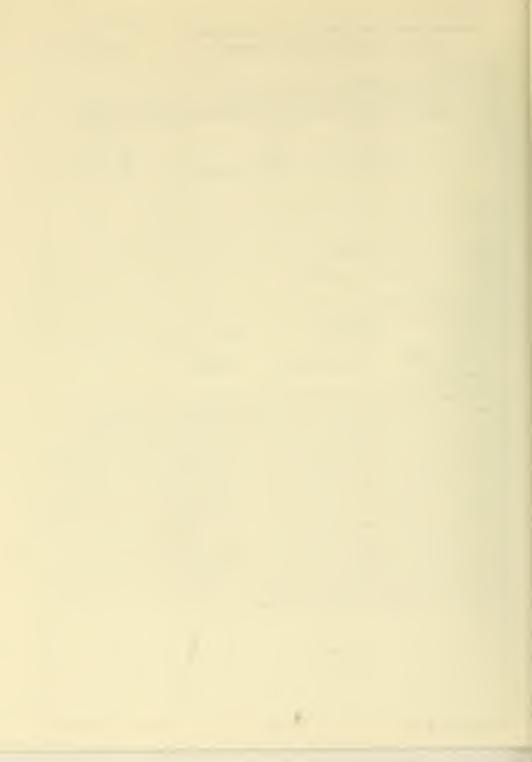
PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

Z01 0P05086-09 LMC

NOTICE OF INTRAMURAL RESEARCH PROJECT

October 1, 1986 to Septe							
Study on Cross-reactivit	y of Monoclon	al Antibodi	ies to Rat				
PRINCIPAL INVESTIGATOR (List other profe P. I.: S. S. F	essional personnel below ark	the Poncipal Invest Expert	ngator) (Neme. title,	laboratory, and instr LIAC	NC I		
Others: H. V. G G. M. S Y. C. L	undaresan	Chief Chemist Guest Resea	archer	LMC LMC LMC	NC I NC I NC I		
		-					
COOPERATING UNITS (If any)							
Univ. of Oulu, Finland (Nashville, TN (F. P. Gue		Vanderbilt	t Univ., Sc	hool of Med	dicine,		
Laboratory of Molecular	Carcinogenesi	S					
Metabolic Control Section	n						
NCI, NIH, Bethesda, MD 2	0982						
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.0		OTHER:	.0			
(a1) Minors	(b) Human tis	sues 🗆	(c) Neither				
(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 numan 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.							
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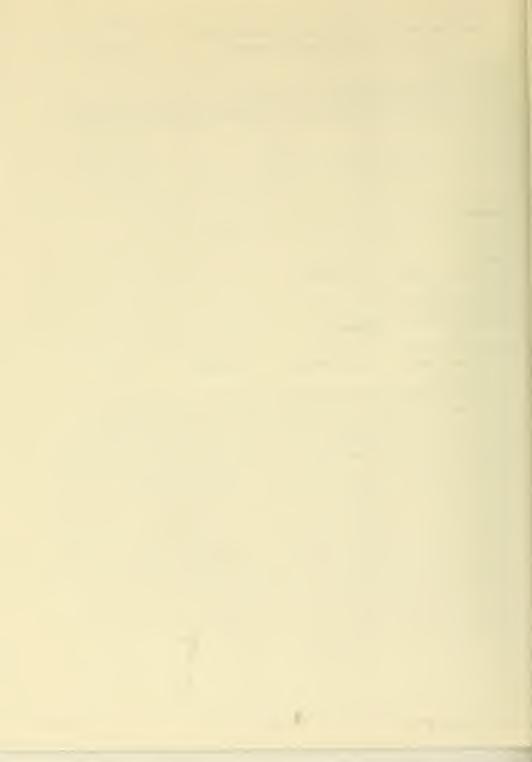
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CP051 25-07 LMC

October 1, 1986 to	Sentember 30 108	7						
TITLE OF PROJECT (80 cherecters 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 effiliation)								
PHINCIPAL INVESTIGATOR (EST	other professional personnel belo-	w the Fincipal investi	gaior./ (reame, mie. rac	Joraiory, and institu	ute enmanony			
PI: S.	S. Park	Expert		LMC	NC I			
Others: H.	V. Gelboin	Chief		LMC	NC I			
		Chemi st		LMC	NCI			
		Guest Resea	rcher	LMC	NCI			
	••							
COOPERATING UNITS (if any)								
Dana-Farber Cancer	Institute, Boston	, MA (D. J.	Waxman)					
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LAB/BRANCH								
Laboratory of Molec	cular Carcinogenes	is						
SECTION								
Metabolic Control S	Section							
INSTITUTE AND LOCATION								
NCI, NIH, Bethesda,	, MD 20892							
TOTAL MAN-YEARS:	PROFESSIONAL:		OTHER:					
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(a) Human subjects	(b) Human ti	issues XX	(c) Neither					
(a1) Minors								
(a2) Interviews								
SUMMARY OF WORK (Use stand	SUMMARY OF WORK (Use standard unreduced type. Do not exceed the spece provided.)							

Cytochrome P-450 (P-450) is a key component of the mixed-function oxidases which metabolize numerous xenobiotics and endobiotics such as prostaglanding, 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-45ORLM5. RIA studies indicated that the expression of P-45ORLM5 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.

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1	NOTICE OF INT	RAMURAL RE	SEARCH PROJ	СТ			
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PERIOD COVERED							
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Phenotyping	of Human Cy	tochrome P-	450				
PRINCIPAL INVEST	IGATOR (List other pro	fessional personnel b	elow the Principal Invest	igator.) (Name, title, la	boratory, and	d institute affiliation)	
PI:	Fred K. Fr	iedman	Research Che	mist	LMC	NC I	
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Others:	Haruko Mil		Bio. Lab. Te	cn.	LMC	NC I	
	Sang S. Pa		Expert		LMC	NC I	
	Harry V. G	elboin	Chief		LMC	NCI	
COOPERATING UN	IITS (if any)			 			
Hebrew Univ	ersity, Jerus	salem, Israe	el (H. Kapitu	lnik)			
University	of Oulu, Fin	land (O. Pe	ikonen)	,			
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LAB/BRANCH							
Laboratory	of Molecular	Carcinogene	esis				
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NCI, NIH, B	Bethesda, Mar	yland 20892	2				
TOTAL MAN-YEARS	S:	PROFESSIONAL:		OTHER:			
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

XX (b) Human tissues

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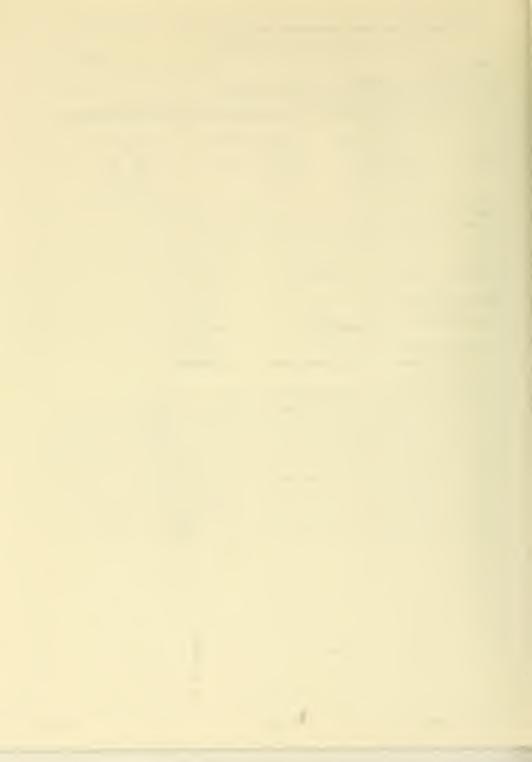
CHECK APPROPRIATE BOX(ES)

(a) Human subjects

(a1) Minors (a2) Interviews

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.

(c) Neither



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CP05318-05 LMC

PROJECT NUMBER

PERIOD COVER October 1	1986 to September	30, 1987				
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PRINCIPAL INVE	STIGATOR (List other professional p	ersonnel below the Princi	pal Investigator) (Na	me, title, laboratory, and	d institute affiliation)	
PI:	Fred K. Friedman	Research	n Chemist	LMC	NC I	
Others:	Richard C. Robin Sang S. Park Harry V. Gelboin	Expert	st ,	LMC LMC LMC	NC I NC I	
	UNITS(#æny) Services University Institute of Aging,			(A. Alvares)	,	
LAB/BRANCH Laboratory	of Molecular Carci	nogenes is				
	Control Section					
NCI, NIH,		20892				
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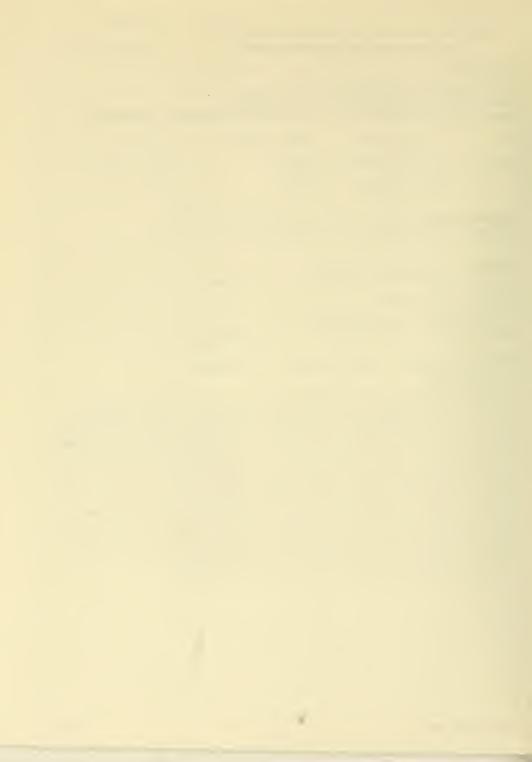
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(a2) Interviews

The cytochromes P-450 metabolize a wide array of compounds, including xenobiotics such as drugs and carcinogens, and endogenous compounds such as steroids. 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.

PHS 6040 (Rev 1/84)

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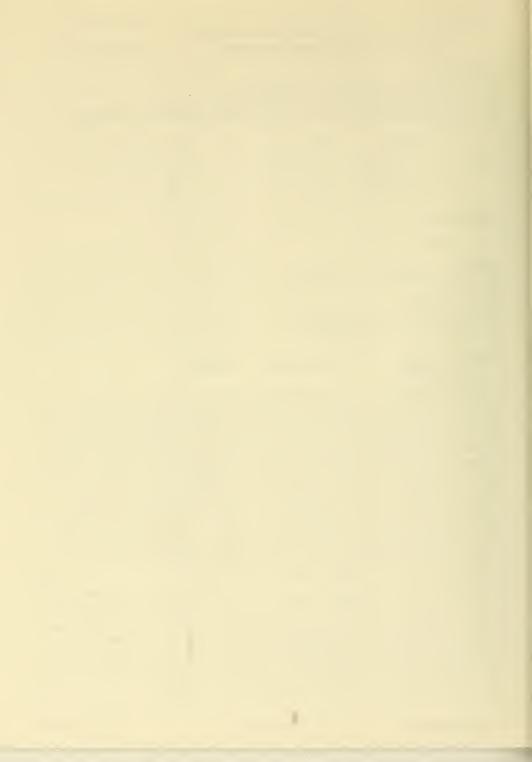
NOTICE OF INTRAMURAL RESEARCH PROJECT Z01CP05436-03 LMC

October 31	1986 thro	ough Sente	mber 30, 1987	7				
TITLE OF PROJECT (80 cherecters or less. Title must fit on one line between the borders.) Expression of Cytochrome P-450 and Their Role in Carcinogenesis								
PRINCIPAL INVESTIG	GATOR (List other	er professional per	sonnel below the Princip	al Invest	igator.) (Name, titi	e, laboratory,	and institute effilietion	,
P.I.:	N. Battul	la	Expert		i	_MC	NCI	
Others:	G. K. Tov	wnsend	Biologist		ı	_MC	NC I	
	F. J. Gor		Senior Staff	Fell.	ow 1	MC	NCI	
	H. V. Gel		Chief			_MC	NC I	
COOPERATING UNIT	S (if any)							
NONE	Ē							
LAB/BRANCH								
Laboratory o	of Molecul	lar Carcin	ogenesis					
SECTION								
Metabolic Co	ntrol							
INSTITUTE AND LOC	ATION							
NCI, NIH, Be	ethesda, N	Maryland	20892					
TOTAL MAN-YEARS:		PROFESSI	ONAL:		OTHER:			
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☐ (a1) Mir	nors							
☐ (a2) Inte	erviews							
SUMMARY OF WORK	(Use stendard	unreduced type. D	o not exceed the space	provided	1.)			
Cytochrome F	-450s are	e a superf	amily of enzy	/mes,	some of v	hich ar	re capable of	f metab-

olizing 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.

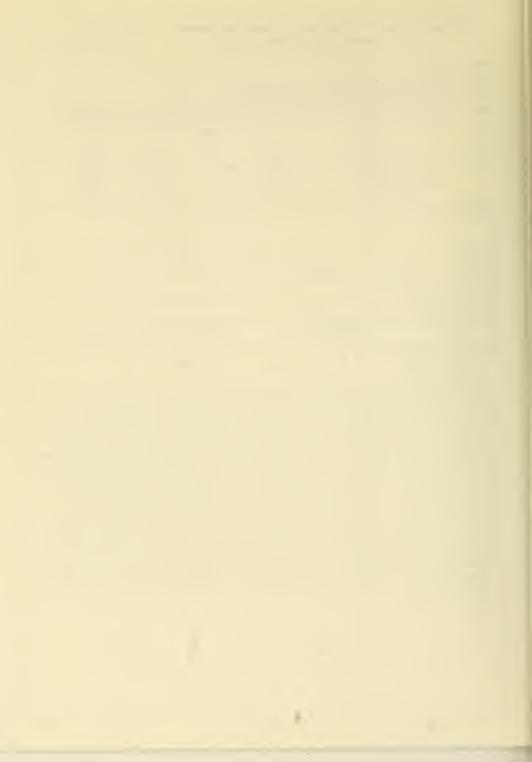
For this purpose, we employed two types of expression systems, namely recombinant vaccinia virus and recombinant retrovirus. We have constructed infectious recombinant vaccinia virus and infectious recombinant retrovirus containing the full length coding cDNA sequences for mouse cytochrome P1-450 and 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.



PROJECT NUMBER

DEPARTMENT OF HEALTH A	DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE							
NOTICE OF INT	RAMURAL RESEA	RCH PROJ	ECT	Z01C	P05519-01 LMC			
October 1, 1986 to Sept								
TITLE OF PROJECT (80 cheracters or less Developmental Regulatio			rs.)					
PRINCIPAL INVESTIGATOR (List other pro			tigator.) (Name, title, le	aboretory, and inst	itute effiliation)			
PI: Frank J. Gonz	alez Sr	. Staff F	ellow	LMC	NCI			
Others: Harry V. Gelb		ief		LMC	NC I			
Tamihide Mats		siting Fe	11 ow	LMC	NCI			
James Gillett		ief		LCP	NHLBI			
Kiyoshi Nagat	a V1	siting Fe	Ilow	LCP	NHLBI			
COOPERATING UNITS (# eny)								
Laboratory of Molecular	Carcinogenesis							
SECTION								
Nucleic Acids Section								
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Mar TOTAL MAN-YEARS:	yland 20892							
2.0	2.0		OTHER:					
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PROJECT NUMBER

Z01CP05520-01 LMC

NOTICE OF INTRAMURAL RESEARCH PROJECT

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, leboratory, and institute effiliation)

PI: Frank J. Gonzalez Sr. Staff Fellow

LMC

Others:

Harry V. Gelboin Mario Umena Tamihide Matsunaga

Chief Visiting Fellow Visiting Fellow

LMC LMC LMC

NC T NC T NC I

NC I

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)

LAR/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Nucleic Acids Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2.0

PROFESSIONAL: 2.0

OTHER:

CHECK APPROPRIATE BOX(ES)

(a) Human subjects

XX (b) Human tissues

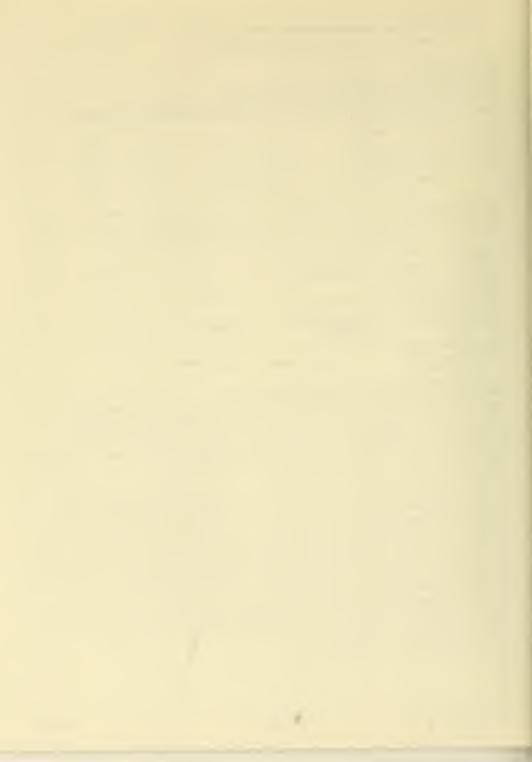
(c) Neither

(a1) Minors (a2) Interviews

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

N-nitrosodimethylamine demethylase (P-450i) 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-450; 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.



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 Debrisoguine 4-Hydroxylase Genes

		el below the Principal Investigator.) (Name, tit		
PI:	Frank J. Gonzalez	Sr. Staff Fellow	LMC	NC I
Others:	Harry V. Gelboin	Chief	LMC	NC I
	Shioko Kimura	Visiting Associate	LMC	NCI
	Morio Umeno	Visiting Fellow	LMC	NC I
	Eiji Matsunaga	Visiting Fellow	LMC	NC I
	Tamihide Matsunaga	Visiting Fellow	LMC	NC I
	Jullia Pastewka	Chemist	LMC	NC I

COOPERATING UNITS (if any)

Argonne National Laboratory, Argonne, IL (James P. Hardwick) Biocenter, University of Basel, Switzerland (Urs A. Meyer)

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				

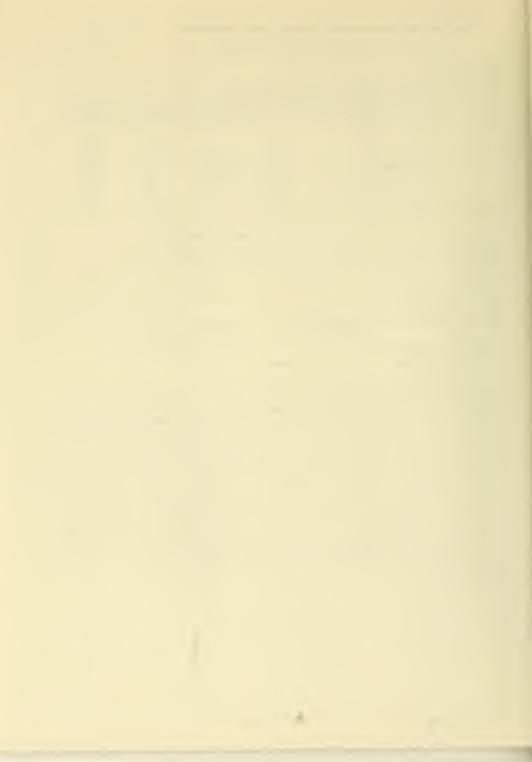
(a) Human subjects	XX (b) Human tissues	(c) Neither
(a1) Minors		

(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 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 debrisoguine 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 debrisoguine 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.

SE SENSE



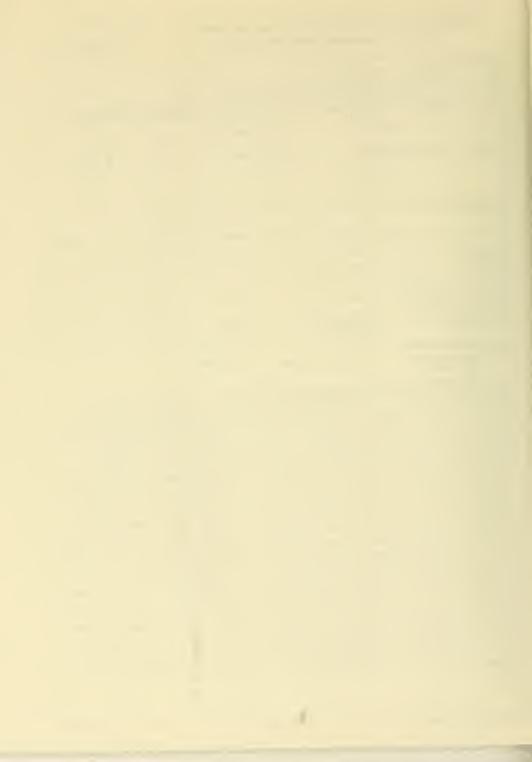
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05522-01 LMC

October 1, 1986 to Septe	ember 30, 1987		
Structure and Character			
PRINCIPAL INVESTIGATOR (List other pro	fessional personnel below the Principal	Investigator.) (Name, title, labora	tory, and institute affiliation)
PI: Shioko Kimura	Visiting	Associate LMC	C NCI
Others: O. Wesley McB	ride Section	Head LB	NC I
COOPERATING UNITS (# any)			
Miyazaki Medical College	e Hospital, Miyazaki,	Japan (Sachiya Oh	ntaki, Tomio Kotani)
LAB/BRANCH Laboratory of Molecular	Carcinogenesis		
SECTION Nucleic Acid Section			
NCI, NIH, Bethesda, Mary	/land 20892		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER:	
CHECK APPROPRIATE BOX(ES) (a) Human subjects	(b) Human tissues	(c) Neither	
(a1) Minors			
☐ (a2) Interviews			
SUMMARY OF WORK (Use standard unred	uced type. Do not exceed the space pri	ovided.)	

Peroxidases represent a group of hemoproteins that are ubiquitous in both the plant and animal kingdoms. They reduce H₂O₂ 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.



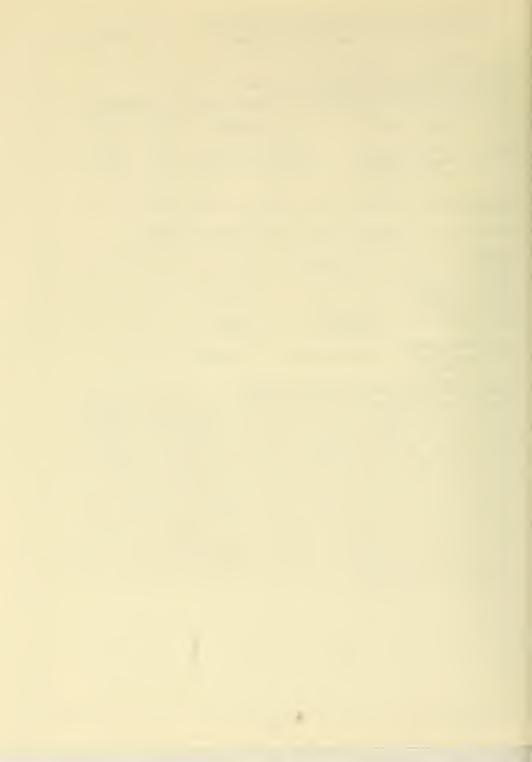
PROJECT NUMBER

Z01CP05523-01 LMC

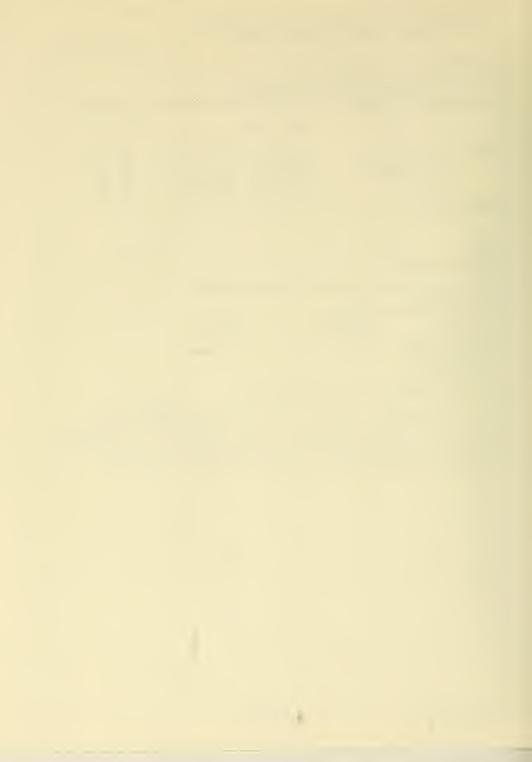
NOTICE OF INTRAMURAL RESEARCH PROJECT

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	Shioko Kimura		sociate	LMC	NC I	
Frank J. Gonz	s: Toshifumi Aoyama Frank J. Gonzalez Harry V. Gelboin		Visiting Fellow LMC NCI Senior Staff Fellow LMC NCI Chief LMC NCI			
COOPERATING UNITS (# any) Argonne National Labora	itory, Argonn	e, Illinois	(James P.	Hardwick)		
Laboratory of Molecular	Carcinogene	sis				
Nucleic Acid Section						
NCI, NIH, Bethesda, Mar	<u> </u>					
TOTAL MAN-YEARS: 2.0						
CHECK APPROPRIATE BOX(ES) (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews						
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DEPAR		D HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT			Z01CP04265-22 BB
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PERIOD COVER	1 1006 to Sont	amban 20 1097	
October	1, 1986 to Sept	EMDER 30, 1907 Title must fit on one line between the borders.)	
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Consulti	ng in Statistic	s and Applied Mathematics assigned personnel below the Principal Investigator.) (Name, title	(aboraton) and institute affiliation)
PRINCIPAL INV	ESTIGATOR (LIST other profe	issignal parsonnal balow tha Principal Investigation, (Name, the	. recorderly, and moneta amount
P.I.:	J. J. Gart	Chief, MS.AMS	BB NCI
Others:	R. E. Tarone	Mathematical Statistici	an BB NCI
others.	H. M. Pettigre		BB NCI
	D. G. Thomas	Mathematical Statistici	an BB NCI
	J. Nam	Mathematical Statistici	
	A. M. Smith	Statistician (Health)	BB NCI
COOPERATING		Statistician (nearon)	
COOPERATING	ONITS (if any)		
NONE			
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LAB/BRANCH	istics Danah		
	istics Branch		
SECTION		and Applied Mathematics Section	
Mathemai	tical Statistics	and Applied Mathematics Section	
INSTITUTE AND		00000	
NCI, NI	H, Bethesda, MD	PROFESSIONAL OTHER:	
TOTAL MAN-YE		11101 2001011112	
3.0		3.0	
	PRIATE BOX(ES)	(b) Human tissues (c) Neither	
	man subjects	(b) Human tissues (c) Neither	
) Minors		
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SUMMARY OF) Interviews WORK (Use standard unred	his study to collaborate with NCI	researchers on
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It is t) Interviews WORK (Use standard unred he purpose of t tical problems	his study to collaborate with NCI related to many areas of cancer re	esearch. Consulting
U (a2) SUMMARY OF It is t mathema assista) Interviews WORK (Use standard unred he purpose of t tical problems nce in statisti	his study to collaborate with NCI related to many areas of cancer re cal methodology and applied mather	natics is provided for s. In general, the study
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It is t mathema assista NCI inv	he purpose of t tical problems nce in statisti estigators and	his study to collaborate with NCI related to many areas of cancer recal methodology and applied mathet to some extent for NCI contractors ting the use of quantitative methors.	natics is provided for s. In general, the study
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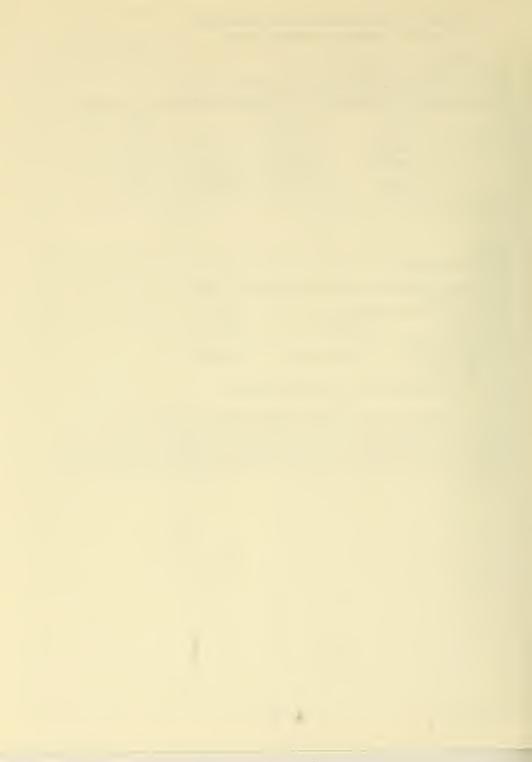


PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

					Z01CP04267	-22 BB
PERIOD COVERE	D					
		tember 30, 1987				
		. Title must fit on one line be				
Research	<u>in Mathematic</u>	<u>al Statistics an</u>	d Applie	d Mathematics	;	
		fessional personnel below the		tigator.) (Neme, title, lei	poratory, and institute affile	ietion)
P.I.	J. J. Gart	Chief,	MSAMS		BB NCI	
Others:	R. E. Tarone	Mathem	atical S	tatistician	BB NCI	
	H. M. Pettig	rew Mathem	atician		BB NCI	
	D. G. Thomas	Mathem	atical S	tatistician	BB NCI	
	J. Nam			tatistician	BB NC I	
	A. M. Smith		tician (BB NCI	
		000013	cician (ilea i cii j	DD NOI	
COOPERATING U	NITS (if eny)					
NONE						
LAB/BRANCH						
	tics Branch					
SECTION	1 0					
Mathemati	cal Statistic	and Applied Ma	thematic	s Section		
		00000				
TOTAL MAN-YEAR	Bethesda, MD	PROFESSIONAL:		OTHER:		
3.0	13.					
CHECK APPROPE	NATE BOY/ES)	3.0		0.0		
(a) Huma		(b) Human tissu	es 🕅	(c) Neither		
☐ (a1) I		(b) (/a///a// //occ		(0)		
_ ` '	nterviews					
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It is the	nurnose of th	nis project to c	anduct r	accarch in ma	thomatical	
ctatictic	nrobabilit	, and applied m	athomati.	esearch in me	ichemacicai	lan nou
statistic	o, probability	, and appried in	achellaci	cs, and espec	lally to deve	rop new
Dantinula	a i methodorog	which is appli	cable to	the blomedic	al 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.						

ALCOHOLD.



PROJECT NUMBER

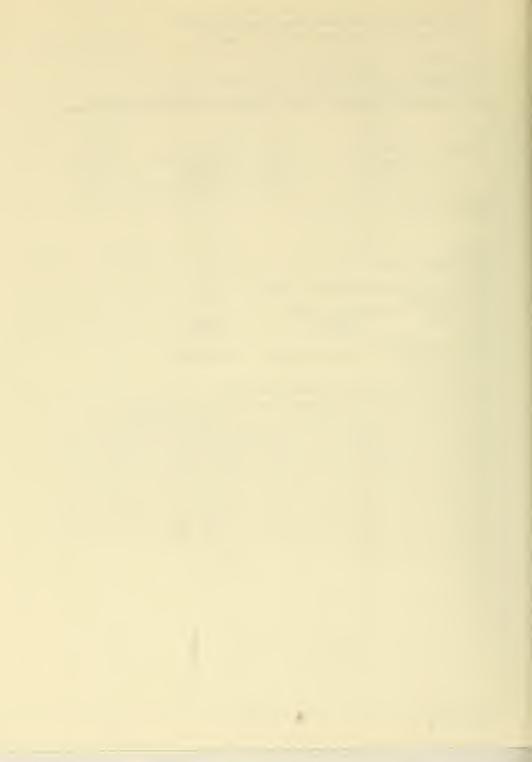
NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP04269-16 BB

								- ·
PERIOD COVER								
			September					
			Title must fit on one					
						and Development,		
PRINCIPAL INVE	STIGATOR (L	List other prof	essional personnel be	low the Principal	nvest	igator) (Nama, titla, laboratory,	and institu	ute affiliation)
P.I.:	J. Mich	nael Stu	qmp	Chief, I	RMS		ВВ	NCI
Others:	D. J. (Grauman		Computer	Svs	stems Analyst	ВВ	NCI
		Ramsbott	om	Computer			BB	NC I
	B. L. S	Stephens	son	Computer			BB	NCI
		Wolfson				grammer/Analyst	BB	NCI
						- g. a		,,,,,
NONE	UNITS (if any))						
LAB/BRANCH								
Biostatis	stics Br	ranch						
SECTION								
Informat	ion Resc	ources M	Management S	ection				
NCI, NIH		sda. Mar	vland 2089	2				
TOTAL MAN-YEA		,	PROFESSIONAL:			OTHER:		
6.0			5.0			1.0		
CHECK APPROP	RIATE BOX(E	ES)						
, ,	Minors Interviews	s	☐ (b) Human			(c) Neither		
SUMMARY OF W	ORK (Use ste	andard unredu	uced type. Do not exc	eed the space pro	videa	1.)		
						mission includes		
conducti	ng resea	arch and	developmen	t work to	imp	prove methodology	in th	ne
application of computers and data processing techniques in support of research					fresearch			
conducted and coordinated by NCI investigators and their collaborators; 2)								
serving as the focal point in the Epidemiology and Biostatistics Program for the								

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.

the contract



PROJECT NUMBER

NOTICE-OF INTRAMURAL RESEARCH PROJECT

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 personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)				
PHINCIPAL INVESTIGATION (LIST direct professional personnel below the Phincipal Investigation), Professional and Professional Company of the Phincipal Investigation, Professional Company of the Phincipal Investigation, Professional Company of the Phincipal Investigation, Professional Company of the Phincipal Investigation, Professional Company of the Phincipal Investigation, Professional Company of the Phincipal Investigation, Professional Company of the Phincipal Investigation, Professional Company of the Phincipal Investigation (Investigation) of the Phincipal Investigation (Inves				
P.I.: J. Scotto Health Services Director BB NCI				
Others: T. R. Fears Mathematical Statistician BB NCI				
COOPERATING UNITS (# 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)				
Biostatistics Branch				
Analytical Studies Section				
INSTITUTE AND LOCATION				
NCI, NIH, Bethesda, Maryland 20892				
TOTAL MAN-YEARS: PROFESSIONAL: OTHER:				
1.25				
CHECK APPROPRIATE BOX(ES) (b) Human tissues (c) Neither				
(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 chlorofluoro-				
carbons 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.



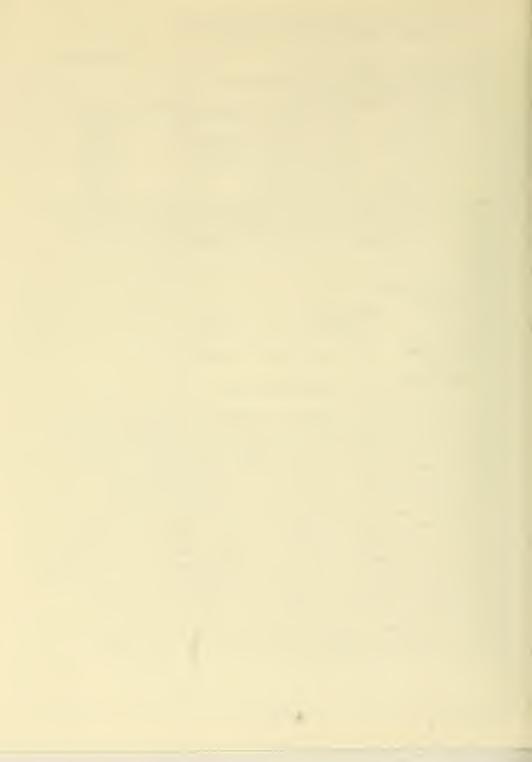
NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP04500-10 BB

PROJECT NUMBER

PERIOD COVERED							
	October 1, 1986 to September 30, 1987						
TITLE OF PROJECT (80 cherecters or less	. Title must fit on one line between the bord	ers.)					
Methodologic Studies of							
PRINCIPAL INVESTIGATOR (List other pro	fessional personnal below the Principal Inve	stigator.) (Name, title, lab	oratory, and institute effiliation)				
P.I. M.H. Gail	Medical Statistical	Medical Statistical Investigator BB NCI					
Others: J. Benichou	Guest Researcher		BB NCI				
R. Brookmeyer	Visiting Biostatistic	ian (IPA)	BB NCI				
W. Blot	Visiting Biostatistic Chief, Biostatistics	Branch	BB NC I				
T. Fears	Mathematical Statisti	cian	BB NCI				
J. Lubin	Health Statistician		BB NC I				
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), Co	ommittee on Biological E	ffects of Ion	izing Radiation of				
the National Academy of	f Sciences, Memphis Stat	e University	(Y. Tan). Chinese				
Academy of Medical Scie	ences (Y. Liu), NIEHS (C	. Weinberg)	,				
LAB/BRANCH		<u>~</u>					
Biostatistics Branch							
SECTION							
Epidemiologic Methods S	Section						
INSTITUTE AND LOCATION							
NCI, NIH, Bethesda, Mar	ryland 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

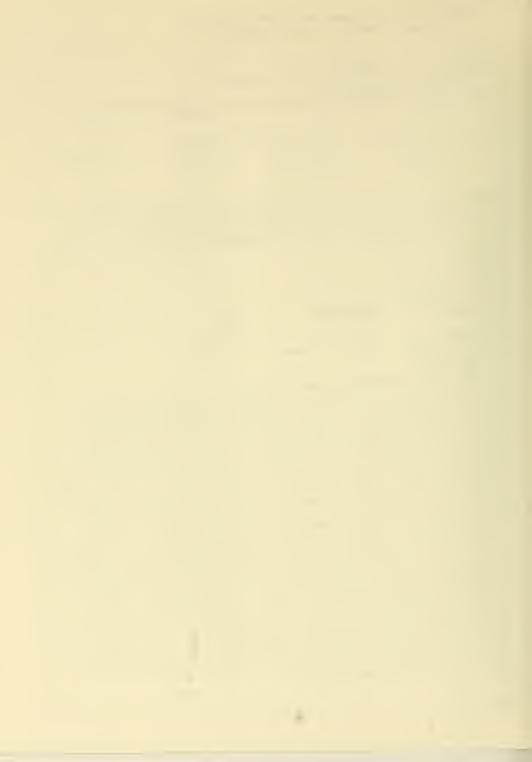
Z01CP04779-11 BB

PERIOD COVER	ED							
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TITLE OF PROJE	ECT (8	O cherecters or less	. Title mus	st fit on one line betw	een the border	s.)		
		es in High						
PRINCIPAL INVE	PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, leboretory, and institute effiliation)							
P.I.:	W.	Blot		Chief		BB	NCI	
Others:		Fraumeni,	Jr.	Associate	Director	E&B	NCI	
		Hoover		Chief		EEB	NC I	
	Τ.	Mason		Chief, PSS		EEB	NCI	
	В.	Stone		Mathematic	ian	BB	NCI	
COOPERATING	UNITS	(if eny) LA St	. Univ	v. (P. Corre	ea); Univ	′• TX (I	P. Buffler); Med. Univ.	
SC (S. S	chun	nan); NJ Dp	t. Hea	alth (A. Sto	emhagen);	Chine	se Acad. Med. Sci. (B.	
Li); Shai	ngha	ni Cancer I	nst.	(Y. Gao); Co	enter Pre	ev. Med.	. (E. Buiatti); Univ. So.	
CA. (S.	Pres	ston-Martin); Em	ory Univ. (R. Greent	erg);	CA. Hith. Dpt. (D. Austin))
LAB/BRANCH								
Biostatis	stic	s Branch						
SECTION								
Analytica	al S	Studies Sec	tion					
INSTITUTE AND	LOCA	TION						
NCI, NIH, Bethesda, Maryland 20892								
TOTAL MAN-YEA	RS:		PROFES	SIONAL:		OTHER:		
7.5			6.5			1.0		
CHECK APPROP								
🛛 (a) Hum	an s	ubjects	X (b)	Human tissue	s 🗆	(c) Neith	her	
(a1)	Mind	ors						

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

(a2) Interviews

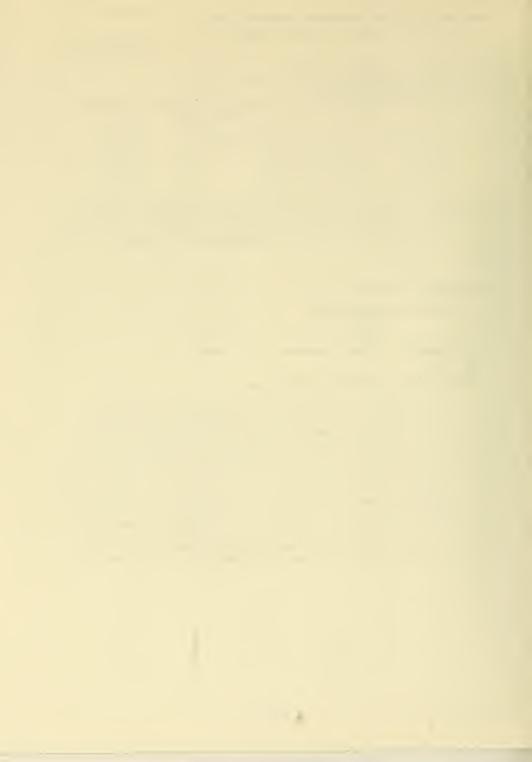
The objectives of this project are to identify and describe environmental and host determinants of cancer in areas at high risk of cancer through the use of analytical epidemiologic and biometric techniques, particularly case-control studies of specific cancers. Completed during the year were case-control 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.



PROJECT NUMBER DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT 701CP05498-02 BB PERIOD COVERED October 1, 1986 to September 30, 1987 TITLE OF PROJECT (80 cherecters or less. Title must lit 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) M.H. Gail Chief, Epidemiologic Methods Section P. I.: BR NCT R. Brookmever Visiting Biostatistician (IPA) BB NCI Others: T. Fears Mathematical Statistician BR NCT J. Lubin Health Statistician BB NCI J. Benichou Guest Researcher BB NCT S. Wacholder Senior Staff Fellow BB NCI COOPERATING UNITS (if eny) 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: 2.1 2.3 0.2 CHECK APPROPRIATE BOX(ES) (a) Human subjects (b) Human tissues X (c) Neither (a1) Minors (a2) Interviews SUMMARY OF WORK (Use stendard unreduced type. Do not exceed the spece 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 implementation 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.

MA SPECE



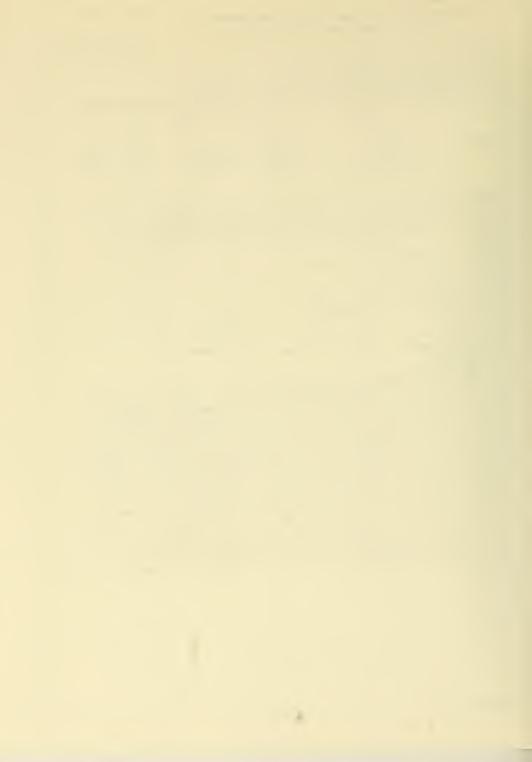
PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

				ZO1CPC	04377-16 CEB
October 1, 1986 to Se					
TITLE OF PROJECT (80 characters or Familial, Congenital,	and Genetic F	actors in N	lalignancy		
PRINCIPAL INVESTIGATOR (List other	professionel personnel bel	ow the Principal Inve	istigator.) (Name, titla, labori	atory, and inst	tute affiliation)
PI: John J.	Mulvihill	Chief, Cli	nical Genetics	CEB	NCI
Others: D. M. Pa	rry	Geneticist		CEB	NCI
P. Madig		Research 1		CEB	NC I
C.A. Col	lins	Research A	Assistant	CEB	NCI
COOPERATING UNITS (if any)					
Atomic Energy of Cana	da. Ltd. (M. P	aterson): 1	ICLA (R. Sparke	s): Biot	ech
Laboratory (S. Tsai);					
Sandberg); Brookhaven	Laboratory (R	. Setlow);	Litton Bionetic	cs (J. I	(vett)
LAB/BRANCH			·		
Clinical Epidemiology	Branch				
SECTION Clinical Genetics Sec	tion				
NCI, NIH, Bethesda, M		2			
TOTAL MAN-YEARS.	PROFESSIONAL:		OTHER:		
2.8 CHECK APPROPRIATE BOX(ES)	2.0		0.8		
(a) Human subjects (a1) Minors (a2) Interviews	☑ (b) Human		(c) Neither		
SUMMARY OF WORK (Use standard un	reduced type. Do not exce	ed the spece provid	ed.)		nou holo
Study of preneoplasti	c genetic dise	ases with a	nigh risk of t	cancer ii	ially whoo
detect environmental and genetic influences in carcinogenesis, especially when appropriate laboratory assays are used. Neurofibromatosis, an autosomal					
dominant disorder wit					
on 12 families show 1					
factor, with a lod so	ore of 4.4 at	a recombina	tion distance of	of 14 ce	entimorgans.
Forty-year follow-up	of 212 neurofi	bromatosis	patients in Der	nmark pe	rmitted
life-table analysis:					
probands, slightly be expected in the gener					

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.

. gill delivert



NOTICE OF INTRAMURAL RESEARCH PROJECT

701CP04400-22 CEB

PERIOD COVERED								
October 1, 1	986 to Septe	ember 30,	1987					
TITLE OF PROJECT (80	TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)							
Clinical Epi	demiology of	Cancer						
PRINCIPAL INVESTIGAT	TOR (List other profes.	sional personnel b	elow the Principal	Investigator) (f	Vame, title, laborat	ory, end instit	ute affiliation)	
PI:	Frederick P	P. Li	Chief,	Clinical	Studies	CEB	NCI	
Othono.	D 11 M411-	_	Obd. 6			٥٥٥	NOT	
Others:	R. W. Mille		Chief			CEB	NCI	
	J. J. Mulvi				Genetics		NC I	
	D. M. Parry	/	Genetic	ist		CEB	NCI	
COOPERATING UNITS	(if any)							
None								
LAB/BRANCH								
Clinical Epi	demiology Br	ranch						
SECTION								
Clinical Stu	dies Section	1						
INSTITUTE AND LOCAT	ION							
NCI, NIH, Be	thesda, Mary	land 20	892					
TOTAL MAN-YEARS:	P	ROFESSIONAL:		OTHER				
1.8		1.0			0.8			
CHECK APPROPRIATE								
🛛 (a) Human sı	ubjects 🗆	🖟 (b) Humar	tissues	☐ (c) N	either			
⟨a1⟩ Mino	rs							
(a2) Inter	views							
SUMMARY OF WORK (Jse standard unreduce	ed type. Do not ex	sceed tha spece p	rovided.)				

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.

in verese



PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

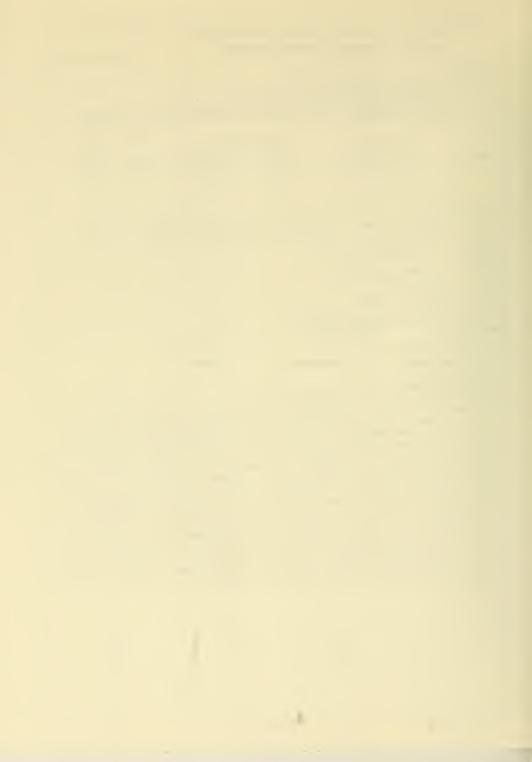
Z01CP05139-08 CEB

October 1, 1	1986 to Se	ptember 30, 19	87			
NIH Interins	stitute Me		Program: The Genetics Cl			
PRINCIPAL INVESTIGAT	TOR (List other pri	ofessional personnel below	the Principal Investigator.) (Name, title, laborato	ry, and institu	uta affiliation)	
PI:	Dilys M.	Parry	Geneticist	CEB	NC I	
Others:	J. J. Mu C. A. Co		Chief, Clinical Genetics Research Assistant	CEB CEB	NC I NC I	
	singer); ID (W. Gah	l, J. Sidbury,	-Kupfer); NIADDK (D. Camer M. Zasloff); NIDR (K. Bro			
Clinical Epi	demiology	Branch				
SECTION Clinical Gen		tion				
NCI, NIH, Be						
TOTAL MAN-YEARS: 0.80		PROFESSIONAL:	OTHER: 0.10			
(a) Human su (a) Human su (a1) Mino	ubjects rs	☑ (b) Human tis	ssues (c) Neither			

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.

: New Arthres



NOTICE OF INTRAMURAL RESEARCH PROJECT

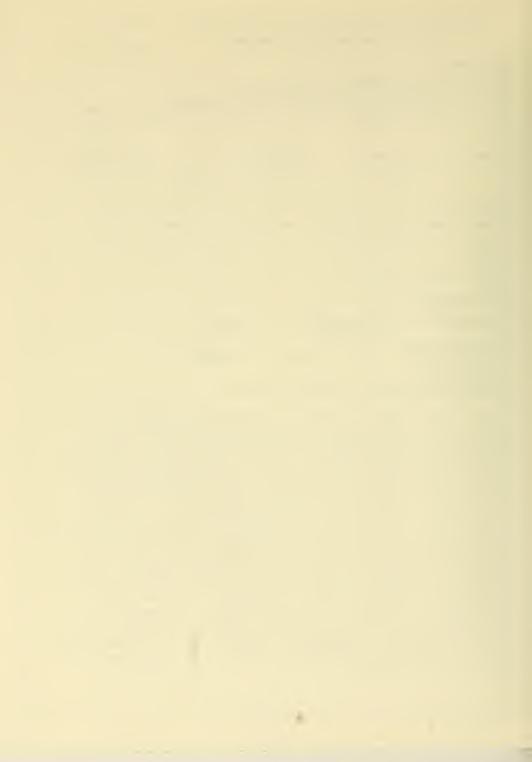
PROJECT NUMBER

Z01CP05146-08 CEB

PEHIOD COVERED				
October 1, 198	6 to September 30,	1987		
TITLE OF PROJECT (80 ch	naracters or less. Title must fit on or	e line between the borders.	.)	
Morbidity in C	hildhood Cancer Sur	vivors and The	ir Offspring	
PRINCIPAL INVESTIGATOR	R (List other professional personnel	below the Principal Investig	ator.) (Name, title, laboratory, and in:	stitute affiliation)
PI: J	ohn J. Mulvihill	Chief, Clinica	al Genetics	CEB NCI
Others: J	. M. Byrne	Epidemiologist	t	CEB NCI
R	. R. Connelly	Statistician	SORB.	DCPC NCI
	. H. Gail		ologic Methods BB,	DCE NCI
		, .,	,	
COOPERATING UNITS (if a	алу)			
NICHD (R. Sher	ins): Oueens Hospit	al. New York.	NY (F. Rosner); VA M	ledical
	t, NY (H. Zarrabi)	,	• • • • • • • • • • • • • • • • • • • •	
33.133.	., (,			
LAB/BRANCH				
Clinical Epider	miology Branch			
SECTION	33			
Clinical Genet	ics Section			
INSTITUTE AND LOCATION				
NCI. NIH. Beth	esda, Maryland 20	892		
TOTAL MAN-YEARS:	PROFESSIONAL:		OTHER:	
1.1	1.0		0.1	
CHECK APPROPRIATE BO				
(a) Human sub	jects (b) Huma	n tissues	(c) Neither	
(a1) Minors				
(a2) Intervie				

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

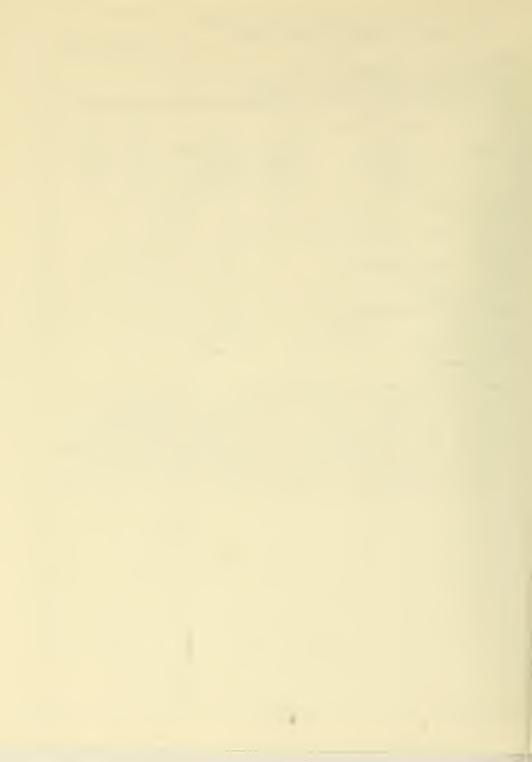
Fertility and reproductive histories of cancer patients, especially of long-term survivors of childhood and adolescent cancer, and of men and women who reproduced during cancer therapy, are studied for information on the gonadal toxicity and possible mutagenicity and teratogenicity of cancer treatment, 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 HE	EALTH AND HUMAN SER	VICES - PUBLIC HEALTH	SERVICE	ROJECT NUM	BER	
NOTICE	OF INTRAMURAL RE	SEARCH PROJECT				
**				Z01CP05	194-06 CEB	
PERIOD COVERED	t - C + 1 - C					_
TITLE OF PROJECT (80 characte	to September 30,	1987				
National Cancer	Mortality Studie	s by Computer				
PRINCIPAL INVESTIGATOR (List	other professional personnel b	selow the Principal Investigato	r) (Nema title Jahorato)	and institute	- Million 1	
	, , , , , , , , , , , , , , , , , , , ,	and the third point in today of the	r / (rreme, title, laborator	y, and institute	emiletion)	
PI: Rob	ert W. Miller	Chief		CEB	NC I	
	W. McKay	Computer Sys	tems Analyst	CEB	NCI	
	E. Tarone	Biostatistic		BB	NC I	
	Madigan	Research Ass		CEB	NCI	
J.	Byrne	Visiting Ass	ociate	CEB	NC I	
COOPERATING UNITS (if any)						
National Center	for Health Stati	stics (R. Israel)			
LAB/BRANCH						
Clinical Epidemi	ology Branch					
SECTION	orogy branch					_
Office of the Ch	ief					
INSTITUTE AND LOCATION						
NCI, NIH, Bethes	da, Maryland 20					
TOTAL MAN-YEARS:	PROFESSIONAL:	ОТН				_
1.4 CHECK APPROPRIATE BOX(ES)	1.3		0.1			
(a) Human subjects	(b) Human	tissues \(\bigcap \) (c)	Neither			
(a1) Minors	_ (=)	(0)	74011101			
(a2) Interviews						
SUMMARY OF WORK (Use stands	ard unreduced type. Do not ex-	ceed the space provided.)				
We have used info	ormation from the	e National Cente	r for Health	Statisti	cs (NCHS)	
and Bureau of the	e Census to creat	te a comprehensi	ve data base	concerni	ng	
mortality and pop	pulation informat	tion at the coun	ty level. Da	ta are a	vailable,	
1950-1981, for ca Population data	dricer mortality,	and 1965-78, To	r deaths from	other c	auses.	
available. Three	will be excended e-dimensional dra	and corrected w	hese data are	census a	ata become	4
the value of the	data collection.	. Under develop	ment are syst	ems for	manning	
counties in black	k-and-white, for	projecting cance	er mortality	in comin	a decades.	
and for grouping	counties by ecor	nomic subregions			,,	
	No. 64 act.					

1266

PHS 6040 (Rev 1/84)



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

701CP05279=05 CFR

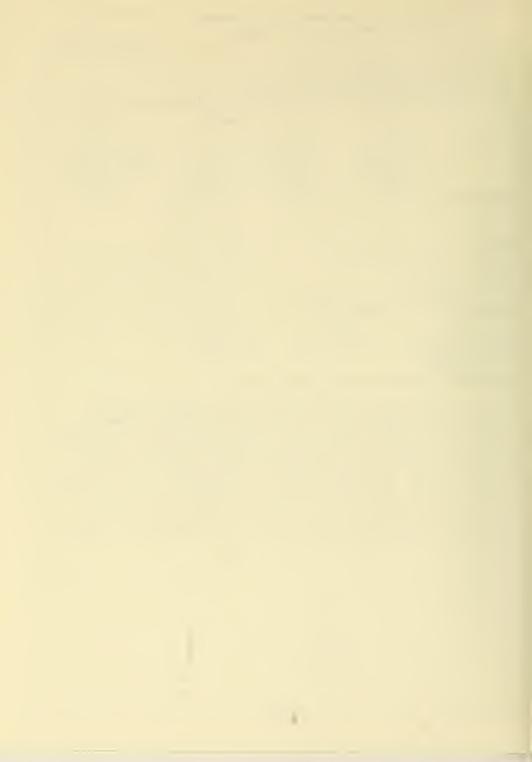
PROJECT NUMBER

October 1, 198						
Development of	racters or less Epidem	Title must lit on one	line between the borde Resources	rs.)		
PRINCIPAL INVESTIGATOR	(List other pro	fessionel personnel be	slow the Principal Invest	tigetor.) (Name, title, leboreto	ory, and institute effiliation	n)
PI:	G. W. Be	ebe :	Statistician	(Health)	CEB,	NCI
<u> </u>	R. Spirt J. D. Bo B. F. Ha T. J. Ma Z. Hrube	ice (nkey E son (Biostatistici Chief Biostatistici Chief, Popula Expert	an	REB, EBP, SORB, DCPC, EEB, REB,	NC I NC I NC I
COOPERATING UNITS (if en			-ybei c		KLD,	1101
None	,,					
Clinical Epide	emiology	Branch				
Office of the	Chief					
NCI, NIH, Beth	nesda, M		0892			
TOTAL MAN-YEARS:		PROFESSIONAL: 0.2		OTHER: 0.5		
CHECK APPROPRIATE BOX (a) Human subje (a1) Minors (a2) Interview	ects	☐ (b) Human	tissues 🗵	(c) Neither		

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.

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PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

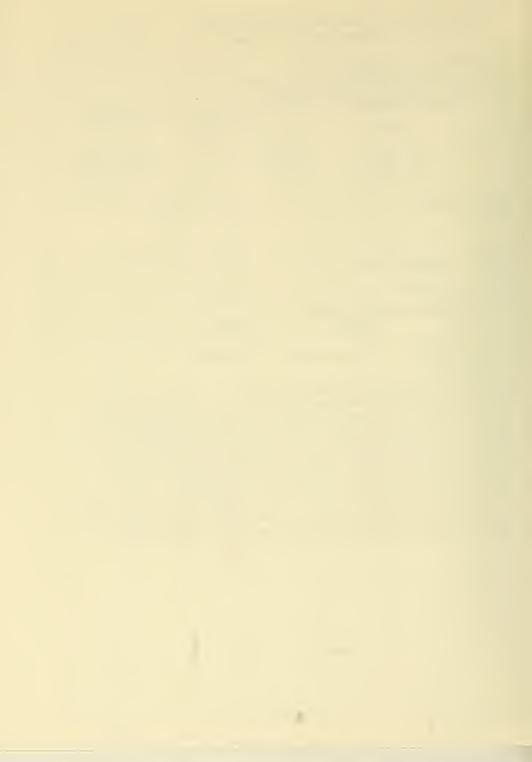
701CP05280-05 CFR

PERIOD COVERED									
	086	t o	September 30	1007					
				·					
Carcinogonic			rless. Title must fit on S of Ionizin		orders.)				
				-					
PRINCIPAL INVESTIGAT	TOR (Li	st oth	er professional personn	al below the Principal Ir	vestigator) (Name, title, lab	oretory, and inst	itute effiliation)	
PI:	G.	W.	Beebe	Statist	ician	(Health)	CEB	NC I	
Others:	C.	Ε.	Land	Statist	ician		RFB.	EBP, NCI	
			Boice	Chief			•	EBP, NCI	
			Wachholz	Chief				PCP, NCI	
	•			011161			KED,	,, ,, ,,	
COOPERATING UNITS	if any)								
None	,,								
110110									
LAB/BRANCH									
Clinical Epi	demi	010	gy Branch						
SECTION			55						
Office of th	e Ch	ief							
NSTITUTE AND LOCAT	ION								
NCI, NIH, Be	thes	da,	Maryland	20892					
TOTAL MAN-YEARS:			PROFESSIONA	L:	ОТН	IER:			
0.8			0.3			0.5			
CHECK APPROPRIATE	BOX(ES	5)							
🗀 (a) Human su	ubject	S	(b) Hum	an tissues	☑ (c)	Neither			
(a1) Minor	rs								
(a2) Interv	views								

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.

with out sports



PROJECT NUMBER

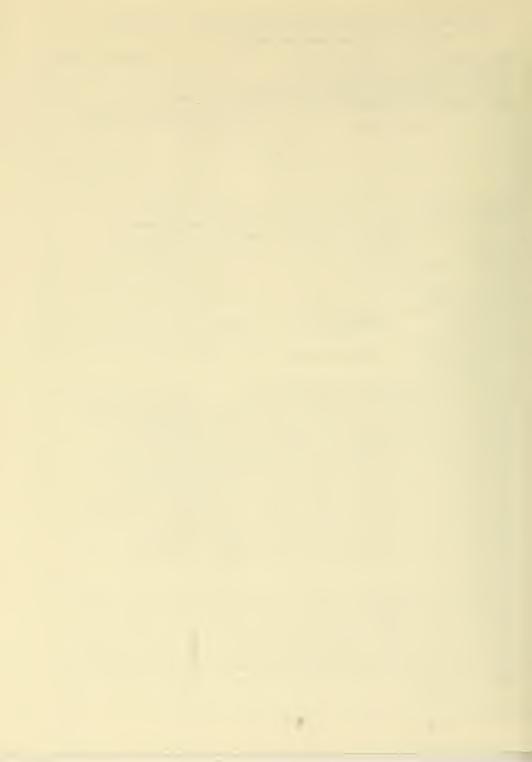
NOTICE OF INTRAMURAL RESEARCH PROJECT

701CD05320 04 CER

PERIOD COVERED October 1, 1986 to Sep			
TITLE OF PROJECT (80 characters or less. Hepatitis B Virus and	Liver Cancer in Army	Veterans of WWII	
PRINCIPAL INVESTIGATOR (List other pro-	essional personnel below the Principal	nvastigator.) (Nama, titla, labora	atory, and institute affiliation)
PI: Gilbert W	V. Beebe Statist	ician (Health)	CEB NCI
COOPERATING UNITS (if any)			
Medical Follow-up Agen	icy, National Research	Council, NAS (J.	. Norman);
Veterans Administration DIR, NIDDK (J. Hoofnag	n, Six Hospitals (L. Jle)	Seeff); Liver Dis	seases Section,
LAB/BRANCH Clinical Epidemiology	Branch		
SECTION			
Office of the Chief			
NSTITUTE AND LOCATION NCI, NIH, Bethesda, Ma	ryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:	
0.4	0.3	0.1	
CHECK APPROPRIATE BOX(ES)		_	
<u></u>	(b) Human tissues	(c) Neither	
(a1) Minors			
(a2) Interviews SUMMARY OF WORK (Use standard unred	0		
The study is based on	**		honatitic in the
The study is based on	the epidemic of 50,00	Cases of Viral	nepacicis 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 vellow 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 (HBsAq+) was identified in Group I, none in Group II or III. The mortality study reveals no excess mortality from circhosis 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.



PROJECT NUMBER

0.25

NOTICE OF INTRAMURAL RESEARCH PROJECT

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) NCI PI: T. Mason Chief, PSS L. Pickle Health Statistician **EEB** NCI Others: EEB NC.T N. Dalager Epidemiologist NCI R. Falk Health Statistician **EFB** NCI B. Stephenson Computer Specialist BB R. Ramshottom 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

3.25

CHECK APPROPRIATE BOX(ES)

(a) Human subjects

(a) Hullian Subjects

(a1) Minors
(a2) Interviews

(a2) Interviews

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

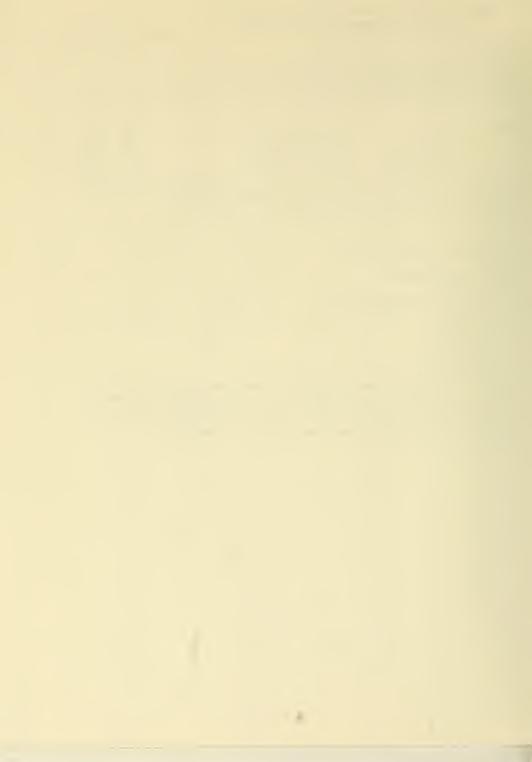
(b) Human tissues

- NET-THE PROPER

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.

OTHER

X (c) Neither



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

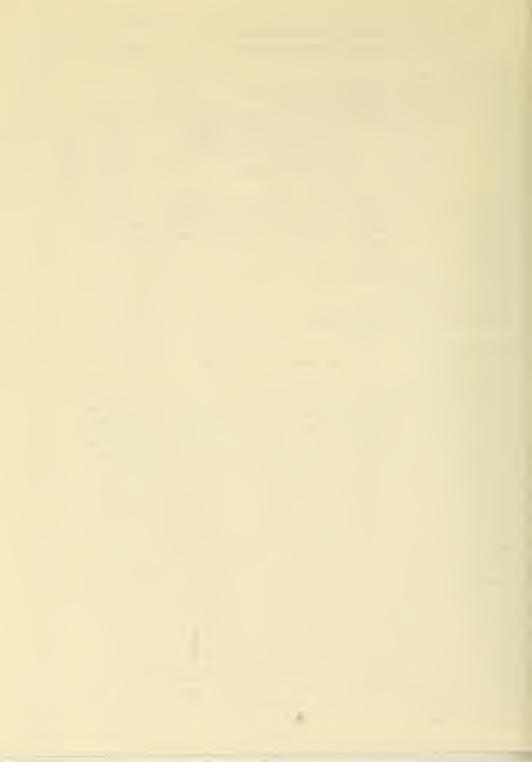
PROJECT NUMBER

Z01CP04410-11 EEB

October 1, 1986 to Se	eptember 30, 1987						
TITLE OF PROJECT (80 characters or less	TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)						
Studies of Persons at							
	fessional personnel below the Principal Investigator) (Name, title, laboratory, and institute	affiliation)					
PI: M.A. Tucke	r Coordinator of Family Studies	EEB	NCI				
Others: W.A. Blatt	ner Chief, Family Studies Section	EEB	NCI				
D.L. Mann	Chief, Biochemical Epidemiology Section	LHC	NCI				
S.J. Bale	Staff Fellow	EEB	NCI				
N. Caporas	o Medical Staff Fellow	EEB	NCI				
Y. Liu		EEB	NCI				
R.C. Young		MB	NCI				
		CEB	NCI				
Biotech (S. VedBrat); Cahill); CSG/ORI (K. B	ogical Research Faculty & Facility (T. Shimada); Biotech Laboratories (A. Bodner); Westat, Inc. oyd/D. Switalski)	, Brate (J.	on				
Environmental Epidem	iology Branch						
SECTION Family Studies Section	n						
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892							
TOTAL MAN-YEARS	PROFESSIONAL OTHER						
7.5	6.2						
CHECK APPROPRIATE BOX(ES)							
(a) Human subjects	☐ (b) Human tissues ☐ (c) Neither						

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.

(a1) Minors (a2) Interviews



PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

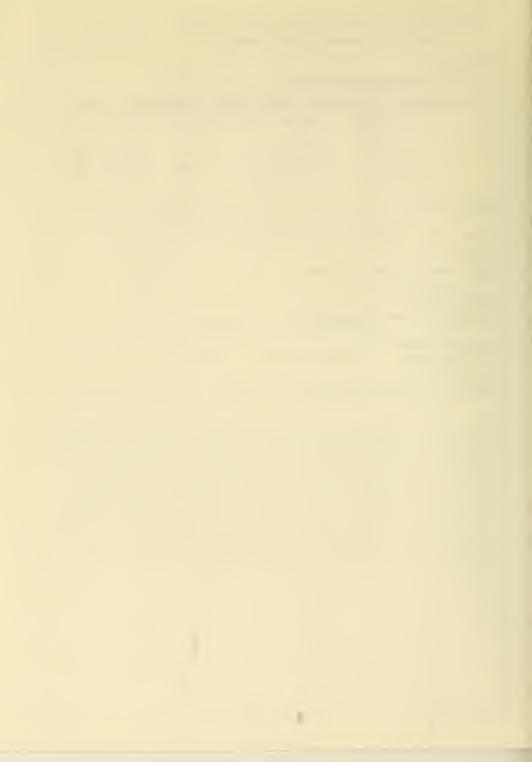
NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP04411-11 EEB

PERIOD COVERED								
	October 1, 1986 to September 30, 1987							
The state of the s		on one line between the borders.)						
Cancer and	Related Conditions	in Domestic Animal	s: Epidemi	ologic Comp	arisons			
PRINCIPAL INVESTIGA	ATOR (List other professional pers	onnel below the Principal Investigator	r.) (Name, title, labora	tory, and institute at	filiation)			
PI:	H. M. Hayes	Veterinary Medical	Officer	EEB	NC I			
0.4	B			EED	NO T			
Others:	R. N. Hoover			EEB	NC I			
	L. W. Pickle	Statistician			NCI			
		Veterinary Medical	Officer	OD, DCE	NCI			
	K. P. Cantor	Epidemiologist		EEB	NCI			
								
COOPERATING UNITS								
		. Anatomy, Ohio Sta	ite Univ. (G	.P. Wilson	, J. Burt);			
Dept. of Me	ed., Cornell Univ.	(B. Tennant)						
LAB/BRANCH								
Environment	tal Epidemiology Bu	ranch						
SECTION								
	tal Studies Section	1						
INSTITUTE AND LOCA								
	Bethesda, Maryland							
TOTAL MAN-YEARS:			IER:					
1.3		.2	0.1					
CHECK APPROPRIATE	BOX(ES)	uman tissues (c)	N. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1.					
		uman tissues \square (c)	Neitner					
(a1) Mind								
(a2) Inter	rviews							

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.



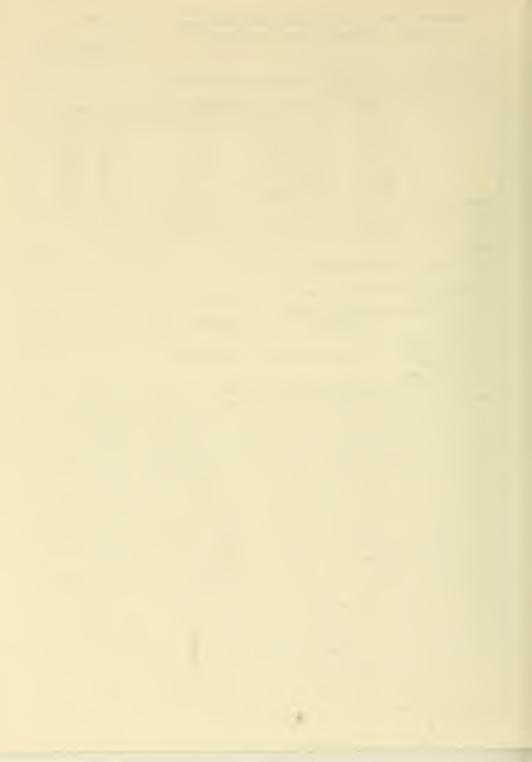
PROJECT NUMBER

I M LIVI OI	HEALTH AND HO	MINIT DESTRICES - 1 OBI	CIO HEALIN SERVICE
NOTIC	E OF INTRAMI	JRAL RESEARCH	PROJECT

701CP04480-11 FFB

PERIOD COVERED							
October 1, 1987 to Sep							
	. Title must fit on one line between the border	3.)					
Studies of Occupationa							
	fessional personnel below the Principal Investi		institute affiliation)				
PI: A. Blair	Chief, Occupational	l Studies Section	EEB NCI				
OTHERS: M. Alavan			E&BP NCI				
K. Cantor	-F		EEB NCI				
M. Doseme			EEB NCI				
R. Hayes	Epidemiologist		EEB NCI				
B. Miller			EEB NCI				
R. Spirta			EEB NCI				
P. Stewar T. Thomas S. Zahm	t Industrial Hygieni Epidemiologist Epidemiologist	st	EEB NSI				
	Epidemiölögist		EEB NCI				
COOPERATING UNITS (# any)	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	11-3\- 11 C C	+ Cuand (T				
Univ. of NE (D. Weisen	berger); Univ. of KS (F.	Holmes); U.S. Coas	t Guara (1.				
Haas); USDA (J. Teske)	; U.S. Air Force (S. Bird	ch); NIOSH (H. Aman	dus, W.				
	ontrol Program (J. Davis)					
LAB/BRANCH							
Environmental Epidemio	logy Branch						
SECTION		_					
Occupational Studies S	ection						
INSTITUTE AND LOCATION							
NCI, NIH, Bethesda, Ma	ryland 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 axceed the space provided.)							
The Occupational Studies Section supports and conducts epidemiologic studies of							
occupational groups to	identify and clarify the	e role factors in t	he workplace				
play in the origin of	cancer. During the past	vear several studi	es were				
pray in the origin or	ake among workers expose	d to posticides A	mortality				
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							
i cancer were noted amon	ig grain workers, particu	iarly those from gr	atti milis 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. PHS 6040 (Rev 1/84)



PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01CP05128-08 EEB

PERIOD COVERED							
October 1, 1986 to Se							
TITLE OF PROJECT (80 cherecters or less			rs.)				
Diet and Nutrition in	Cancer Etiol	logy					
PRINCIPAL INVESTIGATOR (List other pro-		w the Principal Invest	rigetor.) (Name, title, labor	etory, and institut	e effilletion)		
PI: R. G. Ziegler		Cancer Exper	`t	EEB	NC I		
Others: K. E. Brock	1	Visiting Fel		EEB	NCI		
M. H. Schiffm	ian (Clinical Inv	restigator	EEB	NC I		
L. A. Brinton	i (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. Fraumer	ii, Jr. A	Associate Di	rector	E&B	NCI		
COOPERATING UNITS (if any) NO					Austin); Uni		
of HI (A Nomura); USC (B Henderson); Kaiser Hith Plans (A Glass); Walter Reed							
Army Med Ctr (G Quisp							
Res Cen (S Schwartz);							
LAB/BRANCH							
Environmental Epidemi	ology Branch						
SECTION							
Enviromental Studies Section							
INSTITUTE AND LOCATION							
NCI, NIH, Bethesda, M	larvland 20892	2					
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.

PHS 6040 (Rev 1/84)



NOTICE OF INTRAMURAL RESEARCH PROJECT

OTHER:

0.0

(c) Neither

PROJECT NUMBER

Z01CP05319-04 EEB

October	Paul H. Levine Senior Investigator EEB NCI S: D. V. Ablashi Microbiologist LCMB NCI R. W. Biggar Senior Investigator EEB NCI R. Gallo Chief, FSS EEB NCI M. Robert-Guroff Senior Investigator LTCB NCI J. Salahuddin Microbiologist Microbiologist						
TITLE OF PROJE	ECT (80 characters or less. Title must fit of	on one line between the borders.)					
Epidemiologic Studies on Viruses and Genetics in the Etiology of Cancer							
PRINCIPAL INVE	STIGATOR (List other professional person	nnel below the Principal Investigator) (Name, to	tie, leboretory,	and institute effiliation)			
		Senior Investigator	EEB	NCI			
Others:	D. V. Ablashi	Microbiologist	LCMB	NC I			
	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	NC I			
	Z. Salahuddin	Microbiologist	LTCB	NCI			
	W. C. Saxinger	Senior Investigator	LTCB	NCI			
COOPERATING	UNITS (if any)			_			
Univ. of	Ghana, Accra, Ghana (J. Neeguaye, F. Nkrumah);	Gorgas	Memorial			
Chapel H	ill, N.C. (N. Raab-Trad	ub and J. Pagano)					
LAB/BRANCH							
Environme	ental Epidemiology Brai	nch					
SECTION							
Office of	f the Chief						
INSTITUTE AND	LOCATION						

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

PROFESSIONAL:

0.7

(b) Human tissues

NCI, NIH, Bethesda, Maryland 20892

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 antihodies 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.

BEBIOD COVERED

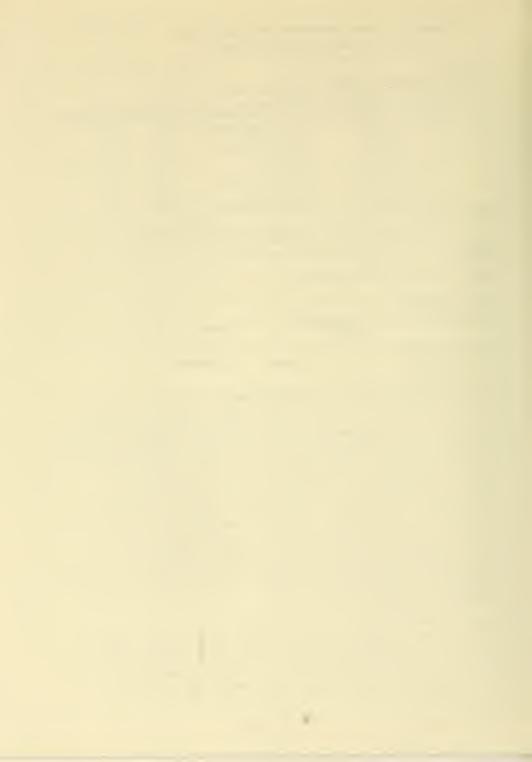
TOTAL MAN-YEARS:

0.7

CHECK APPROPRIATE BOX(ES)

(a) Human subjects

(a1) Minors
(a2) Interviews



PROJECT NUMBER

Z01CP05400-04 EEB

NOTICE OF INTRAMURAL RESEARCH PROJECT

ED							
	0-4-6	1000		20	1007		

October I, 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) W.A. Blattner R.J. Biggar Chief, Family Studies Section Senior Investigator Others: J.J. Goedert S.H. Weiss E. Murphy Coordinator, AIDS Working Group Medical Staff Fellow Medical Staff Fellow EEB Chief, Metabolic Epidemiology Section Chief D.L. Mann R.C. Gallo A. Manns LHC TCB EEB Biotechnology Fellow P.H. Levine Senior Investigator FFR NCT G. Agius Guest Researcher **FFB** 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

PERIOD COVER

Family Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892 TOTAL MAN-YEARS

PROFESSIONAL

8.0 7.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (a1) Minors

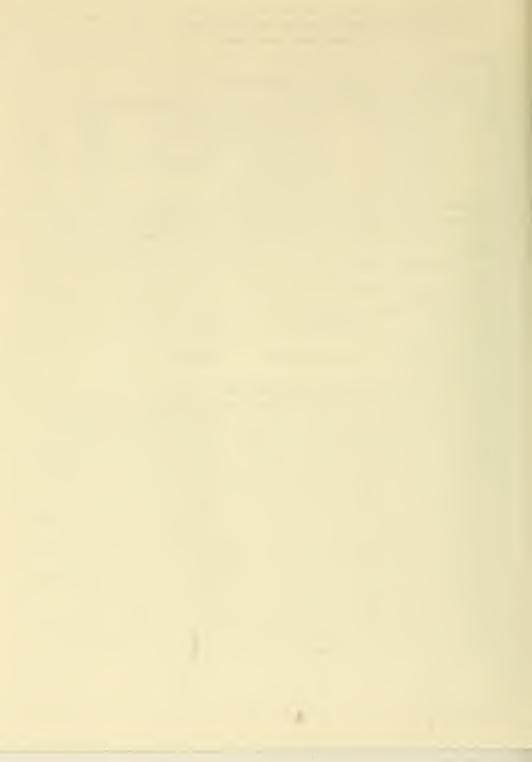
(c) Neither

1.0

OTHER

(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. 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

PROJECT NUMBER

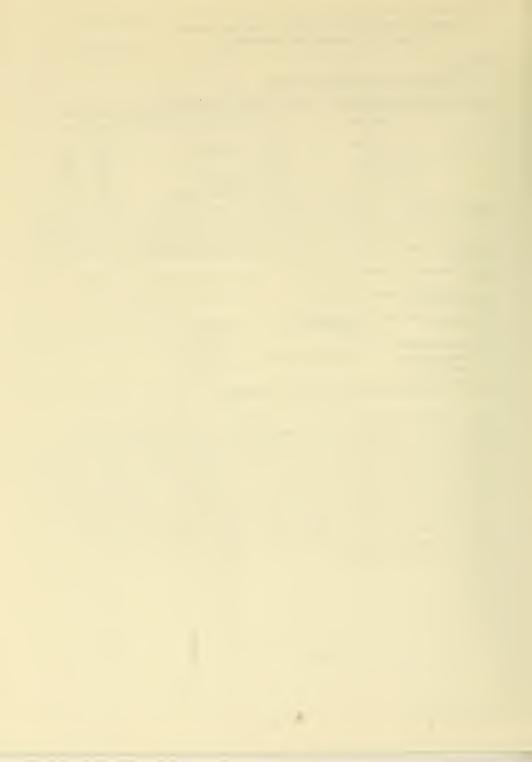
NOTICE OF INTRAMORAL RESEARCH PROJECT					2010	ZUICPU5526-01 EEB			
PERIOD COVERED									
October 1, 1986 to September 30, 1987									
TITLE OF PROJECT (80 characters or less. Title must lit on one line between the borders.)									
Analytical Investigations of Selected Issues in Human Cancer									
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, leboratory, and institute effiliation)									
PI:	L. A. Brint	on	Chief, E	nvironmental	Studies	EEB	NCI		
Others:	J. F. Fraum	neni, Jr.	Associate	Director		E&B	NC I		
	R. N. Hoove		Chief			EEB	NCI		
	K. P. Canto	r	Epidemio'	ogist		EEB	NCI		
	P. Hartge		Epidemio'	ogist		EEB	NCI		
	M. H. Schif	fman		Investigato	r	EEB	NC I		
COOPERATING UNITS (# 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 SFFR 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:									
TOTAL MÁN-YEARS	š: , , , , , , , , , , , , , , , , , , ,	PROFESSIONAL:	_	OTHER:					
8.0		6.0		2.0					
CHECK APPROPRIATE BOX(ES)									
(a) Humar		(b) Human t	issues	(c) Neither					

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

(a2) Interviews

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.

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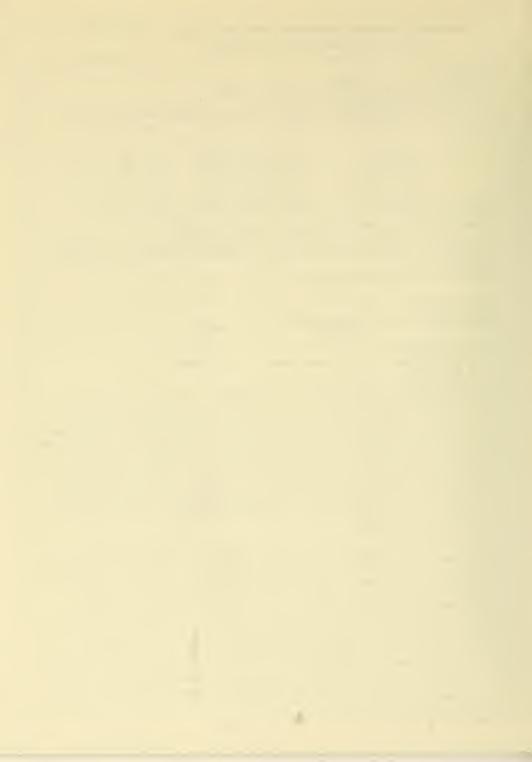


DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

			Z01CP04481-11 REB					
PERIOD COVERED								
October 1, 1986 to Se	ptember 30, 198/							
TITLE OF PROJECT (80 cherecters or less Studies of Radiation-		ders.)						
PRINCIPAL INVESTIGATOR (List other pro	fessionel personnel below the Principal Inv							
PI: J. D. Boice,	Jr. Chief	KER	NC I					
Others: C. E. Land	No. 14b Chahle							
The state of the s	Health Statis		NCI					
G. W. Beebe	Health Statis		NCI					
Z. Hrubec	Expert Statis		NCI					
R. A. Kleine			NC I					
E. Ron	Visiting Asso		NCI					
M. Blettner	Expert Statis	tician REB	NCI					
	and Francisco	/11 1/ 1						
Radiation Effects Res	earch Foundation, Japan	(H. Kato);						
Department of Energy	(R. Goldsmith); Chaim S	heba Medical Cen	ter, Israel					
LAB/BRANCH	versity (M. Kaplan); Ha	rvard University	(G. Hutchison)					
	, Danah							
Radiation Epidemiolog	/ Branch							
SECTION								
INSTITUTE AND LOCATION								
NCI, NIH, Bethesda, M	aryland 20902							
TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:						
11.0	8.0	3.0						
CHECK APPROPRIATE BOX(ES)	0.0	3.0						
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither								
(a1) Minors	` '	, ,						
(a2) Interviews								
SUMMARY OF WORK (Use standard unred	uced type. Do not exceed the space provide	ded)						
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 resonnse								
radiation quality, fractionation of dose, time since exposure, sex, age at expo-								
sure and at observation, and possible modifying influences of other environ-								
mental and host factors; and (3) examines, tests, and formulates models of radi-								
ation 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 sug	inest that (1) suscepti	hility to madica	onic broact cancer					
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								
(3) repeated exposure	to relatively low radia	acton doses pose	s some ruture 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.



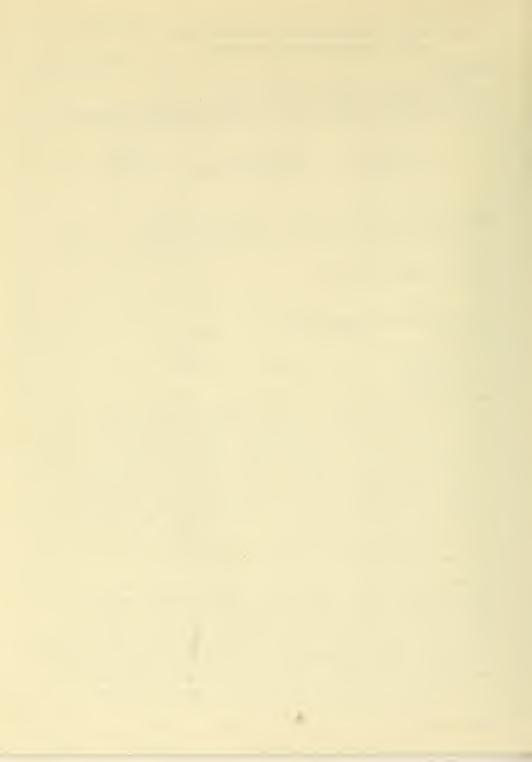
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

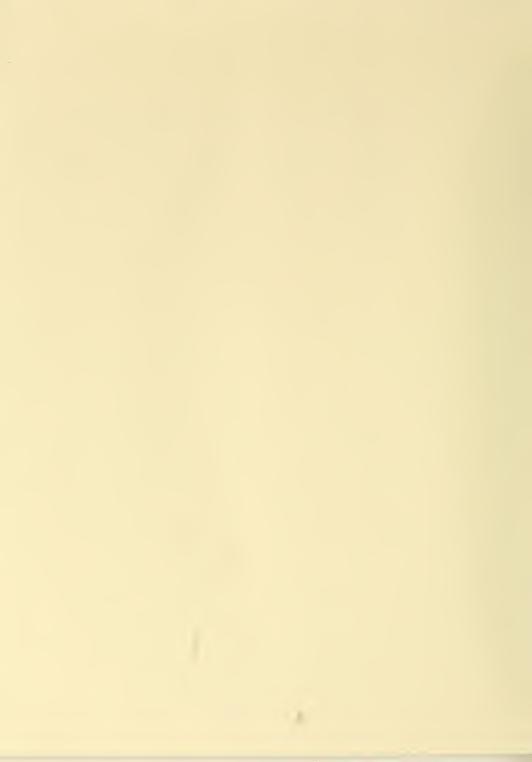
NOTICE OF INT	Z01CP05368-04 REB							
PERIOD COVERED October 1, 1986 to September 30, 1987								
TITLE OF PROJECT (80 characters or lass Titla must lit on one line between the borders.) Studies of Drug-Induced Cancer and Multiple Primary Cancers								
PRINCIPAL INVESTIGATOR (List other professional personnal below the Principal Investigator.) (Name, titla, leboratory, and institute affiliation)								
PI: J. D. Boice,	Jr. Ch	hief	RE	EB NCI				
Others: R. E. Curtis		tatistician	RE	B NCI				
R. A. Kleine	rman Ep	pidemiologist	RE	B NCI				
		linical Invest	igator EE	B NCI				
		·						
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								
NCI, NIH, Bethesda, Maryland 20892								
TOTAL MAN-YEARS.	PROFESSIONAL:		OTHER:					
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 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. ZO1CPO4412-11 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.











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