9102-184

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U.S. DEPARTMENT OF ENERGY
UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM

COVER PAGE

(THIS PAGE MUST BE THE FIRST PAGE OF THE APPLICATION)

1. Name of Institution: The Johns Hopkins University School of Hygiene & Public Health
2. Department: Biochemistry
3. Street: 615 North Wolfe Street 4. City: Baltimore
5. County: N/A 6. State: MD 7. ZIP: 21205 8. Congressional District: 7th
9. Telephone: Area Code 301 Office: 955-3949 Home: [redacted]
10. Title of Application: Molecular Densitometry and Imaging Facility
11. Area of Proposed Research (Select ONE) Biomedical/Environmental*

    A. Biomedical/Environmental
    B. Chemical/Coal Science
    C. Geosciences
    D. Materials Research
    E. Plant Science/Microbiology
    F. Other

12. Research Subcategory (See Section II of DOE/URI FY 91 Announcement) Life Science Studies
13. Total DOE Funding for Research in Selected Area (During the last two fiscal years): $ 1,009,993.00
14. Estimated Purchase Price of Equipment: $ 198,000 15. Amount requested from DOE: $ 178,000

List all Federal agencies which are currently considering proposals from the institution involving the same or similar equipment.

16. Agency: none Agency Proposal Number:
17. Agency: Agency Proposal Number:

NOTE: The institution is responsible for immediately informing the URI program manager in writing if a proposal involving similar or related equipment is submitted to a federal agency prior to the announcement of DOE's URI awards.

18. List and federal agency which has provided funds to the institution during the past two years for the same or similar equipment.

    Agency: none Amount of Funds:

19. Please check one of the following:
    - ☒ I authorize outside peer review of this proposal.
    - ☐ I do not authorize peer review of this proposal.**

Signature of Principal Investigator: Date: December 7, 1990

Name and Title of Institutional Official (President or Designee)
Alan M. Goldberg, Ph.D.
Assoc. Dean for Research

Signature:
Date: 12/07/90
Area Code/Telephone: (301) 955-3343

20. Is Applicant Delinquent on any Federal Debt? ☐ Yes (If, "Yes," attach an explanation) ☒ No

* Note – The application will be evaluated by reviewers in this field.
** Note – May prevent full consideration of this application.

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BOX No. H-182-17 Bldg. 2774-H
FOLDER US/DOE Univ. Research Inst. Program 9102-184
REPOSITORY Oak Ridge Operations
COLLECTION Records Holding Area Documents 1994-1994

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SUMMARY

Nearly every aspect of modern research programs studying DNA damage and repair, or mapping and sequencing of the human genome, or the structure and function of biological macromolecules involves the use of gel electrophoresis techniques, since electrophoretic procedures provide by far the most proficient means for resolving fundamentally similar but distinct macromolecules (i.e., proteins, DNA and RNA) from one another. Quantitative analysis of the kinetics of interactions between such macromolecules or reactions involving macromolecular substrates or products often requires the use of electrophoretic techniques. Historically, quantitation of bands produced by one-dimensional (1D) electrophoresis has been notoriously inaccurate. Errors of 50-100% or more in measurements are not uncommon with traditional 1D-densitometers. Much more accurate data can be obtained by excising the relevant bands by hand and measuring the amount of material in a spectrophotometer or, if the material is radioactively labeled, in a liquid scintillation counter. This approach, however, is extremely laborious and is only used if the number of samples is very limited. Recent technical advances in the capabilities of laser densitometers, storage phosphor technology and computing software now make it possible to conduct accurate two-dimensional quantitation of all data contained on entire gels within a few minutes. The availability of instruments that can rapidly and reliably quantitate electrophoretic data will permit our group of investigators to initiate a wide range of experimental approaches that simply are not feasible with our current equipment.

We are requesting funds for a Molecular Dynamics 400E PhosphorImager and a Molecular Dynamics 300E Computing Densitometer and associated computing and graphics equipment and software. The PhosphorImager provides high-resolution, 2D, direct autoradiographic analysis of radioactive samples that have been resolved by electrophoresis or thin layer chromatography. The sample gel or plate is exposed to storage phosphor screens, which capture the energy of strong β particles (e.g., from ^{32}P, ^{35}S, ^{14}C) or gamma rays (e.g., ^{125}I) with nearly 100% efficiency. The trapped energy is stable until the screen is scanned with a laser beam, which causes the stored energy to be released as blue light. In addition to its capacity to quantify data two dimensionally, the PhosphorImager has several advantages over conventional film autoradiography. The storage phosphor screens have a dynamic range 400 times that of x-ray film, so that both highly radioactive and weakly radioactive samples in the same experiment simultaneously provide a linear response. Furthermore, the higher efficiency of the PhosphorImager means we can greatly increase our productivity, since exposure times can be reduced up to 10-fold, and we can quantify samples containing 100-fold less radioactivity than detectable by film. The Computing Densitometer provides rapid, high-resolution, 2D densitometry of stained, nonradioactive gels or membranes (wet or dry) or x-ray fluorographs of ^{3}H-labeled samples. The entire image of a large 35 x 43 cm gel can be scanned in less than 3 minutes. The ImageQuant software supplied with these machines permits rapid and highly accurate integration of individual bands, whole lanes, or even irregularly shaped objects.

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The research programs described in this application constitute biomedical research, principally in the life sciences area, and focus on studies of (i) DNA damage and repair, (ii) mapping and sequencing of the human genome, and (iii) structure and function of biological macromolecules. Several of the research programs are recognized internationally as being right at the forefront of their respective areas. More specifically, the research programs include: (1) development of 2-D electrophoretic methods for the mapping of large (200 kilobase) human DNA fragments; (2) studies of the molecular mechanisms involved in the initiation, propagation and regulation of chromosomal DNA replication *in vitro*; (3) mechanistic studies of excision repair of damaged nucleotides from duplex DNA; (4) an examination of the role of ATP hydrolysis in bringing about changes in the structure of DNA during pairing and unpairing reactions carried out during the repair of damaged DNA; (5) the design of oligonucleotide analogues which can control gene expression in cultured cells at the mRNA or DNA level; (6) studies of DNA recognition and conformational rearrangements involved in site-specific recombination *in vitro*; (7) studies of the biochemical mechanisms involved in the somatic mutation of antibody variable genes during the development of high-affinity antibodies; and (8) development of rapid DNA sequencing technologies and studies of the crystal structures of restriction enzymes bound to their DNA target recognition sequences.

Acquisition of the PhosphorImager will allow several of our research groups to conduct quantitative kinetic studies of the rates and efficiencies of numerous protein-DNA interactions, of stages of DNA repair, of steps involved in DNA rearrangement and recombination, and of initiation of DNA replication fork propagation. Such analyses are critical if we are to elucidate the fundamental molecular mechanisms that underlie these key metabolic processes. These studies are only made possible by the fact that many of the individual investigators have reconstituted purified protein systems that support the aforementioned complex DNA transactions *in vitro*. The PhosphorImager will also permit us to determine thermodynamic binding isotherms for proteins that recognize regions of damaged DNA, or a series of repeating sequences in chromosomal replication origins, or sequences involved in site-specific recombination. Likewise, this instrument will permit us to measure the affinities of oligonucleotide analogues for RNA and for specific segments of duplex DNA. The Computing Densitometer will be used to quantify the purity of numerous protein preparations, the levels of expression of cloned genes, protein-protein interactions involved in the initiation of chromosomal DNA replication, protein-DNA binding stoichiometries, and 2D patterns of hundreds of restriction fragments during human genome mapping. These instruments are absolutely critical if these various research programs are to progress to the next level of mechanistic understanding of the structure and behavior of the various biological macromolecules being studied.

Approximately 20 graduate students have graduated in the past 5 years from the laboratories compiling this application. It is estimated that acquisition of the PhosphorImager and Computing Densitometer will aid the research of roughly 40 currently enrolled graduate students.

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U.S. DEPARTMENT OF ENERGY
UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM

BUDGET PAGE

ESTIMATED COSTS

Instrumentation	Requested of DOE	Institution's Cost Sharing *(1)*	Other Federal Funds *(2)*	TOTAL
A. Purchase Price *(3)*	$167,250	$10,000	$5,000	$182,250
Maintenance *(4)*	$ 10,750	X X X X X	X X X X X	$ 10,750
Subtotal:	$178,000	$10,000	$5,000	$193,000
B. Other Allowable Costs				
1. Shipping/Handling	X X X X X	$ 500	$1,000	$ 1,500
2. Building/Laboratory	X X X X X	$ 3,200	$ 300	$ 3,500
Subtotal:		$ 3,700	$1,300	$ 5,000
C. TOTAL	$178,000	$13,700 *(5)*	$6,300	$198,000

NOTES:

(1) Non-Federal funds only. (However, may be provided by a third party.)

(2) Estimate funds to be obtained from other Federal agencies for purchasing the instrument, etc.

(3) Only the purchase price of the instrumentation is eligible for DOE funding through this program.

(4) See discussion of eligible maintenance costs on page 11.

(5) Only those costs specified above are eligible as cost sharing. Installation, operation, maintenance, travel and training costs, or faculty and student salaries, etc. are ineligible as cost sharing. Review discussion of eligible and ineligible costs on pages 10 and 11.

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A. **Purchase Price (List components and unit prices.)**

Description/Vendor	Quantity	Total Estimated Unit Price	Requested of DOE	Institution's Cost Sharing	Total
Molecular Dynamics Model 400E PhosphorImager (includes reader module, eraser module, and data system with 80386 processor, data storage, keyboard, Microsoft Windows, Image Quant software and color monitor)	1	$85,000	$76,500	$8,500	$85,000
Molecular Dynamics Large Phosphor Screen Starter Kit (35 cm x 43 cm screens)	2	$11,000	$21,800	$ 200	$22,000
Molecular Dynamics Small Phosphor Screen Starter Kit (20 cm x 25 cm screens)	2	$ 4,850	$ 9,600	$ 100	$ 9,700
Molecular Dynamics Grey Scale Printing	1	$ 7,900	$ 7,825	$ 75	$ 7,900
Molecular Dynamics Advanced Graphics System	1	$ 5,250	$ 5,200	$ 50	$ 5,250
Molecular Dynamics Ethernet Interface	1	$ 900	$ 900	0	$ 900
Microsoft Excel software	1	$ 575	$ 500	$ 75	$ 575
Molecular Dynamics 300E Computing Densitometer (with Laser scanning module and densitometry data system complete with 80386 processor, data storage keyboard, Image Quant programming, color monitor, MS-DOS, and Microsoft Windows)	1	$48,825	$47,925	$ 900	$48,825
NEC P5200 Color Printer and Interfacing	1	$ 2,100	$ 2,000	$ 100	$ 2,100
Subtotal:		$166,400	$172,250	$10,000	$182,250

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OMB CONTROL NO. 1910-1400

DEPARTMENT OF ENERGY
UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM
GRANT AND CONTRACT SUMMARY FORM

Institution Name:

Current Grant/Contract/Subcontract No. (and contractor name if subcontract)	Principal Investigator	Title	Contract Date: From MO	From YR	To MO	To YR	Total Award Value	$ AMT Awarded for FY Period 1988 to 1990	DOE Technical Monitor/Location
DE-FG02-86ER60396	Lawrence Grossman Ph.D.	"Molecular basis for the mutagenic and lethal effects of ultraviolet irradiation"	12	88	11	91	$198,062	$128,707	Chicago Operations Office Argonne, Illinois
DE-FG02-88-ER60636	Paul O.P. Ts'o Ph.D.	"Genes and Gene Expression: Localization, Damage and Control - A Multilevel and Interdisciplinary Study"	02	88	01	91	$1,334,430	$881,286	Chicago Operations Office Argonne, Illinois

Provide information only on those research projects which are directly related to the selected principal research area and which were active during the period from October 1, 1988 to September 30, 1990. Do not include grants or contracts for the following: (1) workshops, (2) education/training projects or other non-research projects, (3) facilities, (4) instrumentation, (5) those projects completed before October 1, 1988.

See discussion on page 8 for additional requirements relating to subcontracts.

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RELATED FEDERAL AGENCY GRANTS AND CONTRACTS

Agency/Agency Contact	Principal Investigator	Grant/Contract No.	Title	Grant/Contract Dates From MO	From YR	To MO	To YR	Total Award Value
NIH - Dr. Stephen Fahnestock	Lawrence Grossman Ph.D.	5 RO1 GM22846	"Enzymatic excision and repair mechanisms"	09	90	08	91	$270,889
NIH - Dr. Stephen Fahnestock	Lawrence Grossman Ph.D.	5 RO1 GM31110	"DNA repair gene expression in mammalian cells"	07	90	06	91	$273,904
NCI - Dr. Colette Freeman	Paul O.P. Ts'o, Ph.D.	2 P01 CA42762	"Oligonucleotide Analogs as Gene Regulation Agents	07	90	05	91	$967,010
NIH - Dr. Stephen Fahnestock	Roger McMacken, Ph.D.	5 RO1 GM32253	"Initiation of Bacteriophage λ DNA Replication in vitro"	07	90	06	91	$315,181
NIH - Dr. Stephen Fahnestock	Roger McMacken, Ph.D.	5 RO1 GM36526	"Host-Virus Interactions in Initiation of DNA Replication"	04	90	03	91	$171,309
NIH - Dr. Warren Jones	Floyd R. Bryant, Ph.D.	2 RO1 GM36516	"Enzymatic Transformation of the DNA Helix"	05	90	04	91	$169,849
NIH - Dr. Michael Rogers	Paul S. Miller, Ph.D.	1 RO1 GM45012	"DNA-Specific Oligonucleotide Analogs"	06	90	05	91	$173,184
ACS - Dr. John Stevens	John Scocca, Ph.D.	MV - 385A	"DNA Recognition in Site Specific Recombination"	01	91	12	91	$ 88,000
NIH - Dr. Marion Zatz	Patricia Gearhart Ph.D.	9 RO1 GM42975	"Rearrangement of VK Genes During Ontogeny"	12	90	11	91	$136,832
ACS - Dr. John Stevens	Patricia Gearhart Ph.D.	IM - 526	"Rearrangement of Immunoglobulin Genes"	07	90	06	91	$139,000
NIH - Dr. James Cassatt	S. Chandrasegaran, Ph.D.	5 R29 GM42140	"Studies on FokI (a Type IIs) restriction enzyme"	4	90	03	91	$108,425

Only provide information on research projects which are directly related to the selected principal research area.

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NARRATIVE

OVERVIEW:

Scientific Merit and Accomplishments of the Research Programs

Although we feel somewhat uneasy being asked to judge the scientific merit of our own research programs for fear of sounding self serving, we, nevertheless, are proud to point out that many of our investigators are internationally recognized as being leaders in their respective fields. For example, Dr. Ts'o and Dr. Miller published in 1976 perhaps the first article describing the application of antisense oligonucleotides to the targeted regulation of cellular gene expression and have remained at the forefront of this rapidly burgeoning field ever since. Dr. Ts'o has more recently been making important contributions to the development of advanced strategies for the mapping of the human genome. Dr. Grossman has for more than two decades been a leading figure in the field of the enzymology of repair of damaged DNA. His group is one of 2 groups in the world that has established the *E. coli uvrABC* excision repair pathway with a set of purified proteins and his laboratory continues to define the cutting edge for mechanistic studies of repair processes. Dr. McMacken and his colleagues have achieved the total reconstitution, solely with purified proteins, of a system that supports the specific initiation of duplex chromosomal DNA replication *in vitro*. This is a highly noteworthy achievement that has only been accomplished (in a different system) by one other laboratory (Nobel laureate Dr. Arthur Kornberg). Dr. McMacken's laboratory has established their replication system as a leading paradigm for the mechanisms involved in the initiation and regulation of chromosomal DNA replication. Additionally, Dr. McMacken has developed the only physiological *in vitro* system, composed of purified proteins, for studying the function and behavior of hsp70 proteins and other heat shock proteins that modulate the behavior of the hsp70 protein. Dr. Bryant is perhaps the world's leading expert in studies of how proteins couple the energy of ATP hydrolysis to carry out transformations of the DNA helix. Dr. Chandrasegaran has developed novel and rapid DNA sequencing strategies that should aid in the sequencing of the human genome. He also has prepared a number of cocrystals of restriction enzymes with their cognate DNA recognition sequences that should provide important new information about the mechanisms available for proteins to achieve sequence-dependent recognition of DNA. Dr. Gearhart is well-known for her pioneering work on the somatic mutagenesis of the variable regions of antibody genes that occurs during development of antibody-producing cells. Dr. Scocca is recognized for his leadership in defining the pathway for transformation of *Haemophilus influenzae*. More recently, Dr. Scocca has established the first *in vitro* system for the site-specific integrative recombination of bacteriophage chromosomes of the non-λ type.

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An independent measure of the merit of the ongoing research programs is that each laboratory is currently funded by federal or private research organizations, ... a real tribute in these days of extreme competition for scarce funds for biomedical research. In fact, each of the investigators listed on this application has been continuously funded by external organizations since he/she was appointed a faculty member in the Johns Hopkins School of Hygiene and Public Health.

The Johns Hopkins University in 1890 was the first American university to establish graduate research training programs in the form that has become the standard today. The university has continued to remain at the forefront of biomedical research. The esteem with which the research programs here are held is in some measure reflected by the fact that The Johns Hopkins Medical Institutions currently receives more external research support than any other medical complex in the United States. The intellectual environment at Johns Hopkins is extremely enriching and extraordinarily stimulating.

Equipment Requested

We are requesting funds for the purchase of a Molecular Dynamics Model 400E PhosphorImager, a Molecular Dynamics Model 300E Computing Densitometer and the associated computers, graphics equipment and software for each. Each instrument is capable of rapidly scanning and quantifying large 2-dimensional arrays of data displayed on a gel or membrane. 2D analysis is inordinately more accurate than 1D scanning densitometry and is the only means by which two dimensional patterns produced by standard high resolution gel electrophoresis protocols can be quantitated. The PhosphorImager replaces standard x-ray film autoradiography and quantifies radioactivity from molecules labeled with ^{32}P, ^{35}S, ^{14}C or ^{125}I (the storage phosphor technology used to quantify β-emissions in the PhosphorImager is described briefly in the Summary section). The storage phosphor screens used with the PhosphorImager, however, are 100-times more sensitive than x-ray film and 10-fold more efficient. Thus, the significantly shorter exposure times permitted by the availability of the PhosphorImager is expected to greatly increase the productivity of the research programs described in this application. Just as importantly, the dynamic range of the PhosphorImager is greater than 100,000 to 1. Film, on the other hand, produces a notoriously nonlinear response that greatly complicates the acquisition of quantifiable data. The Computing Densitometer is critically needed to provide accurate quantification of stained protein bands following polyacrylamide gel electrophoresis in a wide variety of experimental contexts (see descriptions of individual research programs below). The availability of specific antibodies directed against many of the

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purified proteins discussed in this application makes feasible highly sensitive immunological techniques for detecting and measuring the levels of specific proteins, or fragments or domains of these proteins. It is especially useful that the high intensity laser beam of the Computing Densitometer is also capable of precisely quantifying immunoblot (i.e., "Western blot") reactions occuring on nitrocellulose or nylon membranes. The availability of instrumentation that rapidly and reliably integrate two-dimensional patterns of radioactivity or optical density will permit each of our research programs to quantify data from electrophoretic or thin layer chromatographic analyses. This capacity will permit the determination of crucial kinetic constants and binding constants needed to enable our investigators to formulate detailed molecular mechanisms for the complex biological reactions being studied.

Although the capacity of the requested instruments to provide accurate quantitation of electrophoretic data is the overriding need for our research programs, these instruments will help our research in several other ways. We have already mentioned the increased speed and sensitivity of the PhosphorImager for autoradiographic analysis that will significantly enhance our productivity and permit accurate quantification of weakly radioactive bands that cannot be measured with x-ray film. At present, the only method available to us to quantify electrophoretic data is to cut out individual bands or spots, dissolve the gel slice in strong oxidizing acid overnight at 60 °C, and count each individual sample in a liquid scintillation counter. This procedure is extremely labor intensive, requiring several hundred samples and two or three person-days, to complete the analysis of even a standard size agarose gel. Thus, it is extremely difficult to convince a graduate student that quantification of experimental results are worth the effort and repetitive exposure to radiation that is required to carry out such an analysis. The availability of the PhosphorImager will not only reduce human exposure to strong β-ray emissions, but also would significantly reduce the amount of low level radioactive waste that we generate. Just as important, these instruments should provide a real impetus to our researchers to quantify the data from their experiments. Far far too often in the literature, electrophoretic data is left unquantified and is simply described in very qualitative terms that simply aren't sufficiently rigorous to derive convincing mechanistic conclusions. Experiments that currently require several days to quantify can be carried out in a few minutes to an hour with the aid of the PhosphorImager.

It is perhaps worth noting why we selected the Molecular Dynamics Model 400E PhosphorImager over other 2D autoradiographic analysis machines. We also considered the Betagen Betascope and a machine from Ambis. The Ambis machine has somewhat lower resolution than the PhosphorImager, but does have extremely good analysis software. Unfortunately, if the Ambis machine needs service, it must be shipped to California for repair. One of our

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colleagues at the NIH has had to ship his machine to the West Coast twice in the first year, each time causing interruptions of 6-8 weeks in his capacity to quantitate experiments. Both Betagen and Molecular Dynamics have service representatives on the East Coast. The Betagen instrument utilizes direct counting technologies and produces data somewhat faster than the PhosphorImager. However, this machine has several problems. First, the resolution of the electrophoretic data is very poor, owing from the fact that the detectors are positioned more than one cm from the sample. This means that sample bands or spots located closer together than 4 or 5 mm are very poorly resolved. Consequently, the Betagen instrument cannot be used to determine thermodynamic binding isotherms from footprinting experiments or to quantify DNA bands at sequencing gel resolution. Moreover, with the Betagen, only one sample can be counted at a time, meaning that gels with low radioactivity would tie up the machine and impede analysis of other samples. With the PhosphorImager, each individual labs would have their own storage phosphor screens which could be exposed outside the machine until sufficient radioactive emissions had been obtained. Scanning of the screens would only take 20 min, at most, even for a large 35 x 43 cm sequencing gel. If others are waiting, the instrument computer can be freed by transfer of the digitized data over a local ethernet system to a high speed Convex computer system that is available for our use. Thus, the data can be manipulated and quantified off site. Accordingly, the PhosphorImager is the instrument best suited for both multiuser studies and for fulfilling all anticipated experimental requirements.

Graduate Student Involvement in Research Using the Requested Equipment

The Biochemistry Dept. in the School of Hygiene and Public Health is a relatively small department comprised of 14 faculty members, 36 graduate students and 18 postdoctoral fellows. We take great pride in the quality of our graduate training program. Each faculty member invests considerable amounts of time in the training of both his/her own students and the students of other faculty members. Thesis committees for each student meet twice annually to review research progress and to provide informed guidance. Weekly research group meetings, journal clubs, and seminars provide a highly stimulating intellectual environment for student development. The stature of the Biochemistry Department graduate training program is also recognized by NIH review groups which have awarded two training grants to the Department, one from the National Institute of Environmental Health Sciences and one from the National Cancer Institute. In June of 1990, we submitted the competitive renewal application of our NCI training grant. Dr. McMacken, the Chairman of the Biochemistry Department, has recently been informed by the NCI that our

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application received one of the highest priority scores (1.16). Thus, strong external support for graduate training seems assured for the forseeable future.

Of the 36 graduate students in the Department, 17 are currently pursuing Ph.D. thesis research in the laboratories of the 7 departmental investigators listed on this application (Dr. Chandrasegaran of the neighboring Dept. of Environmental Health Sciences currently has 1 graduate student). Moreover, we anticipate that at least four of the eight first-year graduate students will elect to conduct their thesis research in laboratories of the faculty members listed on this application. We anticipate that nearly every single student in the Biochemistry Dept. will benefit at one point or another from the presence of the PhosphorImager and the Computing Densitometer, given the ubiquitous use of electrophoretic techniques in every type of research program. The ongoing DOE research projects currently involve 5 graduate students, with a good possibility that an additional student or two will join these projects in June of 1991, after the first-year students decide in which laboratory to work. The ongoing DOE project in Dr. Ts'o's laboratory is very heavily oriented toward the development of improved 2D electrophoretic strategies for mapping large segments of chromosomes. Students working on this project will benefit immensely from the acquisition of both the PhosphorImager and the Computing Densitometer, especially in terms of speed and sensitivity of analysis of complex arrays of restriction fragments. These instruments would permit Dr. Grossman's students to initiate experiments to determine the thermodynamic binding isotherms and binding stoichiometries for key DNA repair proteins. This type of precise quantitation is not possible with our current instrumentation. It is crucial that this type of information be obtained, since it defines essential fundamental properties of these biologically important repair systems that have not yet been determined by anyone in the DNA repair field.

Experience of Faculty with Similar Equipment

We do not have any previous experience using the requested instrumentation. However, we have seen the instruments in operation and it is clear that the user-friendly ImageQuant software supplied with the instrument will enable an average graduate student to quantify complex patterns after only an hour or two of learning. With this instrumentation, it will not be necessary to hire a full-time research technician to operate the machine.

Life Span, Operation and Maintenance of the Requested Equipment

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It is estimated that the PhosphorImager and the Computing Densitometer should have a useful life span of approximately 10 years. Maintenance costs are expected to be relatively low, although it is strongly advisable that we continue to purchase service contracts for both machines throughout the useful lifetime of the instruments. These service contracts will be paid for from the research grants awarded to the 8 faculty listed on this application. Dr. McMacken, the Chairman of Biochemistry, has committed the Department to pay for such service contracts for a minimum period of 5 years following the termination of DOE support, in the unlikely event that sufficient research funds are not available from investigator-initiated grant awards. The Biochemistry Department will also pay (~ $1000) for the limited renovations of Room 8009 of the Biochemistry Dept. that will be required for the installation of the instruments. Furthermore, the Department also agrees to pay the mandatory space charges to the School of Hygiene and Public Health for the space occupied by the new instruments for the duration of the lifetime of the instruments. During the current fiscal year, this charge would amount to approximately $1750 (for the estimated 100 ft^2 of space necessary to house the two instruments). The aforementioned space is centrally located in the Department in an area contiguous to Dr. McMacken's laboratory. Dr. McMacken has agreed to oversee the use of these instruments to guarantee that these machines are properly cared for and not abused.

Our group expects to use these machines intensively. While we will permit occasional use by outside investigators, we anticipate that insufficient free time will be available to make these machines facility instruments for the cadre of hundreds of researchers located at the Johns Hopkins Medical Institutions. To our knowledge, no other 2D-autoradiographic counters or 2D computing densitometers are available anywhere in the Medical Institutions. The main Arts & Science campus of the Johns Hopkins University is located more than 3 miles away across town, making every day use of their research facilities highly impractical.

Descriptions of Research Projects of Individual Laboratories

LABORATORY OF PAUL TS'0

Research Objectives

The objective of this research program is to develop 2-D enzymatic and electrophoretic analysis to map DNA restriction fragments. These procedures will be applied to studying human DNA sequences by providing restriction fragment mapping/ordering of cosmids and yeast artificial chromosomes (YAC's) containing human DNA from 50-200 kilobases (kb) in size. New methods for improving resolution and sensitivity of this analysis will also be developed.

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This program is supported in part by a grant from DOE (ED-FG02-88ER60636) "Genes and Gene Expression: Localization, Damage and Control - A Multilevel and Inter-Disciplinary Study" - P O P Ts'o P.I. Project II Genome Organization and DNA Rearrangement Paul Ts'o, investigator - $251,776.00 per year direct costs.

Background and Research Accomplishments

A method has been developed for monitoring mammalian genome rearrangements. This method is based on electrophoretic separation of twice digested restriction fragments of genomic DNA in a 2-D pattern. The first digestion is carried out in solution followed by electrophoresis of the restriction fragments in the first dimension. A second restriction enzyme digestion is carried out *in situ* in the gel followed by electrophoresis in a second dimension perpendicular to the first electrophoresis. The DNA fragments are then transferred to a filter (Southern blotting) and the blot is probed with a suitable DNA probe. This method has been used to study the rearrangement of Intracisternal A Particle (IAP) genes and their neighboring sequences in rodent cells. The 2-D mapping procedure was able to resolve approximately 370 distinct spots.

A probe-free method has also been developed to detect DNA rearrangement in bacteria based on the electrophoretic separation of twice digested restriction fragments of genomic DNA into a 2-D pattern. The first restriction enzyme digestion was done in solution, followed by electrophoresis of the restriction fragments in the first dimension. A second restriction enzyme digestion was carried out *in situ* in the gel, followed by electrophoresis in a second dimension perpendicular to the first electrophoresis. The 2-D pattern resolves 300-400 spots which are defined and indexed by an "x,y" coordinate system with size markers. This approach greatly increases the resolution power compared to conventional 1-D electrophoresis. To study DNA rearrangement, a 2-D pattern from a test strain was compared with the 2-D pattern from a reference strain. After the first digestion, genomic DNA fragments from the test strain were labeled with ^{35}S while those from the reference strain were labeled with ^{32}P. This was done in order to utilize the difference in the energy emission of ^{35}S and ^{32}P isotopes for autoradiography when two x-ray films were exposed simultaneously on top of the gel after the second dimension electrophoresis. The irradiation from the decay of ^{35}S only exposed the lower film, whereas the irradiation from the decay of ^{32}P exposed both the lower and upper films. By comparison, different DNA fragments existing in the test DNA and the reference DNA can be identified unambiguously by the differential two 2-D patterns produced on two films upon exposure to the ^{35}S and ^{32}P fragments in the same gel. An appropriate photographic procedure further simplified the process, allowing only the difference in

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DNA fragments between these two patterns to be shown in the map. We have utilized the difference map obtained from *E.coli* HB101 and *E.coli* HB101 [lambda (λ)] genomic DNA to show the incorporation of one copy of λ DNA without the use of a λ DNA probe.

A matrix approach developed by Fitch, et al., [Fitch, W.M., Smith, T.F. and Ralph, W.W., (Gene, 22:19-29, 1983)] can be used to order DNA restriction fragments obtained by double or two single digestions. This method is based on the accurate molecular weight measurements (in bp unit) of all fragments and the uniqueness in the relationship of the sum of all molecular weights for the doubly digested fragments to the presumptive, parental restriction fragments obtained after the single digestion (i.e., digestion by use of enzyme only). The uncertainty of assignment is totally eliminated by our current approach of two enzymes - 2-D gel treatments and display. The origins of all the doubly digested restriction fragments to the parental restriction fragments for the first/single digestion are completely and unambiguously displayed in the 2-D gel map. The only requirements are that: all the restriction fragments obtained for the first/single digestion can be completely resolved in the 1-D electrophoresis; and all the fragments must be contained and displayed within the gel.

The 2-D gel display provides a fixed set of coordinates for each fragment obtained from the single and double digestion, thus, the various possible permutational computation for relating the doubly digested fragments to the single digested fragments as proposed by Fitch, et al., are eliminated. Our current studies indicate that with a larger gel (34cm x 34cm) we should be able to separate all (30-some) fragments from a 200kb human fragment in the YAC using a single restriction enzyme in the first dimension gel. Thus, this method can be applied to restriction fragment ordering/mapping for YAC's up to 200kb in size. In the case of YAC's having one megabase fragment size, this YAC will be partially digested and then electrophoresed using pulsed-field gel electrophoresis. The partially digested fragments can be ordered by the end labeling experiment and then these partially digested fragments can be picked up from the agarose gel for 2-D gel restriction mapping. This partial digestion-end labeling approach is outlined below. For this approach, we can provide the restriction fragment mapping for YAC's up to one megabase in size by the stepwise procedure in reducing these YAC's down to 200kb through partial digestion.

Future Experiments

We are currently using the 2-D restriction mapping procedure to provide an order and mapping of restriction sites for the restriction fragments of human DNA sequence cloned in cosmid and yeast artificial chromosomes (YAC's).

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The objective is to produce a facial and reliable method which can be used for sequencing of any DNA pieces (including those containing human sequences) approximately 50-200 kb in size. In addition, we are utilizing this approach in conjunction with a Computer Microscopic Imaging System (CMIS) to align the cosmids and the YAC's along human chromosome #21. Hopefully, this tactic will provide us with a systematic approach for human genome sequencing or sequencing of other organisms. Once the general procedure is established, we can then consider the application of this method for DNA rearrangement. A possible study could be on Drosophila genome and the yeast genome.

We plan to work with the Department of Pediatrics (Stylianos E. Antonarakis, M.D.), to study the variations and rearrangements of "line" elements in human genome using the method which we have successfully employed in our paper to be published in Analytical Biochemistry. Using the "line" elements as probes and using the improved method of 2-D mapping, we hope to ask the question concerning polymorphism and rearrangement of "line" elements in the genome of individual humans.

Another approach is to improve the current method of resolution by the use of different electrode geometry and electrode area so as to increase the mobility of the large DNA fragments and decrease the mobility of the small DNA fragments. The results of this technology will allow us to increase resolution of large DNA fragments, while preventing the escape (loss) of small DNA fragments in the 2-D gel map. By such techniques, it is possible to increase resolutions of our 2-D gel from 2,000 fragments per gel to 3,000 fragments per gel.

Analysis of the 2-D gels and Southern blots of the 2-D gels will be greatly facilitated by the availability of the PhosphoImager and 2-D computing densitometer. As described above we are currently using standard autoradiographic methods to detect radioactivity in the gels and blots. This procedure often requires time consuming multiple exposures. The PhosphorImager will enable direct analysis of the blots and the gels derived using the probe-free method employing ^{32}P- and ^{35}S-labeled restriction fragments. The greater sensitivity of the PhosphorImager will reduce the time required to analyze the gels and blots and the associated software will enormously simplify the analysis procedure.

LABORATORY OF ROGER MCMACKEN

Current Research Projects and Overall Objectives

Our laboratory investigates the fundamental biological, biochemical and biophysical mechanisms involved in the initiation and regulation of chromosomal DNA replication. Using the genome of bacteriophage λ as a model

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chromosome, we have reconstituted, solely with purified proteins, a system that supports the initiation, propagation, termination and regulation of λ DNA replication *in vitro*. This important accomplishment has enabled us to begin to define many of the underlying molecular mechanisms involved in these complex processes. The molecular events identified in the reconstituted λ replication system serve as a valuable paradigm and guide for the still rudimentary field of eukaryotic chromosomal DNA replication, since the available data suggests that many of the critical features of DNA replication have been conserved from bacteria to man. The primary objectives of our research are to elucidate the structures and functions of the 15-20 virus and bacterial proteins required to carry out and regulate λ DNA replication. Our reconstituted multiprotein replication system will permit us to study a plethora of fundamental biological processes whose molecular mechanisms have not been solved in any system. For example, we are actively pursuing studies aimed at understanding the following basic questions: (i) what elements of protein structure enable initiator proteins to bind specifically to replication origins?; (ii) how does the binding of multiple copies of an initiator protein to a chromosomal origin cause the opening of the DNA double helix in adjacent regions?; (iii) what molecular events are involved in the transfer of replicative DNA helicases between the two DNA chains at an origin?; (iv) how do rapid and processively moving replication fork proteins respond to damaged or modified nucleotides in the DNA?; (v) why is supercoiling of the DNA template necessary for the initiation of chromosomal DNA replication?; (vi) how are bacterial histonelike proteins involved in the regulation of the initiation of DNA replication?; and (vii) why is transcription of the template DNA at or near replication origins required for the initiation of DNA replication? Research on these problems is supported by grant 5 R01 GM32253-08 from the National Institutes of Health. The grant is titled "Initiation of Bacteriophage λ DNA Replication in Vitro" and receives funding of $315,181 in the current budget year.

Our laboratory is also actively engaged in research into the molecular mechanisms by which viruses recruit cellular replication proteins and direct the host proteins to participate in the duplication of the invading viral chromosome. This research is supported by National Institutes of Health grant 5 R01 GM36526-05. This grant is titled "Host-Virus Interactions in the Initiation of DNA Replication" and receives funding of $171,309 in the current budget year. We have been examining the early stages of the initiation reaction. We have found that the λ-encoded P replication protein seeks out the bacterial DnaB helicase and forms a very tight complex with it. This P•DnaB complex subsequently binds to a nucleosomelike assembly of λ O initiator protein that forms at the λ replication origin (*ori*λ).

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The intrinsic helicase activity of each DnaB molecule located at *ori*λ is suppressed at this stage by tight interactions with λ P. The virus relies on three evolutionarily conserved bacterial heat shock proteins, DnaJ, DnaK and GrpE, to release DnaB from its protein restraints. DnaK is the hsp70 homologue of *E. coli* and shares many structural and functional features with eukaryotic hsp70, hsc70 and BiP proteins. DnaJ and DnaK bind sequentially to the *ori*λ • O • P • DnaB nucleoprotein structure to form a complete preinitiation complex. In the presence of ATP, DnaK mediates the partial disassembly of the preinitiation complex, ejecting the P and DnaJ proteins, and freeing DnaB protein to initiate localized unwinding of DNA near the viral origin. This is the first committed step in the replication of the viral genome.

The protein disassembly steps mediated by the DnaK and DnaJ heat shock proteins closely resemble reactions carried out by members of the eukaryotic heat shock protein family in normal cellular metabolism, e.g., disaggregation of protein aggregates that form in the nuclei of cells following heat shock, disassembly of clathrin coated vesicles during receptor-mediated endocytosis, import of cytoplasmically-synthesized proteins into mitochondria or the endoplasmic reticulum, etc. Studies of the apparently common underlying molecular mechanisms responsible for these processes can perhaps be examined most deeply in the λ replication system, since only this reaction has been reconstituted wholely with purified proteins. And only in this system can the effects of other heat shock proteins on hsp70 function be determined *in vitro*. In this regard, we have recently discovered that the DnaK protein has an intrinsic peptide-dependent ATPase activity that is modulated by each the DnaJ and GrpE heat shock proteins. DnaJ acts to increase the affinity of small peptides for DnaK, whereas GrpE functions to increase the catalytic efficiency of DnaK, both in ATP hydrolysis and in λ DNA replication. Furthermore, small peptides that interact with DnaK, when present at sufficient concentration, inhibit λ DNA replication *in vitro*. This and other data suggests that the peptide-binding site on DnaK is also used in the DnaK-mediated disassembly of preinitiation nucleoprotein structures. The objectives of our research on the *E. coli* heat shock proteins include: (i) elucidation of the molecular mechanism by which DnaK protein mediates the ATP-dependent disassembly of nucleoprotein structures formed at *ori*λ; (ii) identification of the location and structure of the peptide binding site in the DnaK polypeptide; (iii) determination of the role of DnaJ protein in targeting DnaK protein to act on specific nucleoprotein structures; and (iv) examination of the mechanism by which GrpE protein increases the catalytic efficiency of DnaK.

Specific Experiments to be Conducted with the Molecular Dynamics Model 400E PhosphorImager

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Very little is known about the properties of the interaction of initiator proteins with specific chromosomal replication origins. We are interested in determining the binding affinity of both wild type and a large series of mutant λ O initiator proteins to the O recognition site under a variety of physiological conditions. We will use gel mobility shift assays to measure the strength of the O protein-DNA interaction. Short DNA fragments containing a single O recognition sequence will be end-labeled with ^{32}P and incubated with varying amounts of purified wild-type or mutant O protein. After binding equilibrium is achieved, the protein-DNA complexes are resolved from free DNA by electrophoresis through neutral polyacrylamide gels. The PhosphorImager will be used to quantitate the amount of free DNA and bound DNA formed at each concentration of protein so that the equilibrium binding constants can be determined. This type of analysis will ultimately require thousands of samples and only can be practically and accurately carried out with the aid of an automated 2D radioisotope counter such as the PhosphorImager.

In vivo, formation and activation of preinitiation complexes at *ori*λ occurs on supercoiled DNA. A determination of the energetics of O protein binding to its multiple recognition sites (iterons) in a supertwisted *ori*λ should help elucidate whether or not cooperative protein interactions play a critical role in the initiation of DNA replication as they do in the regulation of transcription by the λ repressor. To measure the thermodynamic binding isotherms for O interaction with each origin iteron, we will use a supercoil footprinting methodology, which we recently modified to include repetitive primer extension with *Taq* DNA polymerase from ^{32}P-labeled primers. The extended DNA chains will be resolved by electrophoresis in polyacrylamide sequencing gels and the footprint patterns obtained quantitated using the PhosphorImager. This advanced technology will make it possible for us to rapidly and accurately measure the % occupation of individual binding sites at each O protein concentration so that quantitative thermodynamic binding isotherms for interaction of the O initiator with the viral replication origin on supercoiled DNA can at last be determined. This would represent the first determination of the binding isotherms for interaction of any initiator with its origin.

Similar supercoil footprinting technology will be used to assess changes in the template DNA structure at *ori*λ as the initiation proceeds through its multiple stages. Chemical or enzymatic probes (e.g., $KMnO_4$, dimethyl sulfate, diethylpyrocarbonate, P1 nuclease, etc.) that detect unwound DNA, bent DNA or other non-B DNA structures, will be used to modify DNA after specific nucleoprotein prepriming complexes are formed at *ori*λ. The modified DNA will be separated from protein, cleaved at the positions of modification and subjected to primer-extension analysis. The

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availability of the PhosphorImager will be particularly valuable in these experiments, since it can be used to measure the frequencies of modification at individual nucleotide positions so that the conformational state of the origin DNA at each residue can be monitored throughout the initiation process. This type of analysis has never been conducted for any replication origin.

In other experiments, we are investigating the affect of template superhelicity and histonelike proteins on the initiation of λ DNA replication. Plasmid templates with varying topological linking numbers will be prepared and subjected to 2-dimensional agarose gel electrophoresis before and after they are incubated in the complete λ replication system. The level of replication of each plasmid topoisomer will be determined with the aid of the PhosphorImager following hybridization of each topoisomer band with ^{32}P-labeled probes. We have also established a rolling-circle replication system to study the speed and processivity of λ replication forks. In this system, we will use ^{32}P-labeled primers to monitor the effect of ultraviolet-induced pyrimidine dimers and ionizing radiation damage on the movement of a physiological and highly processive replication fork. The PhosphorImager will enable us to rapidly quantitate the degree to which replication forks halt at or proceed past damaged sites. This instrument will also provide a very convenient and rapid method for determining the kinetics of ATP (or other nucleotide) hydrolysis (by either DnaB, DnaK or DNA polymerase III holoenzyme proteins) in the thousands of such assays we expect to employ each year. [α-^{32}P]ATP is resolved from its hydrolysis product (usually ADP) by thin layer chromatography on PEI strips and the levels of product and substrate present at a given time will be directly quantitated in the PhosphorImager.

Specific Experiments to be Conducted with the Molecular Dynamics 300E Computing Densitometer.

Several other types of experiments will be greatly facilitated by the availability of the computing densitometer. This instrument will be used to quantitate all electrophoretic analyses that employ nonradioactive substrates or ^{3}H-labeled substrates, since the weak β-ray emitted by ^{3}H cannot be detected with the PhosphorImager instrument. For experiments involving ^{3}H, polyacrylamide or agarose gels containing resolved ^{3}H-labeled substrate or product molecules are first soaked in a fluor. This enables β emissions to be "captured" and converted into photons that can be detected by standard x-ray film and quantitated with a densitometer. To determine the mechanism by which processive replication forks are established at a chromosomal origin, we have synthesized model circular DNA substrates that can be used to monitor the assembly of a physiological replication fork apparatus. Once established on this substrate, a processive replication fork produces extensive rolling-circle DNA synthesis, generating DNA molecules that are over a

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megabase in length in roughly 20 min. The availability of the Computing Densitometer will enable us to measure the kinetics of initiation of replication fork assembly under a wide variety of conditions and protein combinations. In this experiment, the substrate DNA, which had been labeled with ^3H during its synthesis, is electrophoresed in a neutral agarose gel at different time points following the beginning of the reaction. Substrate molecules that have initiated rolling-circle DNA synthesis migrate as a broad smear in the gel (at a position determined by the length of the newly synthesized DNA tail) behind the position of unreplicated template. The computing densitometer will permit us to determine the number of substrate molecules remaining as well as the number of replicated substrate molecules.

Replication proteins are uniformly expressed at very low levels *in vivo*. Biochemical and biophysical analysis of this group of proteins has been greatly facilitated by the use of PCR and recombinant DNA technologies to achieve enormous amplification of the production of individual proteins. Protein resolution by SDS-PAGE, followed by staining of polypeptides with Coomassie Brilliant Blue dye, is standard procedure for monitoring expression of individual proteins *in vivo* and for judging purity of purified protein preparations. The computing densitometer will permit us to analyze Coomassie stained polyacrylamide gels directly so that we may accurately and quickly quantitate the levels amplified proteins and the purities of our numerous protein reagents. We also will be attempting to use a variety of protein-protein crosslinking reagents to map the many protein-protein contacts present in the large multiprotein preinitiation complexes that are assembled at *ori*λ prior to the initiation of λ DNA replication. The analysis of the crosslinked protein pairs will utilize two dimensional polyacrylamide gel electrophoresis, followed by quantitation of the level of crosslinking using densitometric analysis of the protein spots (which will be stained with Coomassie or silver or with specific antibodies and immunological reagents). A densitometer with two-dimensional capabilities is absolutely imperative for these type of analyses.

LABORATORY OF LAWRENCE GROSSMAN

PROJECT 1 - Structure Function Studies of Nucleotide Excision. Nucleotide excision in all organisms is a multigenic and regulated process which appears to be highly conserved in going from *Escherichia coli* to humans. We have cloned most of the genes which have, as a consequence, been mapped, sequenced and amplified. In so doing reagent quantities of high quality protein have been generated. Because of the stability of the separate Uvr-nucleoprotein complexes it has been possible to isolate partial reactions and to study them as separate biochemical steps. The

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incision, and pre-incision steps are intrinsically the most interesting because they are both the rate-limiting stage and because they include damage recognition as a prerequisite for repair.

In order for productive dual incision to take place at a damaged site in DNA the component proteins of the UvrABC endonuclease system must effectively perform a number of unique functions. It is through directed, deletion and directed-random mutagenesis that the role of putative structural motifs can be effectively specified. Identification of these roles through investigation of the molecular phenotypes of the mutant proteins include a prescribed series of reactions, or molecular phenotypes, that are followed with routine methods demanding quantitation which would be enormously facilitated by both phosphorimaging and microdensitometry. In order to be reactive with DNA, UvrA must first dimerize in the presence of ATP or ATP-γ-S (first ATP-dependent-step). By determining the Coomasie extinction coefficient of the purified Uvr proteins we follow the stoichiometry of nucleoprotein formation by both microdensitometry and phosphorimaging. In its reaction with DNA, (UvrA) causes topological unwinding during initial nucleoprotein formation in the presence of ATP. The extent of DNA unwinding, in the presence of UvrB, is approximately a single helical turn - the extremes of the dual incision sites. Quantitation of such molecular phenotypes and the kinetics of such reactions can only be achieved with the equipment requested. From mutagenesis, kinetic and thermodynamic studies the formation of the UvrAB-nucleoprotein complex at an undamaged site is the first binding event and is an obligate intermediate in the translocation of the UvrABC complex to a damaged site. To study the mechanisms for such a reaction we need K_D from polymeric absorption isotherms derived from kinetic footprinting techniques. These experiments cannot be accomplished without multiple gel data analyzed by automated phosphorimaging. It is during this part of the repair process that the UvrA component of the $Uvr(A)_2B$ complex senses the presence of damage by coupling the hydrolysis of ATP that is required for the dissociation of the protein complex. (a second ATP dependent step measured by the requested equipment). From the same mutation studies, the energy required for translocation is derived from ATP hydrolysis (third ATP-dependent step) catalyzed by the UvrB-associated cryptic ATPase. Translocation by $Uvr(A)_2B$ is accompanied by a wave of supercoiling whose rate and level are significantly increased by damage. In order to determine the kinetic parameters of supercoiling, we desperately need a phosphorimaging system. This pre-incision complex is able to accommodate to the phosphodiesterase properties of the UvrC protein leading to dual incision of the damaged sites. The excision of the oligonucleotide fragment containing the damage requires the coordinated action of the UvrD associated helicase and

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DNA polymerase under polymerizing conditions. Polynucleotide ligase restores the integrity to the excised resynthesized DNA.

PROJECT II DNA Repair as a Risk Factor in Environmentally-Induced Diseases.

The molecular epidemiology of environmentally induced cancer and the development of assays for measuring the genotoxicity of environmentally generated damage (including radiomimetic agents) is being validated using an inexpensive, non-invasive assay which is highly sensitive, specific and reproducible assay. In its biological element this assay is an extension at the molecular level of host cell reactivation. When a damaged virus infects a cell, its ability to produce normal levels of progeny is dependent on the ability of the host cell to repair the damage to the DNA of the infecting virus. This assay depends on the use of a DNA molecule rather than a virus, which acts as a shuttle vector and which contains the a bacterial (*Escherichia coli*) *cat* gene encoding an enzyme, unique to bacteria, that is able to diacetylate chloramphenicol from acetyl CoA. The vector contains viral enhancer sequences that allows this bacterial gene to be expressed in human cells and the elements of pBR322 for its perpetuation in bacteria. Whole blood is fractionated using Ficoll gradients, and the buffy coat containing mostly lymphocytes is treated with mitogen to metabolically activate the T-cell lymphocytes. After 36 hours the T-cell lymphocytes are amenable to the physical introduction of the damaged or undamaged DNA facilitated by DEAE-dextran. Thirty six hours later the level of chloramphenicol acetyltransferase (CAT) is assessed. CAT activities are measured by thin-layer chromatography, which because of the large number of patients, can only be quantitated by a rapid and automated instrument phosphorimaging using ^{14}C-labeled reagents. For each patient three doses in triplicate are assayed generating over 4000 samples in our current study of 450 out-patients.

The level of expressed enzyme between undamaged and damaged states is a direct measure of the repair capacity of the individual donor. By introducing such dose modified DNAs into human lymphoid lines derived from patients with DNA repair deficiencies permits an extremely sensitive estimate for the presence of a genotoxic modification of the target DNA. The target DNA can be used to act directly with the damaging agent in aqueous solution, with airborne materials soluble in aqueous medium or indirectly with agents requiring the metabolic activation of lipophilic agents.

This biological phenomenon has been extended to the physical introduction of a damaged gene into the peripheral lymphocytes of a patients blood (20 cc.). The ability of the lymphocyte to restore full activity to that gene is a direct

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reflection of the repair capacity of that individual. Feasibility studies were executed with fibroblast, lymphoid and peripheral blood from patients with the DNA repair deficiency disease xeroderma pigmentosum.

Cellular Studies - Initial feasibility studies were carried out with established xeroderma pigmentosum (XP) fibroblast and lymphoid cell lines from the NIGMS Human Genetic Mutant Cell Repository using lines from out-patients in the Baltimore vicinity. The D_0 or D_{37}, for the XP cell lines were the same as published data validating the assay as a measure of DNA repair. These studies were extended to the peripheral lymphocytes of an outpatient XP family from West Virginia. Not only were the D_0 values the same as the established lines from the same individual but also the presumed heterozygotes (siblings and parents) were somewhat lower than normals.

Pilot Population Studies - In collaboration with Dr. Evan Farmer, Associate Professor of Dermatology, The Johns Hopkins School of Medicine, we identified a population of out-patients with basal cell carcinoma and a group of matched controls visiting the clinic for non-cancerous skin diseases. The demographics of the population includes subjects below the age of fifty to avoid age-related problems and those subjects who have been life-long Baltimore residents and who work indoors. A stratification of the population data reveals unexpected findings. To begin with, an examination of the control data reveals the minimal variance (4-5%) of the repair levels between groups giving biochemical confidence to these values compared to the levels of repair against the cases. The biochemical differences between group starts to become apparent. Young women of Celtic origin (northern Scotland and Ireland) who have a propensity to sunburn and blister have a consistently lower repair capacity. These data are further borne-out when the data are subject to a multivariate analysis. The gender orientation, observed in malignant melanoma and in *lupus erythematosis* may be hormonally induced and deserves further study.

In collaboration with Dr. Johan Hannson, of the Radiology Department of the Karolinska Hospital in Sweden, we plan to use this to assess the capacity of individual patients undergoing radiotherapy to tolerate ionizing radiation. Dosimetry is normally determined from geometric considerations rather than on an individual's personal tolerance. We will examine the ability of the lymphocytes of such patients to repair transfecting DNA containing differing doses of ionizing radiation.

LABORATORY OF FLOYD R. BRYANT

Grant Title: Enzymatic Transformations of the DNA Helix

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Sponsor: National Institute of General Medical Science, 5 RO1 GM36516, 05/01/89 through 04/30/94, Total direct costs $629,886

The overall objective of our research program is to determine the molecular mechanisms by which enzymes utilize the free energy of ATP hydrolysis to bring about changes in the structure of DNA. Our research focuses on the DNA strand pairing and unpairing reactions that are carried out during the repair of damaged DNA. The mechanism of action of two proteins from *Escherichia coli* are currently under investigation: the recA protein and the uvrD protein (DNA helicase II).

The recA protein is an ATP-dependent DNA pairing enzyme that is responsible for the formation of heteroduplex joints during recombinational DNA repair in *E. coli*. We are currently studying the mechanism of the recA protein-promoted pairing of complementary single-stranded DNA molecules in order to determine how the recA protein brings about the ATP-dependent alignment of complementary DNA sequences. We have recently developed a quantitative agarose gel electrophoresis assay for the recA protein-promoted renaturation reaction that allows us to monitor the time-dependent disappearance of the starting complementary single-stranded molecules, as well as the appearance of various duplex DNA pairing intermediates that are formed on the reaction pathway. This assay has revealed that the recA protein-promoted renaturation reaction proceeds in two kinetically-distinct stages. The first stage results in the formation of unit length duplex DNA as well as a distribution of other initial products. In the second stage, the initial products are converted to a variety of complex multipaired DNA structures. The kinetics of formation for the various paired intermediates and the distribution of the final reaction products are determined by the nature of the nucleotide cofactor (ATP or various ATP analogs) that is included in the reaction. In order for us to develop mechanistic models for the recA protein-promoted DNA strand pairing reaction, we must carry out extensive studies of the kinetics of the reaction under a wide variety of solution conditions. The PhosphorImager and Computing Densitometer will allow us to individually monitor specific reaction products that are produced transiently in low yield or are only narrowly resolved on agarose gels, and thus will greatly enhance the precision with which we will be able to analyze our agarose gel kinetic data.

DNA helicase II is an ATP-dependent DNA unwinding enzyme that is involved in DNA recombinational, excision, and mismatch repair in *E. coli*. In order to determine how DNA helicase II couples the hydrolysis of ATP to the

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unwinding of duplex DNA, we are currently examining the kinetics of the ATP hydrolysis reaction that occurs during a DNA helicase II-promoted duplex DNA unwinding event. We have found that ATP hydrolysis by DNA helicase II in the presence of duplex DNA exhibits a transient lag phase before a linear phase of ATP hydrolysis is attained. In contrast, ATP hydrolysis by DNA helicase II follows a linear time course in the presence of single-stranded DNA. In order to explain the nonlinear kinetics of ATP hydrolysis that are obtained with duplex DNA, we have developed a model in which DNA helicase II undergoes a time-dependent transition from an ATPase-inactive state to an ATPase-active state. We have also developed an agarose gel electrophoresis assay for DNA helicase II-promoted DNA unwinding reaction that allows us to measure both the time-dependent disappearance of the starting duplex DNA molecules, as well as the concurrent appearance of the separated complementary single-stranded DNA molecules. This assay shows that unwinding of the duplex DNA begins during the lag phase of the duplex DNA-dependent ATP hydrolysis reaction, and is completed during the linear phase of ATP hydrolysis. This suggests that the transient lag phase of ATP hydrolysis of the duplex DNA-dependent ATP hydrolysis reaction may correspond to the progressive unwinding of the duplex DNA. The PhosphorImager and Computing Densitometer will greatly facilitate our ability to analyze the agarose gel DNA unwinding data, and thus will enable us to more precisely determine the correlation between the instantaneous kinetics of ATP hydrolysis and the progress of duplex DNA unwinding by DNA helicase II. This, in turn, will lead to the development of detailed mechanistic models for the role of ATP hydrolysis in the DNA helicase II-promoted DNA unwinding reaction.

LABORATORY OF PAUL MILLER

Research Objectives

The overall objective of this research program is to design oligonucleotide analogs which can control gene expression in cells at the mRNA or DNA level. The program is supported in part by the following grants: DOE (ED-FG02-88ER60636) "Genes and Gene Expression: Localization, Damage and Control- A Multilevel and Inter-Disciplinary Study" P O P Ts'o P.I. - Project III Antisense Oligonucleotide Analogs, P S Miller, Investigator - $ 21,729 per year; NCI (CA 42762) "Oligonucleotide Analogs as Gene Regulating Agents" P O P Ts'o P.I. - Project I - Chemistry of Oligonucleotide Methylphosphonates, P S Miller, Project Leader - $ 138,041 per year; and NIH (GM-45012) "DNA-Specific Oligonucleotide Analogs", P S Miller P.I. - $ 105,013 per year.

Background and Research Accomplishments

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The expression of normal and altered genes can be studied and controlled by antisense oligonucleotides and oligonucleotide analogs which are complementary to functional regions of cellular or viral mRNAs. Our laboratory has studied the properties of novel antisense oligonucleoside methylphosphonates. These nonionic oligomer analogs which contain nuclease resistant methylphosphonate internucleotide linkages are taken up intact by mammalian cells in culture. We and others have demonstrated that suitably designed antisense oligonucleoside methylphosphonates can selectively suppress mRNA expression *in vitro* and in living cells. The efficacy of methylphosphonate oligomers can be considerably improved by conjugation with psoralen which allows the oligomer to cross-link to its mRNA target upon irradiation with long wavelength UV light. Such derivatization enables detection and characterization of oligomer binding to its target nucleic acid. We have demonstrated that psoralen-derivatized oligonucleoside methylphosphonates cross-link *in vitro* to single stranded DNA, RNA and to mRNA in a sequence specific manner and that they can function as specific inhibitors of protein synthesis in cells in culture.

Although antisense oligomers are usually designed to inhibit mRNA, it should be possible to design analogs which can inhibit gene expression directly at the DNA level *via* formation of triple stranded complexes. We are presently studying triple strand formation between d-CTT·CTT·TTT·TXT·TTT and a model DNA duplex (d-GAA·GAA·AAA·ATA·AAA/d-TTT·TAT·TTT·TTC·TTC) in which the purine-rich tract of the DNA is interrupted by a pyrimidine. The oligomer contains thymidine, 5-methylcytidine (C) and a single modified base (X) which is designed to hydrogen bond with the interrupting T-A base pair in the A-T tract of the DNA.

Initial Experiments

Interaction of Oligonucleotide Analogs with RNA and DNA The interaction of oligonucleoside methylphosphonates with model RNA targets which contain stem/loop structures will be studied by gel mobility shift analysis on non-denaturing polyacrylamide gels. Various concentrations of the methylphosphonate oligomer are added to solutions of 5'-[^{32}P]-end labeled RNA target and after incubation, the solutions are electrophoresed on a non-denaturing gel. Interaction of the oligomer with the RNA target molecule is indicated by a shift to lower mobility by the target. The amounts of free and bound target are determined and the binding constant of the oligomer calculated by observing the effect of oligomer concentration on the amount of bound target.

The interaction of psoralen-derivatized oligonucleoside methylphosphonates with the RNA target molecules will also be studied. In these experiments, an excess of the oligomer is mixed with the target and the solution is irradiated at 365 nm. The reaction mixture is then subjected to electrophoresis on a denaturing gel. Target RNA which is cross-

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linked with the oligomer has a lower mobility on the gel than does non-cross-linked target. The effects of temperature, oligomer chainlength and target structure on cross-linking will be determined.

The interaction of d-CTT CTT TTT TXT TTT with model dsDNA targets will be studied by the gel mobility shift assay. In this case, one of the strands of the duplex is 5'-end labeled with [^{32}P]-phosphate. The experiments are carried out by adding varying concentrations of d-CTT CTT TTT TXT TTT to the labeled duplex. The binding constants can be determined from an analysis of the effect of concentration on the extent of triple-strand formation.

In all these experiments, the availability of the PhosphorImager will allow direct analysis of the gels and rapid quantitation of the extent of interaction or cross-linking between the oligomers and the target RNA or DNA. The ability to easily analyze the results of binding experiments carried out under a wide variety of conditions will allow rapid determination of binding constants between the oligomers and their targets.

Effect of Antisense Oligonucleoside Methylphosphonates on VSV Protein Synthesis The effects of psoralen-derivatized oligonucleoside methylphosphonates on viral protein synthesis in VSV-infected mouse L-cells will be studied. Mouse L-cells are infected with VSV and concurrently treated with the methylphosphonate oligomer. After incubation for six hours, the cells are irradiated at 365 nm for 5 min. The proteins in cells are then labeled with [^{35}S]-methionine for 30 min. The cells are lysed and the lysate subjected to polyacrylamide gel electrophoresis under conditions which separate the five VSV proteins. The PhosphorImager will enable us to directly quantitate the levels of the VSV proteins. This will allow us to rapidly survey a large number of oligomers for their ability to inhibit protein synthesis and to optimize oligomer concentration and irradiation time in these experiments.

LABORATORY OF S. CHANDRASEGARAN

The recognition by a protein of a specific sequence of bases along a strand of double helical DNA is a key element in many biological phenomena. We have used restriction-modification enzymes as simple model proteins for the study of sequence specific DNA recognition. Our focus is on determining the structure of the enzyme-DNA complexes using x-ray crystallography and NMR spectroscopy, with the goal of understanding the ways in which these different proteins recognize a specific DNA sequence. We have cloned, sequenced and overproduced the products of Hha II, Hinf I and Fok I restriction-modification systems. Standard purification techniques yield large amounts of these enzymes. We have obtained protein crystals of Hha II and Fok I restriction endonucleases and co-crystals of

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Hha II and Hinf I restriction endonucleases with their respective DNA recognition sites. We have also obtained co-crystals of Hha II methylase.

The requested instrument 2D computing densitometer will enable us to determine binding constants by quantitating coomassie stained SDS-PAGE gels and preflashed x-ray films leading to an understanding of the chemical dynamics of these biological macromolecules. Similar experiments will be performed on mutant enzymes and the DNA binding domains of these biological macromolecules. This instrument will also be very useful in our DNA foot-printing experiments. Once, the structure of restriction enzyme-DNA complexes is fully understood, we might be able to design chimeric restriction enzymes with altered specificities that could be useful in mapping and sequencing of large genomes.

The development of rapid techniques for DNA sequence analysis has led to major advances in the field of molecular biology. Nucleotide sequence information is so valuable that projects to determine the nucleotide sequence of the entire human genome ($3x10^9$ bp) and the genomes of other organisms have been initiated.

We have developed a technique for rapid sequencing of DNA that removes the need for cloning and primer synthesis. Recently, an article on a related topic was published by Sorge and Blinderman (December, 1989). The idea is based on these simple facts: (a) The 4 bp restriction enzyme sites are expected to occur once every 256 bp while the 5 bp sites (partially degenerate at the central bp) are expected to occur once every 512 bp in a random distribution of these sites on a DNA fragment. (b) Most restriction enzymes cleave sites only in double-stranded DNA and not single-stranded sites or hemimethylated sites. This method is based on the partial exo III digestion of duplex DNA from one end (the other end being blocked by a restriction cut producing a 3' overhang). High ratios of exo III to DNA ends results in relatively synchronous digestions such that long 5' overhangs are produced from the unblocked end. After digestion with a restriction enzyme recognizing 4 bp sites, a DNA molecule is produced which consists of a short duplex region and a long single-stranded region which serves as a template for the sequencing reaction. The duplex region has the necessary fixed-end for the sequencing procedure, and also serves as the primer for the standard Sanger dideoxynucleotide chain termination reaction.

We have also successfully modified the method by carrying out the sequencing reactions with 5-methyl dCTP instead of dCTP. The newly synthesized hemimethylated region of the DNA will be resistant to digestion by restriction endonucleases inhibited by 5-methyl dCTP. Since, in this approach, cutting follows the sequencing reaction, we can

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utilize all restriction enzymes even those that leave 5' overhangs. We use these procedures to sequence 3 kb DNA fragments routinely.

The PhosphorImager from Molecular dynamics will greatly facilitate the analysis of the DNA sequencing gels and is much quicker than film. It would save considerable time for the graduate student (Lin Li) and the postdoctoral fellow (Cynthis Skoglund) in my lab.

LABORATORY OF PATRICIA GEARHART

Objectives and Accomplishments of the Research Program

Somatic mutation of variable (V) genes of antibodies is a remarkable process that introduces genetic diversity for the purpose of creating variant genes to encode proteins with higher affinity for antigen. My research is directed towards determining the structural and enzymatic requirements for mutation. The first goal is to identify potential *cis* DNA sequences around rearranged V genes encoding heavy and light chains that target them for mutation. We have identified a two kilobase region surrounding and including V genes that contains mutations at a frequency of 1%. Mutations are centered around the rearranged V gene, suggesting that secondary structures may template mutation by mismatch repair. In order to identify the actual DNA sequences that cause mutation, we devised an assay in which a plasmid containing a V gene and an adjacent reporter gene was used to make transgenic mice. The mice were immunized and the plasmid was rescued. Mutation was scored on an indicator strain of bacteria and confirmed by nucleotide sequencing. The ability to manipulate genes in a system where they underwent mutation allowed us to analyze several different constructs to determine why mutation is targeted to the V gene. Our results showed that mutation in the reported gene (i) is tissue specific, (ii) is dependent on its position proximal to the V gene, and (iii) requires transcription of the V gene.

The second goal is to determine the molecular mechanism of mutation, which is likely to occur during DNA repair or replication. Previously, we identified a time window of 3-7 days after immunization when mutation occurs *in vivo*. For *in vitro* studies, mice are immunized, and splenic B cells are taken during this time period to prepare nuclear extracts that potentially contain mutation enzymes. The extracts are assayed for enzymes involved in DNA repair of plasmids containing V gene substrates. Potential enzymes include proteins binding to the substrate (assayed

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by gel retardation), nicking enzymes (assayed by specific nicking in the V gene), and DNA polymerases. These structural and biochemical approaches should give insight into this extraordinary mechanism for generating diversity.

The project, "Antibody Diversity", is being performed by Patricia J. Gearhart as Principal Investigator, and is funded by NIH for 3 years at $279,000 direct costs and by the American Cancer Society for 2 years at $111,740 direct costs.

Use of the Phosphorimager and Computing Densitometer-

Initial experiments using the Phosphorimager and Computing Densitometer would take advantage of the rapid and quantitative features of the machines. For example, we plan to construct plasmids containing deletions of the V gene to identify *cis* sequences that target DNA for mutation and repair. Since transcription is necessary for mutation to occur, each plasmid must be assayed for transcription in B cells. In a recent publication, we used nuclear run-off assays to determine the rate of transcription. The low specific activity of RNA labeled *in vitro* forced us to expose the X-ray film for 3 weeks. It would obviously be a great advantage to speed up the exposure time. Furthermore, each plasmid will be used to make transgenic mice and to transfect B-cell lines. The number of genes integrated into chromosomal DNA must be assayed by Southern analysis and quantified in order to know how many substrate molecules are present. The Phosphorimager can rapidly quantify bands within five orders over a linear range; we currently have to run gels with different concentrations of genomic DNA and marker plasmids and perform multiple exposures to approximate the number of copies per genome. Stable secondary structures in the V gene, such as palindromes that are generated by transcription, are measured by relaxation of super-coiled DNA, which is then analyzed by gel electrophoresis and stained with ethidium bromide. The Computing Densitometer would permit quantitative analyses of conformer distribution of each supercoil, which is not possible with the current densitometer in the department.

For studies of enzymes involved in DNA repair, we are collaborating with Dr. L. Grossman, who is an expert in the field. We have identified a 3' to 5' exonuclease that is present in B cells but not in fibroblast cells. The enzyme is assayed by excision of nucleotides from a 5' ^{32}P-labeled DNA substrate, and analyzed on gels. The Phosphorimager would permit sequential exposures of gels to determine the rate and specificity of the enzyme, which is not possible with conventional radioautography. The imager would also be used to detect proteins bound to the V gene, as assayed by mobility shift gels. The small amount of protein bound to the substrate currently requires exposure of X-ray film

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for 3-4 days. The analysis of proteins recognizing truncated V genes, which would be less, would only be feasible with an instrument like the Phosphoimager, which is 100 times more sensitive than X-ray film.

In summary, these enzymatic and structural studies of mutation and repair in antibody V genes complement studies by Dr. Grossman on enzymes in DNA repair from humans, which is funded by the DOE. Availability of the requested instruments would accelerate the analysis of data that is currently obtained from X-ray films, and would enable us to perform experiments that we cannot do now, such as detection of proteins bound to V genes.

LABORATORY OF JOHN SCOCCA

Project Title: DNA Recognition in Site-specific Recombination

Funding Agency: American Cancer Society

Sponsor Number: MV385B

Period of Award: 1-1-88 to 12-31-92 (additional year also committed)

Amount of Award: $90,000 per annum total

Site-specific recombination systems are involved in producing a wide spectrum of functionally important rearrangements in both prokaryotic and eukaryotic genomes. The reaction common to all these systems is the reciprocal conservative breakage and reunion of DNA segments at specific sites which have only limited stretches of sequence homology, and which therefore must come into apposition by processes other than base-pairing. Site specific recombination reactions are of considerable experimental interest in their own right and as models for other processes in which multiple specific DNA-protein interactions must be established at separated sites on the target DNA in order for reaction to occur. Our laboratory has established an in vitro site-specific recombination system derived form the lysogenic phage HP1 of *H. influenzae* Rd. The integration of the HP1 genome occurs at a site which is part of the structural gene for leucyl tRNA, and which lies within an operon of three *H. influenzae* tRNA genes. This property of insertion within a tRNA gene sequence establishes that the HP1 integration system is related to insertion systems of a diverse group of phages and other insertible elements, all of which use tRNA genes as insertion targets.

Our studies have demonstrated that specific recombination between *H. influenzae* (*attB*) and HP1 (*attP*) sites is produced by a HP1-encoded integrase. The efficiency of recombination is increased by an accessory protein, and the recombination is directional. The components of the reaction, HP1 integrase, the accessory factor, and the DNA substrates, each been purified and characterized. We have defined the interactions of the integrase and the accessory

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protein with the DNA substrates, and have identified the precise points at which strand exchanges occur on recombination. These results have identified two different sequence elements which are recognized by the HP1 integrase. The distribution of these two types of site suggests that important mechanistic differences wxist between HP1 integration and λ integrative recombination. The accssory protein from *H. influenzae* has been purified to homogeneity: it has considerable similarity to integration host factor (IHF) of *E. coli*.

Efficient recombination requires the assembly of a multiprotein complex containing both substrates: this assemblage is held together by bridged protein-DNA interactions, by protein-protein interactions, and by intrinsic and protein-induced bends in one DNA substrate. A biochemical analysis of the individual binding steps is fundamentally important for understanding the self-assembly and reactivity of the complex. Studies aimed at achieving this analysis are in progress, and will make substantial use of the instrumentation requested in this proposal. We are defining the quantitative interaction of integrase with sites within *attP* by determining binding constants for synthetic oligonucleotides containing individual elementary binding sites. These experiments presently rely on 'cut and count' methodology for quantitation, and direct instrumental measurements will improve the quality of the data as well as increase the efficiency and sensitivity of the assays. The extent of bending induced by *H. influenzae* IHF and by HP1 integrase with binding sites will be assessed using several methods which again will be more readily quantitated and analyzed with direct 2-D estimation instrumentation.

BUDGET

The Department of Biochemistry is located in the School of Hygiene and Public Health of the Johns Hopkins University. The School of Hygiene and Public Health is an academically and fiscally independent division of the University. Relative to the Medical School and to the School of Arts and Sciences, our School is by far the poorest unit in the University. Our School, which depends almost entirely on overhead generated by research grants and contracts (i.e., is a "soft-money" institution), is undergoing difficult financial times currently, mostly due to increased competition for dwindling NIH funds in the past couple of years and because of large losses of aid from the State of Maryland occasioned by the downturn in the economy in this region of the country. We have been informed by our new Dean, Dr. Al Sommer, who was appointed to his position September 1, 1990, that our School presently has a $4-5 million deficit in committed capital projects. He accordingly, has placed a moratorium on the initiation of new capital

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projects until this fiscal crisis is resolved. Thus, we, unfortunately, were not able to obtain any commitment from the School for cost sharing in the purchase of the requested instruments.

In view of the fact that the requested instruments are critical to the research needs of a significant portion of the faculty and graduate students in the Biochemistry Dept., the Department Chairman, Dr. Roger McMacken, has committed $10,000 of extremely limited Department general funds (non-Federal money) towards the purchase price of the PhosphorImager and the Computing Densitometer. Although the Department wishes it could provide additional financial support, $10,000 represents a significant sacrifice for the Department and may mean the Department will have to postpone recruiting a new faculty member for another year. The Department will also contribute $500 towards shipping and handling and an estimated $1000 towards renovation of the room where the instruments would be located. The Biochemistry Department has, as discussed earlier, agreed to pay mandatory space charges to the School for the space occupied by the equipment for the useful lifetime of the two instruments. The Biochemistry Dept. has also agreed to pay for service contracts for the instruments (for a period of 5 years following termination of DOE support for maintenance), if sufficient funds are for any reason unavailable from the collective research grants of the investigators.

Additionally, the participating faculty have agreed to provide a total of $5000 from their NIH research grants towards the purchase price of the two instruments. They will also provide a minimum of $1300 from their limited equipment funds of their grants to help defray shipping and installation expenses. This brings the total institutional and investigator support to $20,000. We certainly realize this limited cost sharing could weaken our proposal, but we simply do not have the resources to do more at the present time.

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Curriculum Vitae

Name: Paul O.P. Ts'o

Date of Birth: [redacted]

Education:

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Research and Professional Experience:

1955-1961 Research Fellow, Biology Division, California Institute of Technology

1961-1962 Senior Research Fellow (ranked as Associate Professor) Biology Division, California Institute of Technology

1962-1967 Associate Professor of Biophysical Chemistry, Department of Radiological Sciences, Johns Hopkins University

1967-1973 Professor of Biophysical Chemistry, Department of Radiological Sciences, Johns Hopkins University

1987-1988 Interim Director, Division of Toxicological Sciences, Department of Environmental Health Sciences, Johns Hopkins University, School of Hygiene and Public Health

1973-1990 Professor and Director, Division of Biophysics, Department of Biochemistry, Johns Hopkins University, School of Hygiene and Public Health

1973-present Professor, Department of Biochemistry, Johns Hopkins University, School of Hygiene and Public Health

1980-present Professor, Department of Environmental Health Sciences (Joint Appointment), Johns Hopkins University, School of Hygiene and Public Health

Publications:

1. Au, L-C., Ts'o, P.O.P.: *"A general method for detecting rearrangements in a bacterial genome."* Proc. Natl. Acad. Sci. 86:5507-5511, 1989.

2. LeBlond, G., Ts'o, P.O.P.: *"Discriminatory 32P 3'-end labeling of restriction endonuclease co-digested DNA fragments."* Analytical Biochemistry 177:413-418, 1989.

3. Yi, M., Au, L-C., Ichikawa, N., Ts'o, P.O.P.: *"Enhanced Resolution of DNA Restriction Fragments: A Procedure by Two-Dimensional Electrophoresis and Double-labeling."* Proc. Natl. Acad. Sci., 87(10):3919-3923, 1990.

4. Kulka, M., Smith, C., Aurelian, L., Fishelevich, R. Meade, K., Miller, P.S., Ts'o, P. O.P.: *"Site specificity of the inhibitory effects of oligonucleoside methylphosphonates complementary to the acceptor splice junction of herpes simplex virus type 1 immediate early mRNA 4."* Proc. Natl. Acad. Sci. 86:6868-6872, 1989.

5. Yu, Z., Chen, D., Black, R., Blake, K., T s'o, P.O.P., Miller, P.S., Chang, E.: *"Sequence specific inhibition of in vitro translation of mutated or normal ras p21."* J. Exper. Path., 4:97-108, 1989.

Curriculum Vitae

Name: McMacken, Roger

Date of Birth: [redacted]

Education:

[redacted]

Research and Professional Experience:

1990-	The E.V. McCollum Professor of Biochemistry and Chairman, Department of Biochemistry, The Johns Hopkins University School of Hygiene and Public Health
1986-1990	Professor, Dept. of Biochemistry, The Johns Hopkins University
1983-1986	Associate Professor, Dept. of Biochemistry, The Johns Hopkins University
1977-1983	Assistant Professor, Dept. of Biochemistry, The Johns Hopkins University
1974-1976	Postdoctoral Fellow, Dept. of Biochemistry, Stanford University (Advisor: Dr. Arthur Kornberg)
1970-1974	Postdoctoral Fellow and Research Associate, Dept. of Radiobiology, Yale University and Dept. of Biochemistry, University of Florida (Advisor: Dr. Richard Boyce)
1965-1970	Graduate Research Assistant, Dept. of Biochemistry, Univ. of Wisconsin (Advisor: Dr. H. Echols)
1964-1965	Honors Undergraduate Research, Dept. of Chemistry, Univ. of Washington (Advisor: Dr. W. Scott Chilton)

Honors and Awards:

1964	Elected to Phi Lambda Upsilon (National Chemistry Honor Society)
1966-1970	National Aeronautics and Space Administration Predoctoral Fellowship Awardee
1970-1973	National Institutes of Health Postdoctoral Fellowship Awardee
1989-	Elected to Publications Committee of the Amer. Soc. for Biochemistry and Molecular Biology
1986	Meeting organizer of UCLA Symposium on DNA Replication and Recombination
1988	Meeting organizer of EMBO international workshop on "Gene Organization and Expression in Bacteriophages
1989	Session chairperson of Gordon Conference on Nucleic Acids
1991	Keynote Speaker, FASEB-ASBMB Symposium on DNA Replication

Editorial Boards and Review Panels:

1985-	Editorial board of *Nucleic Acids Research*
1989-	Editorial board of *The New Biologist*
1989-	Member: NIH Microbial Physiology and Genetics Study Section

Publications:

1. Mensa-Wilmot, K., Seaby, R., Alfano, C., Wold, M. S., Gomes, B. and McMacken, R. (1989) "Reconstitution of a Nine-protein System That Initiates Bacteriophage λ DNA Replication". *J. Biol. Chem.* **264**, 2853-2861.

2. Alfano, C. and McMacken, R. (1989) "Ordered Assembly of Nucleoprotein Structures at the Bacteriophage λ Replication Origin During the Initiation of DNA Replication". *J. Biol. Chem.* **264**, 10699-10708.

3. Alfano, C. and McMacken, R. (1989) "Heat Shock Protein-Mediated Disassembly of Nucleoprotein Structures is Required for the Initiation of Bacteriophage λ DNA Replication". *J. Biol. Chem.* **264**, 10709-10718.

4. Mensa-Wilmot, K., Carroll, K., and McMacken, R. (1989) "Transcriptional Activation of Bacteriophage λ DNA Replication *in Vitro*. Regulatory Role of Histonelike Protein HU of *Escherichia coli*." *EMBO J.* **8**, 2393-2402.

5. Mallory, J.B., Alfano, C. and McMacken, R. (1990) "Host-Virus Interactions during the Initiation of Bacteriophage λ DNA Replication: Recruitment of *Escherichia coli* DnaB Helicase by the Bacteriophage λ P Replication Protein." *J. Biol. Chem.* **265**, 13297-13307.

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CURRICULUM VITAE

NAME: LAWRENCE GROSSMAN

Department of Biochemistry
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Status:
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PROFESSIONAL EXPERIENCE:

1989-	University Distinguished Service Professor Department of Biochemistry, The Johns Hopkins University
1975-89	The E.V. McCollum Professor and Chairman The Johns Hopkins University
1967-75	Professor - Biochemistry - Brandeis University
1962-67	Associate Professor - Biochemistry - Brandeis University
1957-62	Assistant Professor - Biochemistry - Brandeis University
1956-57	Scientist - National Institutes of Health
1954-56	Postdoctoral Fellow - The Johns Hopkins University
1942-45	U.S.Naval Aviator

AWARDS AND FELLOWSHIPS:

1944-45	Distinguished Flying Cross (2), Air Medal
1951-54	Predoctoral Fellow of the National Institutes of Health
1954-56	Postdoctoral Fellow of the National Institutes of Health
1964-74	Research Career Development Awardee of the NIH
1964-65	Commonwealth Fund Fellow at L'Institut Biologie Moleculaire, L' ecole du Medicin, Universite de Geneve, Switzerland
1973-74	Guggenheim Fellow - University of Tel-Aviv, Israel
1987-Summer	Burroughs Wellcome Fellow to the Imperial Cancer Research Fund, London, UK
1989-Fall	Burroughs Wellcome Fellow to the Imperial Cancer Research Fund, London, UK
1989-1999	Merit Award from NIH

EDITORIAL BOARDS:

Cancer Research
Methods in Enzymology
Critical Reviews of Biochemistry
Journal of Biological Chemistry
Chemtracks

PUBLICATIONS (114 - 5 representative references)

Oh, E.Y. and Grossman, L., Characterization of the Helicase Activity of the Escherichia coli UvrAB Protein Complex. (1989), J. Biol. Chem. **264**, 1336-1343

Seeley, T.W., and Grossman, L., Mutations in the Escherichia coli UvrB ATPase motif compromise excision repair capacity (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 6577-6581.

Hansson, J., Grossman, L., Lindahl, T. and Wood, R.D. Complementation of the xeroderma pigmentosum DNA repair synthesis defect with Escherichia coli UvrABC proteins in a cell-free system. (1990) Nucleic Acids Res. 18:35-40.

Seeley, T.W., and Grossman, L., (1989). Role of Escherichia coli UvrB in Nucleotide Excision Repair, J. Biol. Chem., Vol. 13, 265:7158-7165.

Grossman, L., Yeung, A.T. (1990) The UvrABC endonuclease system of Escherichia coli - A View from Baltimore. Mut. Res. **236**, 213-221.

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Curriculum Vitae

Name: Floyd R. Bryant

Title: Assistant Professor

Birthdate: [redacted]

Education:

[redacted]

Professional Experience:

1986-present	Department of Biochemistry, Johns Hopkins University
1982-1986	Department of Biochemistry, Stanford University School of Medicine Bank of America-Giannini Foundation Postdoctoral Fellow (1984-1985) Damon Runyon-Walter Winchell Cancer Fund Postdoctoral Fellow (1982-1984)

Recent Publications:

Menge, K.L. and Bryant, F.R. (1988) "ATP-stimulated hydrolysis of GTP by the recA protein: kinetic consequences of cooperative recA protein-ATP interactions." Biochemistry 27, 2635-2640.

Bryant, F.R. (1988) "Construction of a recombinase-deficient mutant recA protein that retains single-stranded DNA-dependent ATPase activity." J. Biol. Chem. 263, 8716-8723.

Bryant, F.R., Menge, K.L., and Nguyen, T.T. (1989) "Kinetic modeling of the recA protein promoted renaturation of complementary DNA strands." Biochemistry 28, 1062-1069.

Muench, K.A. and Bryant, F.R. (1990) "An obligatory pH-mediated isomerization on the [asn-160]recA protein-promoted DNA strand exchange reaction pathway." J. Biol. Chem. 265, 11560-11566.

Muench, K.A. and Bryant, F.R. (1990) "Disruption of an ATP-dependent isomerization of the recA protein by mutation of histidine-163." J. Biol. Chem. (in press).

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Curriculum Vitae

Name: Paul S. Miller

Date of Birth: [redacted]

Education:

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Research and Professional Experience:

1969 - 1971	American Cancer Society Postdoctoral Fellow, Department of Radiological Sciences, The Johns Hopkins University
1971 - 1973	Instructor, Division of Biophysics, Department of Biochemistry, The Johns Hopkins University
1973 - 1979	Assistant Professor, Division of Biophysics, Department of Biochemistry, The Johns Hopkins University
1980 - 1988	Associate Professor, Division of Biophysics, Department of Biochemistry, The Johns Hopkins University
1988-present	Professor, Department of Biochemistry, The Johns Hopkins University
1989-present	Associate Editor, Bioconjugate Chemistry
1990-present	Editorial Advisory Board, Antisense Research and Development

Publications

1. Lee, B.L., Murakami, A., Blake, K.R., Lin, S-B., P.S. Miller.: "Interaction of psoralen derivatized oligodeoxyribonucleoside methylphosphonates with single-stranded DNA, Biochemistry, 27:3197-3203, 1988.

2. Lee, B.L., Blake, K.R., Miller, P.S.: "Interaction of psoralen-derivatized oligodeoxyribonucleoside methylphosphonates with synthetic DNA containing a promoter for T7 RNA polymerase." Nucleic Acids Research, 16:10681-10697, 1988.

3. Kean, J.M., Murakami, A., Blake, K.R., Cushman, C.D., Miller, P.S.: "Photochemical cross-linking of psoralen derivatized oligonucleoside methylphosphonates to rabbit globin messenger RNA." Biochemistry, 27:9113-9121, 1989.

4. Bhan, P., Miller, P.S. "Photo Cross-Linking of Psoralen Derivatized Oligonucleoside Methylphosphonates to Single Stranded RNA", Bioconjugate Chemistry, 1: 82-88, 1990.

5. Miller, P.S., Bhan, P., Cushman, C.D., Kean, J.M., Levis, J.T.: "Antisense Oligonucleoside Methylphosphonates and their Derivatives." Nucleosides and Nucleotides, 1990 (In press).

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CURRICULUM VITAE

Srinivasan Chandrasegaran

PERSONAL DATA

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Professional Experience:

1981 - 1984	Postdoctoral Fellow, The Johns Hopkins University, Department of Biophysics
1985 - 1986	Research Associate, The Johns Hopkins University, School of Medicine, Department of Molecular Biology and Genetics
1986 to date	Assistant Professor, The Johns Hopkins University, School of Hygiene and Public Health, Department of Environmental Health Sciences.

Publications:

Scholl, P., Li, L., Skoglund, C.M., and Chandrasegaran, S. Rapid Sequencing of DNA (in preparation).

Skoglund, C.M., Smith, H.O , and Chandrasegaran, S. Construction of an efficient overproducer clone of HinfI restriction endonuclease using PCR technique. **Gene 88**:1-5 (1990).

Smith, H.O., Annau, T., and Chandrasegaran, S. Finding Sequence Motifs in Groups of functionally related proteins. **Proc. Natl. Acad. Sci.** USA **87**:826-830 (1990).

Chandrasegaran, S., Wu, L.P., Valda, E., and Smith, H.O. Overproduction and purification of M. HhaII methyltransferase from Haemophilus Haemolyticus. **Gene 74**:15-21 (1988).

Chandrasegaran, S., Lunnen, K.D., Smith, H.O., and Wilson, G.W. Cloning and sequencing the HinfI restriction and modification genes. **Gene 70**:387-392 (1988).

Prive, G.G., Heinemann, U., Chandrasegaran, S., Kan, L.-S., Kopka, M.L., and Dickerson R.E. A mismatch decamer as model for general-sequence B-DNA. **In Structure and Expression**, Vol. II: DNA and its Drug Complexes (eds.) R.H. Sarma and M.H. Sarma, Adenine Press, Guilderland, NY (1988), pg. 27-47.

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CURRICULUM VITAE

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Born: [redacted] Illinois
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EDUCATION

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RESEARCH AND PROFESSIONAL EXPERIENCE

1988-present	Associate Professor, Department of Biochemistry, The Johns Hopkins University, School of Hygiene and Public Health
1982-1988	Assistant Professor, Department of Biochemistry, The Johns Hopkins University, School of Hygiene and Public Health
1978-1982	Staff Associate, Department of Embryology, The Carnegie Institution of Washington, Baltimore, MD.

AWARDS

1967-1968	Cornell University Fellowship for graduate school
1975	Sigma Xi Graduate Research Award
1976-1978	Helen Hay Whitney Postdoctoral Fellowship
1982-1984	Basil O'Connor Fellow of the March of Dimes Birth Defects Foundation

REFERENCES

Levy, N. S., Malipiero, U. V., Lebecque, S. G. and Gearhart, P. J. 1989. Early onset of somatic mutation in immunoglobulin V_H genes during the primary immune response. J. Exp. Med. 169, 2007-2019.

Lawler, A. M., Kearney, J. F., Kuehl, M. and Gearhart, P. J. 1989. Early rearrangements of genes encoding murine immunoglobulin κ chains, unlike genes encoding heavy chains, use variable gene segments dispersed throughout the locus. Proc. Natl. Acad. Sci. USA 86, 6744-6747.

Gearhart, P.J. and Levy, N.S. Kinetics and molecular model for somatic mutation in immunoglobulin variable genes. In "Somatic Hypermutation in V-Regions" (E. Steele, ed.) CRC Press, in press.

Lebecque, S.G. and Gearhart, P.J. Boundaries of somatic mutation in rearranged immunoglobulin genes: 5' boundary is near the promoter and 3' boundary is approximately one kb from V(D)J gene. J. Exp. Med., in press.

Umar, A., Schweitzer, P.A., Levy, N.S., Gearhart, J.D. and Gearhart, P.J. Mutation in a reporter gene depends on proximity to and transcription of immunoglobulin variable transgenes. Submitted.

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CURRICULUM VITAE

PERSONAL DATA

Name: John J. Scocca
Social Security No.: [redacted]
Citizenship: USA

EDUCATION

[redacted]

PROFESSIONAL EXPERIENCE

Current:	Professor, Department of Biochemistry, School of Hygiene and Public Health, The Johns Hopkins University.
1987:	Visiting Professor, Centre for Advanced Molecular Biology, University of the Punjab, Lahore, Pakistan.
1975-1986:	Associate Professor.
1968-1975:	Assistant Professor
1966-1968:	NIH Postdoctoral Fellow, McCollum-Pratt Institute.

HONORS AND AWARDS

National Merit Scholar, 1958-1961
Predoctoral Fellowship, NIGMS, NIH, 1961-1965
Postdoctoral Fellowship, NCI, NIH, 1966-1968
Research Career Development Awardee, NIGMS, NIH, 1970-1975

RECENT PUBLICATIONS

Astumian, J. H., Waldman, A. S., and Scocca, J. J. 1989. Site-specific recombination between cloned *attP* and *attB* sites from the *Haemophilus influenzae* phage HP1 propagated in recombination-deficient *Escherichia coli*. J. Bacteriol. **171**: 1747-1750.

Goodman, S. D., and Scocca, J. J. 1989. Nucleotide sequence and expression of the gene for the site-specific recombination protein from bacteriophage HP1 of *Haemophilus influenzae*. J. Bacteriol. **171**: 4232-4240.

Scocca, J. J. 1990. Minireview: The role of transformation in the variability of the *Neisseria gonorrhoeae* cell surface. Molecular Microbiology **4**: 321-328.

Hwang, E. S., and Scocca, J. J. 1990. Interaction of integration host factor from *Escherichia coli* with the integration region of the *Haemophilus influenzae* bacteriophage HP1. J. Bacteriol. **172**: 4852-4860.

Hauser, M. A. and Scocca, J. J. 1990. Location of the host attachment site for phage HP1 within a cluster of *Haemophilus influenzae* tRNA genes. Nucleic Acids. Res. **18**: 5305.

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