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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: Johns Hopkins University, School of Hygiene and Public Health
2. Address: City Baltimore 3. State MD 4. ZIP 21205
5. Principal Investigator: P.C. Huang, Ph.D, Professor
6. Department: Biochemistry
7. Telephone: Area Code 301 Office: 955-3044 Home: 433-0408
8. Title of Application: Purchase of Protein-Peptide Synthesizer and Sequencer (deleted version)
9. Research Area: 5
10. Total DOE Funding for Research in Selected Area, (During the last two calendar years): $
11. Estimated Purchase Cost of Equipment: $ 263,870
12. Amount Requested from DOE: $ 263,870

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

13. Agency: National Science Foundation Agency Proposal Number: DMB 8501471
14. Agency: Agency Proposal Number:

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

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

Agency: Amount of Funds:

16. Please check one of the following :

[X] I authorize external review of this proposal.

[ ] I do not authorize external review of this proposal.

Signature of Principal Investigator: P.C. Huang Date: Dec. 12, 1984

Name and Title of Institutional Official (President or Designee)

Edyth H. Schoenrich, M.D.,M.P.H.

Associate Dean

Signature: Edyth H. Schoenrich

Date: 12-19-84

Name and Title of Financial Officer

Donald I. McLeod, A.B.

Director of Business Management

Signature: Donald I. McLeod

Date: 12-19-84

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U.S. DEPARTMENT OF ENERGY
UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM

BUDGET PAGE

ESTIMATED COSTS

Instrumentation	Requested of DOE	Institution's Share *(1)*	Other Federal Funds *(2)*	TOTAL
A. Purchase Price *(3)*	263,870	-	-	263,870
B. Estimated Cost Sharing	X X X X			
(Shipping, installation, etc.)				
C. TOTAL	263,870	-	-	263,870

NOTES: *(1) Non-Federal funds only*

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

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

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A. **Purchase Price (Detail)**

Description	Quantity	Total Estimated Unit Price	Total
Applied Biosystems Gas-Phase Protein Sequencer (470-A)	1		$108,000
Beckman Peptide Synthesizer Model 990	1		89,600
Preparative reaction vessel kit (350673)	1		3,200
Beckman 346 HPLC binary system with Model 165 detector	1		23,100
Model 450 Data System	1		15,900
Second channel for data system and 128K RAM	1		1,750
Serial communications and software for above system	1		1,750
Autosampler - Model 504	1		9,750
Post column derivatization unit	1		9,000
Column temperature controller	1		1,820
Subtotal:			$263,870

B. **Estimated Cost Sharing (Detail)**

	Institution's Share	Other Federal Funds	Total
1. **Shipping/Handling**			
2. **Installation**, operation & maintenance	27,800		27,800
3. **Building/Laboratory Renovation**			
Subtotal:	27,800		27,800

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DEPARTMENT OF ENERGY
UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM
CONTRACT SUMMARY FORM

Contract/Subcontract No.	Principal Investigator	Title	Contract Dates FM MO	FM YR	TO MO	TO YR	Total Award Value	$ AMT Awarded for period 19 to 19	DOE Technical Monitor/Location
(DOE) EY75022814 A003	L. Grossman	Mol. basis mutagenic and effects of UV irradiation	12	84	11	85	41,333		

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RELATED FEDERAL AGENCY CONTRACTS

Agency	Grants Contract No.	Title	Contract FROM MO YR	Dates TO MO YR	Total Award Value
Gearhart, P. (NIH)	P01ES03131	Cellular response to exogenous agents (30%)		03/31/86	$ 89,745
(NIH)	R01CA34127	Antibody diversity: comparison of genes and proteins (60%)		07/31/85	88,730
(MD)	BOCRSRG5-359	Expression of Ab variable gene during development (10%)		06/30/85	16,667
Grossman, L. (NIH)	P01ES03131	Cellular response to exogenous agents (25%)		03/86	44,163
(NIH)	R01GM31110	DNA repair gene expression in mammalian cells (25%)		06/85	106,182
(NIH)	R01GM22846	Enzymatic excision and repair mechanisms (25%)		05/85	126,841
Huang, P.C. (NIH)	P01ES03131	Cellular response to exogenous agents (25%)		03/31/86	51,849
(NIH)	R01GM32606	Site specific mutation in metallothionein (20%)		11/30/85	87,712
(NIH)	S10RR02410	Gene/gene product synthesizer/sequencer module (20%)		11/30/85	87,500
(NSF)	PCM8104369	Genetics of metallothionein gene sequence analysis (7%)		05/31/85	40,000
U. Med. Sea		Metallothionein gene in striped bass and white perch		12/31/87	11,542

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RELATED FEDERAL AGENCY CONTRACTS

Agency	Grants Contract No.	Title	Contract Dates FROM MO YR	TO MO YR	Total Award Value
Krag, S. (NIH)	K04CA00640	Mutants altered in glycosylation of membrane proteins	04/01/80	03/31/85	$ 39,511
(NIH)	R01CA20421	Mutants altered in glycosylation of soluble and membrane proteins	12/10/76	11/30/87	87,406
Center for Alt. Animal Testing		Potential inflammatory agents; hydrolytic enzymes in culture		12/31/85	19,999
Milman, G. (NIGMS)	1 P01 ES 03131-01	Human DNA Repair Genes This is a joint grant with Dr. L. Grossman as Co-Principal Investigator (30%)	04/01/83	03/31/86	211,184
(NIGMS)	1 R01GM32950	Cloning the Human Folylpolyglutamate Synthetase Gene. Dr. Barry Shane as Co-Prinicipal Investigator	07/01/84	06/30/87	368,467
McMacken, R. (NIH)	P01ES03131	Cellular response to exogenous agents (25%)		03/31/86	57,065
(NIH)	R01GM32253	Initiation of bacteriophage DNA replication in vitro (50%)		06/30/86	104,998

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RELATED FEDERAL AGENCY CONTRACTS

Agency	Grants Contract No.	Title	Contract FROM MO YR	Dates TO MO YR	Total Award Value
Scocca, J. (NIH)	P01SI16959	Sexually transmitted disease research		07/31/85	$ 50,495
(NIH)	R01GM21890	DNA interaction in bacterial transformation		(pending)	80,423
Shane, B. (NIH)	K04CA00697	Regulation of folate-gamma-glutamate synthesis (100%)		08/31/87	36,000
(NIH)	R01CA22717	Regulation of folate-gamma-glumate synthesis (30%)		01/31/89	102,438
(NIH)	R01CA22717	Cloning of human gene for folylpolyglutamate synthetase (20%)		06/30/87	95,498

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4. Summary

A module consisting of a peptide sequencer, a peptide synthesizer, and a common, HPLC plus data system is requested to aid in the research programs of investigators in the Department of Biochemistry, Johns Hopkins School of Hygiene and Public Health.

This equipment is needed for the synthesis of specific antigens, enzyme substrates and inhibitors, and for the characterization of gene products.

The range of projects that will be benefitted include enzyme (dihydrooratase, DNA repair enzymes and folypolyglutamate synthetase) mechanism and function of specific proteins (metal binding protein and DNA replication factors).

A total of nine faculty members and their students will actively use the instruments requested. The department has graduated on the average of three to four doctoral students every year in the last decades. Among those whose are currently enrolled, the majority are involved in projects for which availabilty of the requested instruments become increasingly important to the degree of sophistication of their training and contribution.

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6. Narrative

A. Merit and Accomplishments

(1) Background

The Johns Hopkins University Ph.D. program in Biochemistry is designed to prepare qualified students for a career in basic research and teaching. The Biochemistry Department was founded in 1917 by E.V. McCollum, the discoverer of the first vitamins (vitamin A in 1913, the B complex in 1915, and vitamin D in 1922). We have a young faculty who work in the laboratory and interact closely with their graduate students and postdoctoral fellows every day. At present, our department consists of ten faculty members, twenty-three graduate students, and thirteen postdoctoral fellows working in contiguous quarters on the eighth floor of The School of Hygiene and Public Health. We expect the number of faculty, graduate students and postdoctoral fellows in our department to remain constant in order to preserve a cohesive department with a friendly atmosphere.

The Biochemistry Department in the School of Hygiene and Public Health is one of the departments in this university engaged in basic biochemical and molecular biological research.

Current areas of research include:

1. Enzymology of repair of damaged DNA
2. Mechanisms of glycoprotein and glycolipid biosynthesis
3. Biochemical genetics of animal and bacterial cells
4. Bioorganic chemistry of enzymatic catalysis
5. Biochemistry of environmental carcinogens
6. Enzymology and regulation of DNA replication
7. Biochemistry of genetic tranformation
8. Biochemical nutrition and metabolic control
9. Biochemistry of mutations in animal cells and viruses
10. Design and synthesis of anti-cancer agents
11. Maintenance of chromosome integrity, gene organization and function
12. Membrane function
13. Nutritional factors in carcinogenesis
14. Cloning and expression of recombinant DNA
15. Molecular control of antibody diversity
16. Parasite immunology

These studies have been well recognized through publications see section 6.B.) and funding support (see section 3).

The traditional strength of this group in the study of genetic replication, repair, transformation, and expression is complemented by several other departments at Hopkins such as; Physiological Chemistry, Molecular Biology and Genetics, and Cell Biology and Anatomy at the School of Medicine, the Biology and the Chemistry Departments at the Homewood Campus as well as the Human Genetics and Environmental Health training Programs, two

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university wide activities. Graduate students in the Divisions of Medicine and Public Health take the same course work. Features of our graduate program include laboratory rotations, unique format for the written preliminary exam, and our presence in the School of Hygiene and Public Health, which acquaints us with problems in Public Health to which biochemistry and molecular biology can be applied. With the concerted and dedicated efforts of the faculty, and commitment of the university, the Biochemistry research and teaching activity is expected to maintain its momentum and high quality in the years to come.

(2) General Need

This application is for equipment for the synthesis and sequencing of peptides and proteins, to aid in the research efforts of nine faculty members and thirty-six pre and postdoctoral fellows in the Department of Biochemistry at Johns Hopkins University School of Hygiene and Public Health. The research interests of the applicants in this grant are varied, ranging from DNA replication to nutrition. However, in each case, sequencing and/or synthetic techniques for peptides and proteins play a fundamental role in the conduct of their research; the proposed uses involve the synthesis of peptides for the production of antibodies or as enzyme inhibitors and the sequencing of proteins or peptides for the study of enzyme mechanisms, and the sequencing of proteins as a prelude to the synthesis of specific gene probes. These research efforts, which are outlined in section 6.B. have been greatly hampered by the lack of modern equipment.

Protein and peptide sequencers aid in the rapid indentification of proteolysis sites, the characterization of substrate binding sites and functional domains of proteins. The sensitivity of modern equipment allows the sequencing of proteins isolated from gels which permits the characterization of small amounts of protein and also provides the data required for the synthesis of specific DNA probes for the genes coding for these proteins. Peptide synthesizers allow the convenient synthesis of modified polypeptides which can be used to map substrate binding sites of proteins, as potential inhibitors of some enzymes, as models for DNA-protein or metal-protein interactions, and for the generation of antibodies to specific regions of proteins (Hunkapiller et al. 1984 Nature 310:105-111).

Some of these techniques are currently being employed by the investigators listed in this application. However, the equipment and methodologies current available have severely limited their studies in terms of time and convenience. For instance, peptide derivatives are being synthesized by tedious manual methods and peptide analysis is limited to proteins that can be isolated in larger amounts. No facilities are available for the analysis of small amounts of polypeptides. The lack of the equipment requested in this application has directly impaired the research efforts of the investigators, both in their current research

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endeavours and in their abilities to enter new areas of research.

(3) Description of Instrument

Because of the multi-user nature of this proposal, instruments were selected, where appropriate, because of their simplicity of operation and their ability to be programmed for different operations. The different operating parameters required by different investigators can be easily set and stored, thus allowing an efficient changeover in operation conditions as required by any investigator without disturbing the settings of any other user.

(i) Peptide synthesizer (estimated cost $92,800)

The Beckman peptide synthesizer is more expensive than the synthesiser distributed by Vega. However, it has the advantage of allowing greater flexibility in the scale of synthesis. The scale required by the individual investigators will range from a few micromoles for the generation of potential antigens to about 10-20 mmoles for the synthesis of enzyme substrates and inhibitors.

The Model 990B allows the operator to program a large variety of potential synthetic strategies. Mixing is accomplished by a magnetic coupled stirrer, as opposed to the 180°C tumbling action of the Vega instrument. This allows a savings in solvents and reduces the loss of resin, especially with small scale syntheses. The ability to use more concentrated solutions and still effect efficient mixing increases the yield as the coupling steps are concentrated dependent, and also reduces the generation of side products. The Beckman synthesizer contains one reaction vessel, two mixing vessels, and can deliver combinations of up to nine different reagents and two coupling reagents. A preparative reaction vessel (20g resin capacity) for large scale synthetic work will be required for the larger syntheses.

(ii) Protein-Peptide sequencer (estimated cost $108,000)

The Applied Biosystems model 470A gas-phase protein sequencer automatically performs Edman degradation of proteins and peptides and converts the products to stable PTH amino acid derivatives, which are collected in vials for subsequent HPLC analysis.

A gas phase sequencer is necessary because of the high sensitivity required for the sequencing of proteins and peptides isolated from gels. The high sensitivity also allows extended sequencing with larger amounts of protein.

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(iii) <u>High Performance Liquid Chromatography unit</u>
(estimated cost $63,070)

An HPLC system is required for the identification of PTH amino acids, and the seperation and purificaion of proteins and peptides.

The Beckman 346 system is a gradient system that includes a scanning UV-VIS detector. A modular system was chosen as this is more versatile than single pump systems, such as the Varian, and allows the separation of pumps for isocratic elutions. The Beckman system is similar to the Waters system, and is comparable in price. There does not seem to be any obvious advantages or disadvantages between the Beckman and Waters systems.

An HPLC autosampler and a data system are required for the unattended identificaition of PTH amino acids. A post column derivatization system, consisting of pumps and a fluorometer, and a column heater are required for amino acid analyses.

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(4) Overall objective of the Program

The equipment and facilities for protein synthesis and analysis requested in this proposal will be used to further the research objectives of the participating investigators. In general, the objectives of the group focus on understanding fundamental mechanisms involved in the duplication, maintenance, repair and recognition of DNA, and studies on key enzymes active in metabolism of nucleic acids and their precursors. Most investigations underway within the group either currently employ molecular cloning methodology or will involve these approaches in the immediate future. Several of the investigations, specifically those concerning DNA repair enzymes, metallothionein, and the enzymatic machinery of DNA replication, have reached the stage at which characterization of protein products becomes imperative. Since it is critical to establish that the gene products isolated by cloning techniques are identical to those which participate in the systems under study, it is necessary to compare the sequences of cloned products (available in substantial quantities) with those of naturally occurring gene products (which are usually present in extremely low amounts). The capacity to sequence small amounts of protein is central to these studies, and the needed instrumentation is part of this request.

The synthesis of defined peptides will be required in several projects. These include the investigations on the mechanism of action of metallopeptidases, for which model substrates will be synthesized; studies on the immune response to parasitic infections, in which model peptide antigens will be required; and studies on the detoxification and sequestering of heavy metals, in which predictions concerning the modes of metal binding by specific proteins need to be explored. The possibility of synthesizing peptide analogs which may mimic specific DNA-protein interactions is in the more distant future, but we expect that studies of this type will be forthcoming. Both the preparation of proteins for microscale sequencing and the synthesis of defined peptides will require sophisticated fractionation capability, for which a state-of-the art HPLC system is required.

(5) Impact of DOE areas

The research interest and contributions of this group will exert impact in the understanding energy-related factors on biological systems. The studies concerning gene product structure and function, regulation of gene expression, replication and repair of genes and fundamental aspects of cell transformation are specified in catagory 5 of DOE supportive areas.

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(6) Major Accomplishments in last five years.

a. DOE supported activity

Grossman:

Cloned and characterized UVr A,B,C, and D genes essential for E. coli DNA repair; demonstrated through catalytic and biochemical evidence the interaction of these gene products in the repair of ultraviolet irradiation induced damages in DNA; through genetic transfection experiments showed that coli repair genes can complement human genetic defects for DNA repair in cultures of cells from Xeroderma pigmentosum; completely sequenced UVr A and C genes. Determination of UVr B gene primary sequence and X-ray crystallography of UVr A are in progress. These studies are fundamentally important to energy effect on biological systems, including man.

b. Supported by other sources

Collins:

Successfully cloned a gene for E. coli dihydroorotase, a zinc containing metalloamidase which is important in pyrimidine biosynthetic pathway. With appropriate vector/host, milligram quantity of this enzyme in active form has been obtained. This success has allowed extensive study of the catalytic mechanism of dihydroorotase by means of protein sequence modification, steady state kinetics, isotopic effects, as well as other physical characterization including proton and 13C NMR, EPR. magenetic circular dichroism and X-ray crystallography.

Gearhart:

Determined that heavy and light chains of Ig G and Ig A anthodies underwent frequent mutations, in contrast to Ig M which mutated rarely and, proposed a model for immunoglobulin variable gene diversity; undertook detailed DNA sequence analysis flanking these genes and identified that frequent changes during ontogeny are localized in a small region. Lymphocytes are being used to detect signals and mechinery that regulate this developmental process.

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Huang:

By recombination in vitro and by site specific mutagenesis, created a repertoire of mutants for the coding sequence of metallothionein, a transition metal binding protein. The mutated sequences have been cloned into high expression vectors with the purpose of obtaining a large quantity of mutated proteins for structure-function study.

Other accomplishments include induction and establishment of a collection of metal resistant CHO mutants, many of which show either transport alteration or amplification of metallothionein genes. Also successfully prepared metallothionein antibody and used it in histochemical localization of metallothoinein in differentiated cells and in identification of in vivo and in vitro metallothionein gene products.

Krag:

Isolated and established mutants of Chinese hamster ovary cells which are either defective in mannosylphorsphoryl dolichol synthesis or shown resistance to tunicamycin. These mutants allowed studies in two parts: the former to correlate mannosylphorsphoryl dolichol with altered mannose 6 phosphate receptor and to understand protein glycosylation.

McMacken:

With concerted efforts on the study of phage DNA replication and its regulation, a series of protein factors which are involved in the initiation step has been identified and characterized. Two λ -coded initiator proteins, have been studied in detail, through cloning their genes. Their interaction with other essential replication proteins and DNA is being further studied.

Milman:

While earlier accomplishments center around the study of E. coli guanine-xanthine phosphoribosyl transferase, more recent achievements include the cloning of genes for:

(a) Herpes type 1 and 2 and Epstein - Barr viral proteins.
(b) antigens for a parasite *D. immitis* which causes dog heart worm.
(c) human folypolyglutamate synthetase (in collaboration with B. Shane)

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Scocca:

Identified the sequence essential in gonococcal transformation; refined DNA recognition events in Hemophilus transformation; obtained evidence for the existence of receptor proteins which act in these specific recognition events; characterized a bacteriophage of H. influenzae with respect to its cohesive termini and its site of integration, and are currently studying the mechanism of site-specific recombination by use of molecular cloning techniques.

B. Qualification of faculty

Research activities of the users described below are grouped into three parts:

a. extended description of work in users' laboratory;
b. brieff statement of additional major users and
c. a list of representative projects by the pre and postdoctoral fellows that will be benefited by the instrument.

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(1) Major Users

a. Kim D. Collins

DIHYDROOROTASE: CATALYSIS AND INHIBITION

Dihydroorotase (L-5,6-dihydroorotate amidohydrolase, E.C. 3.5.2.3) is an enzyme in the biosynthetic pathway of the pyrimidines which cyclizes N-carbamyl-L-aspartate to form L-5,6-dihydroorotate. We have undertaken a systematic program to determine the catalytic mechanism of action of the E. coli and hamster kidney enzymes, and to develop effective mechanism-based inhibitors for them; these inhibitors may be useful as anti-proliferative drugs. Dihydroorotase is an especially appealing object of study for at least two reasons:

1. Being a single substrate enzyme, productive enzyme-substrate complexes can be studied by the techniques described below. [This is not true of the other carboxypeptidase-type metallo-amidases.]

2. Of all the enzymes in the biosynthetic pathways for the purines and pyrimidines, dihydroorotase and the subsequent dehydrogenase are the only two which have non-phosphorylated substrates. Thus any mechanism-based inhibitors that we develop have a good chance of penetrating mammalian cells.

We have purified milligram quantities of the E. coli enzyme and shown it to be a dimeric enzyme of subunit MW 41,000 containing 1 atom of tightly bound Zn (II) per subunit (Washabaugh and Collins, 1984). We have also shown that the solutions containing the enzyme must be free of trace contaminants (Washabaugh and Collins, 1983). This is an important enzyme about which very little is known. Although the E. coli and hamster kidney enzymes have different structures, preliminary evidence suggests that they have similar catalytic mechanisms. The gene for E. coli dihydroorotase has a minimum molecular weight of 1.1kb. We have now cloned a 1.7kb E. coli DNA fragment into the high copy number vector pKC16, which is pKC7 containing the lambda origin of replication and a temperature sensitive lambda repressor gene (CI-857); when the temperature is raised from 30° to 42°, the plasmid makes many copies of itself. We have been getting 25% of the soluble protein as dihydroorotase in one liter cultures, and we are now preparing for large scale production of the enzyme. The E. coli pyr c gene is being sequenced by Lennent G. Lundberg in Sweden andd the x-ray structure of the enzyme will be determined by David Evans of Wayne State University.

We are using or soon will be using the following techniques to determine the mechanism of action of dihydroorotase (the experiments described below will initally be done on the E. coli enzyme).

a. To prove that the overproduced enzyme is identical in structure to the "wild type" that we have already purified, we will characterize peptide maps of both enzymes by HPLC and sequence any peptides that appear to differ between the preparations.

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b. We have developed three affinity labels which, in preliminary experiements, inactivate the enzyme in the absence but not the presence of the substrate: bromoacetate, N-(bromoacetyl)-glycine, and N-(acryloyl)-glycine. Using radioactive affinity labels, we will modify the protein, digest with proteases, and sequence the resulting peptides to identify amino acid residues at the active site of the enzyme.

c. The enzyme as isolated appears to have structural Zn^{+2} ions removed, exposing a sulfhydryl (which can be modified chemically) which makes the apoenzyme very susceptible to air oxidation. Using cleavage and sequencing procedures described above, we will identify this sensitive sulfhydryl.

d. We will also obtain functional evidence for a general acid-base at the active site by examining proton exchange with the solvent from substrate analogues in the presence of the enzyme.

e. The rate limiting step of the reaction will be determined by steady state kinetics using solvent isotope effects and by using fluorinated substrate analogues.

f. The metal ion's role in the catalytic mechanism, its protein ligands, and its geometric relationship to the substrate will be determined by substituting paramagnetic Co (II) and Mn (II) for the active site Zn (II), and then examining the substrate or substituted enzyme by ^{1}H and ^{13}C nuclear magnetic resonance, electron paramagnetic resonance, magnetic circular dichroism, and visible electronic spectroscopy. Much of this work will be done in collaboration with Al Mildvan of the School of Medicine.

g. The above information will be used as a guide to develop mechanism-based inhibitors of this enzyme.

In addition to the above work on dihydroorotase, we are interested in developing inhibitors for the mechanistically related metal-dependent carboxypeptidases, and an automated synthesizer would be a great help in making these peptide derivatives.

Access to an amino acid analyzer, a protein sequenator, and an HPLC unit (for peptide separation) is essential for the completion of this work. At present there is only one HPLC unit available to the department as a whole, and it is difficult to obtain time on it. Our amino acid analyses and sequenator runs are presently done on antiquated instruments 4 miles away in the laboratory of Dennis Powers. It is very difficult to obtain time on these machines and long delays are common. It would facilitate our work enormously to have available within this department the amino acid analyzer (HPLC), sequenator, and synthesizer which are requested in this application.

Washabaugh, M.W. and Collins, Kim D., Dihydroorotase from Escherichia coli. Purification and Characterization. J. Biol. Chem. 259 3293-3298 (1984).

Washabaugh, M.W. and Collins, Kim D., Purification of Aqueous Ethylene Glycol, Analyt. Biochem. 134 144-152 (1983).

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b. Patricia J. Gearhart
Rearrangement of Antibody Variable Genes During Ontogeny

Mechanisms that control the developmental expression of genes in a multigene family are not understood. One of the goals of my laboratory is to determine if there is a developmental order to the rearrangement of antibody variable genes. There are several hundred variable genes that code for antibody heavy and light chains. In pre-B lymphocytes from murine fetal liver, the genes for heavy chains rearrange in mid-gestation, and the genes for light chains rearrange in late gestation.

We have made pre-B cell lines from murine fetal liver which have been transformed with Abelson virus. The rearranged variable genes for the heavy chain from each transformed cell line have been cloned into viral vectors for nucleotide sequencing. There is evidence that seven out of eight fetal cell lines have rearranged the <u>same</u> germline gene. This finding of a bias towards the expression of one gene during early development is incredible since one knows that there are several hundred other variable genes that are not selected. Because this particular gene is not expressed in the adult mouse, it is suspected that it may be non-functional because (a) it may not be translated by the cell, or (b) it may contain an amino acid substitution that may not allow it to join with a light chain to form an antibody molecule. It will be necessary to sequence the heavy chain protein because both parental chromosomes contain rearranged heavy chain genes, and it is not known which one is being translated. B lymphocytes commonly contain two rearranged heavy gene alleles, but only one allele is expressed by the cell. Therefore, we would like to sequence the protein to determine if the putative non-functional gene is being translated. If it is, the heavy chain can be isolated to see if it can pair with a light chain to form an immunoglobulin molecule.

A protein sequenator would be used to sequence the immunoglobulin heavy chains that are made by pre-B cells to determine which rearranged genes produce protein. It is expected that one milligram of protein can be purified from the ascites fluid of three mice that have been injected with pre-B cells. The amino-terminal sequence up to residue 30 would confirm which gene was being translated. I have had extensive experience in sequencing antibody proteins in Dr. Leroy Hood's laboratory (Gearhart, P.J., Johnson, N., Douglas, R., and Hood, L. 1981. IgG antibodies to phosphorylcholine exhibit more diversity than their IgM counterparts. Nature 291, 29-34).

c. LAWRENCE GROSSMAN

THE ROLE OF THE E. coli uvr PROTEINS IN DNA REPAIR

The intitial stages in the repair of DNA damaged by UV, or other agents causing large distortional perturbations in DNA, are controlled by three unlinked Esherichia coli genes, the uvrA, uvrB and uvrC genes. These three genes have been cloned, mapped, amplified when linked to other promoters and their respective gene products isolated in milligram quantities as homogeneous proteins. The uvrA protein is the largest with a molecular weight of 117,000 which in the presence of ATP and Mg^{2+} binds to damaged DNA and releasing P_i and ADP. The hydrolysis of ATP is accompanied by a large conformational change in the uvrA protein. The binding of the uvrA protein is further stabilized by the uvrB protein, which has an 80,000 molecular weight. These two proteins neither separately nor in combination are sufficient to catalyze incision of damamged DNA, but require the presence of the uvrC protein (Mr=62,000). These three proteins comprise a uvrABC complex which in the presence of ATP specifically leads to two breaks in UV damaged DNA hydrolyzing 7 nucleotides 5' and 3-4 nucleotides 3' to the same UV-induced pyrimidine dimer (1-5).

From DNA binding data the uvr proteins do not turnover after the incision reaction, nor is there any DNA fragment released suggesting an in vitro system which is limiting and devoid of necessary proteins for the complete repair reaction. This proved to be the case in which the gene product of uvrD (helicase II), DNA polymerase I (polA gene) under polymerizing conditions and polynucleotide ligase (lig gene) are limiting in the reaction and are required to carry out the complete repair processes of incision, excision, resysnthesis and ligation in a coordinated manner as part of a protein complex we refer to as a "repairosome" (6).

One of the major reseach goals for the laboratory is to investigate the roles for each of the uvr proteins, their structure and to correlate their structures with their functionality. To approach this problem each of the uvr genes is being sequenced, their most likely amino acid sequences determined and their probable secondary structural determinations predicted as first approximations for the location of structural domains. The uvrA protein has been crystallized, and in collaboration with Dr. E. Latman of the Department of Biophysics in the School of Medicine more detailed structural relationships are being sought.

The general research strategy involves sequencing each of the uvr genes and from the predicted amino acid sequence and from protein prediction models determine those likely structural domains which should be modified for functionality assays. The uvrA protein is an ATPase, binds to damamged DNA and provides sites for uvrB and uvrC protein binding as well as being required for catalysis. The uvrB protein stablizes the uvrA protein binding to DNA and is required for catalysis, whereas the uvrC protein initiates catalysis of the incision event.

We have sucessfully sequenced the uvrC gene, almost fully sequenced the uvrA gene and plan to complete the primary structure analysis of the uvrB gene. In accordance with the identification of structural domains in each of the uvr proteins we are in the process of proteolyzing each one of the proteins with both exo- and specific endopeptidases to identify both active and inactive polypeptide fragments for functional analyses.

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Knowing the sequence of the genes those regions encoding potentially active sites will be mutagenized at specific sites. The mutagenized gene will be amplified and the mutant proteins tested for their unique activities. The actual sequences of the mutant proteins must be verified through the isolation of those active peptide regions for complete amino acid sequence analysis. It is this aspect of our research as well as the proteolysis experiments which can best be accomplished with an amino acid sequenator. Such an instrument will also be needed to identify sites of modification, the effects of inhibitors and cross-links with both other uvr proteins, but with damaged DNA as well. Because we anticipate that there will be limited quantities of some of the proteolytic fragments a gas phase model sequenator will be needed for some of the experiments.

Although the stoichiometric relationship between each of the uvr proteins is close to unity it is not clear what the nature and level of these proteins are in a complicated macromolecular structure. For this reason this complex structure will be examined by physical means using labeled proteins, crosslinking agents, ATP analogs and specific antibodies for determining which of the uvr proteins are exposed to antibody and those which are masked when in complexed structure.

We plan to use a reversible crosslinking agent which can be cleaved after the isolation of a crosslinked complex so that the identity of a complex can be ascertained not only by its molecular weight but also by that of its components. This approach has been useful in the analysis of other complex structures, including ribosomes. Secondly we plan to exploit our current antibody technology to purify crosslinked complexes into various classes dependent upon their containing one common protein. It is our plan to synthesize the antigens for monoclonal and polyclonal antibody production so that antibodies can be prepared against specific kinds of regions of each of the uvr proteins. It is for this reason that a peptide synthesizer will be invaluable for synthesizing oligopeptides of defined sequence for use in antigenicity studies.

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d. P.C. HUANG

GENES FOR METAL BINDING PROTEINS

Heavy metals damage DNA as well as render enzymes inactive or error prone. Our long term goal is to understand cellular defense mechanisms with which a cell maintains its genetic integrity against heavy metals. With genetic and recombinant DNA techniques, we are undertaking four projects:

A. Inductive response to transition metals -- Nontoxic levels of zinc and cadmium induce the synthesis of specific proteins, such as metallothionein, which have a high affinity to these metals. Is there a family of genes whose expression is responsive to induction by the stress of these metals? Poly A plus-mRNA from induced rat liver cell was used to prepare cDNA by reverse transcription. Direct sequence analysis of these resulting cDNAs and their putative gene products are being done.

B. Organization and expression of the metallothionein genes -- Metallothionein is a metal binding protein, consisting of 61 amino acids, of which one third is cysteine. It is ubiquitous in plants and animals. Its function may involve metal detoxification, transport, storage and homeostasis. Our aim is to test the hypothesis that metallothionein genes have evolved through endoduplication of a basic stretch of DNA, followed by mutations and insertion of introns and regulatory sequences and that they undergo amplification under stress. Metallothionein gene seqeunces from different genomes and metal resistant mutants are being compared for their structure and expression with immuno and chemical assays. Progress has already been made in this laboratory in the identification of specific genomic DNA fragments, resulting from restriction enzyme digestion, which contain the metallothionein genes. As there are two major metallothionein genes in rat genome and as they may be distantly linked, our current work involves a shotgun approach to randomly sequence the subclone of one 8.1 Kb fragment from rat genome by the standard dideoxynucleotide synthesizer technique. The limitation of this approach is time consuming; only 250-300 nucleotides can be determined at a time. With an oligonucleotide synthesizer, we plan to streamline the procedure by sequence walking using the last readable stretch of DNA as a synthetic primer and extending the reading downstream. This is theoretically feasible and can be readily adapted to other metallothionein isogene and pseudogene sequence analyses. The synthetic oligonucleotides will also serve as probes in the detection of control and coding sequences related to the metallothionein genes.

C. Site-specific mutagenesis of metallothionein -- As a protein, each molecule of metallothionein binds seven gram-atoms of transition IB and IIB metals. It apparently consists of two domains with the N-terminal end binding three and the C-terminal four metals. All metallothionein thus far identified lacks aromatic amino acids and contains 20 cysteines in the same positions. Our study is designed to address the question whether there is an evolutionary constraint such that it requires a high degree of conservation in the amino acid sequence. We have isolated a repertoire of site-specific mutants with specific codons in the metallothionein coding sequence replaced. Each of these mutants has been cloned and DNA sequence determined. Of the 264 mutants characterized to date, they project changes in single or multiple (up to six) amino acids, in substitution of polar to nonpolar residuals, in replacing nonaromatic amino acids by aromatic ones

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and in amino acid replacements confined to domain 1 and/or 2. We plan to extend the size of this mutant library to include elimination or replacement of specific cysteines and other alterations, which will include recombinational mutants constructed with a single or multiple exon sequences. By inserting these coding sequences in suitable expression vector/hosts, we shall be in a unique position to study the function and properties of metallothionein.

Two expression vector systems are being used for this purpose: a) a modified tryp promoter and b) a modified lambda promoter coupled with a temperature sensitive repressor. The latter has yielded cloned metallothionein gene product equivalent to about 1% of the total soluble protein of the host, E. coli. The level of synthesis is sufficient for routine assays of 2 ml cultures by radioimmuno reagents, by radioactive metal binding and by pulse polarography. Each of these assays requires only 2.5 to 50 ng of metallothionein. A large quantity of mutant gene product of interest, however, will be produced by scaling up to as much as 120 liters, if needed, in our departmental fermentor, for the intended NMR study which will require 10-20 mg of this protein purified to homogeneity. Here the protein/peptide sequencer is crucial as it will facilitate analysis of the mutated metallothionein, ascertaining the precise changes predicted from the DNA sequence data.

D. Evolution of metallothionein gene sequences -- metallothionein, a protein which binds transition metals is found ubiquitously among mammals. Fish and lower vertebrates also contain metal binding protein, related but different from mammalian metallothionein. We are in the process of comparing their genic organizational differences as well as similarity in their products. Synthetic polypeptides designed to mimic common features of these protein species should aid our studies in elucidating the structure-function relationship during the evolution of this gene family. For instance, if the cloned single domain can be demonstrated to be functional for metal binding, it would be reasonable to chemically synthesize polypeptides, the sequences of which are so modified, that the affinity for specific metals is increased or decreased. Structural alterations, replacing nonaromatic for aromatic, non-polar for polar, are examples which would complement the efforts decribed above using mutated cDNA. Similarly, synthetic segments of the metallothionein sequence will be useful in learning whether antigenicity of this protein is localized. Synthetic peptides will be used to elicit antibody and to test which segment will compete for the Ab/Ag reactive sites. The quantitative aspect of these approaches is being studied. The sequence specificity for antibody determinants and for metal binding are just two major areas upon which we intend to focus our attention.

As a matter of scheduling, it should be noted that the need for amino acid composition analysis capacity is imminent; several purified native metal binding proteins from fish are waiting to be analyzed. Sequencer is desired in approximately six months. It will be used for the anlaysis of some of the fish proteins and the mammalian metallothioneins which are being used as standards or being cloned and synthesized in E. coli. It is envisioned that for this study the peptide synthesizer will not be actively used until the physico-chemical properties of several mutants are determined, which we expect to achieve in about one year's time.

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In short, in three out of four projects in this study, the requested instruments will be beneficially used. The peptide synthesizer will aid the study of metallothionein evolution. The protein/peptide sequencer will be needed for the determination of cloned coding sequence gene products on a routine basis. The HPLC systems are essential to the compositional and size analyses of the synthesized and/or native peptides as well as in preparative work. While these instruments requested here will be used heavily in our study, we recognize that they will be served more efficiently on a shared basis.

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e. Roger McMacken

"BIOCHEMICAL MECHANISMS IN THE INITIATION OF DNA REPLICATION"

The initiation of bidirectional DNA replication starting at specific chromosomal replication origins is a complex biochemical phenomenon requiring the concerted action of multiple proteins. The actual molecular mechanism of DNA chain initiation is currently not understood for any organism. Because of the tremendous complexity of the events that occur in the initiation of DNA replication, we have chosen to study this process in a model system in which the genetically well-characterized chromosome of bacteriophage lambda is used as the template. Initiation of bidirectional DNA replication at the viral origin requires the action of two phage-coded initiator proteins, the λ O and P gene products, as well as the function of numerous Escherichia coli replication proteins. The host proteins required include primase, DNA polymerase III holoenzyme, RNA polymerase, DNA gyrase, single stranded DNA binding protein (SSB), and the bacterial dnaB, dnaJ, and dnaK proteins.

Within the last two years our laboratory has established two different soluble in vitro replication systems that will ultimately enable the elucidation of the biochemical events that occur in the initiation of bidirectional λ DNA replication. Both systems rely on the capacity of the lambda O and P initiators to commandeer the bacterial replication apparatus and direct these enzymes to carry out DNA chain initiation reactions. In one system supercoiled λdv-like plasmids, carrying a cloned phage λ replication origin, are replicated in a bidirectional fashion from the λ origin to produce daughter supercoiled molecules. The properties of the in vitro DNA synthesis are precisely those expected for authentic λ DNA replication.

In the other system single-stranded, circular phage M13 or ϕX174 DNA is replicated to a duplex, supercoiled form. Although this reaction is not dependent upon the presence of λ DNA sequences in the single-stranded template, its properties suggest it is relevant to strand initiation events that occur in the replication of the phage λ chromosome in vivo. The single-strand replication system has been used to establish physiological assays that have enabled the purification of the bacterial dnaJ and dnaK replication proteins. This second replication reaction has recently been reconstituted using eight highly purified protein fractions. Our studies of this system indicate that initiation of DNA synthesis on single-stranded templates is preceded by a complex, ATP-dependent step in which the E. coli dnaB protein is transferred onto the SSB-coated DNA through interactions with the viral O and P protein initiators and the bacterial dnaJ and dnaK proteins. The primosome-like complex containing the dnaB protein serves as a locus for primer synthesis and subsequent DNA chain elongation.

Studies of these in vitro replication systems, together with studies of individual purified proteins, have led to the identification of numerous specific protein-protein interactions required for the initiation of λ DNA replication. We have found that the λ P protein forms a specific complex with the host dnaB protein, and in so doing displaces the bacterial dnaC protein from a dnaB-dnaC protein complex. The bound P protein suppresses all of the known enzymatic activities of the dnaB protein, including its ATPase, priming, and replication activities. We have also demonstrated that each of the viral

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initiators apparently interacts with the *E. coli* dnaK protein, judged by the capacity of each protein to modulate the ATPase activity of the bacterial protein. Preliminary biochemical evidence for interactions between the λ O and P proteins has also been obtained. Moreover, we and others have found that the phage O protein binds specifically to a series of tandem repeating sequences present at the λ replication origin and arranges the origin DNA into a specific globular nucleoprotein structure. Via proteolytic digestion studies, we have also accumulated evidence that the λ O initiator protein is divided into two structural domains: an amino-terminal domain that specifically interacts with the λ replication origin and a carboxyl-terminal domain that is involved in specific protein-protein interactions. Structural studies of the proteins required for λ DNA replication have been greatly aided by our success in cloning the essential replication genes and in amplifying the intracellular levels of these gene products. Multiple milligram quantities of each of the essential proteins can be readily prepared.

Our studies of the mechanism of initiation of λ DNA replication are currently moving into a critical and difficult phase. An understanding of this complex process at the molecular level depends on studies of many protein-protein and protein-DNA interactions, several of which may be transient in nature. Because many of the proteins involved have no associated enzymatic activities that can be followed to monitor such interactions, it is clear that we will need to employ sophisticated, state-of-the-art, biochemical technology to effectively carry out this study. Delineated below are selected examples of specific studies our laboratory will perform that are highly dependent on the requested instrumentation.

Initially, we will focus our efforts on studies to elucidate the nature of the interactions between the normal wild type versions of the replication proteins. Later mutant versions of these proteins will be used to pinpoint particular structural features that are essential for the interaction. Interactions between P protein and dnaB protein, O protein and P protein, P protein and dnaK protein, and between O protein and dnaK protein will all be examined. Reversible bifunctional protein crosslinking agents will be used to probe for surface contact regions in various protein-protein complexes. The amino acid residues modified on each polypeptide will be identified by digesting the cross-linked complex with proteases (generally trypsin) and subsequently isolating and characterizing the modified peptides. This type of analysis will require the routine use of HPLC instrumentation for isolation and identification of the modified peptides, as well as the use of a protein sequenator for unequivocal identification of the modified residue(s). Perhaps it should be noted that primary amino acid sequences are known for all of the replication proteins that we will be investigating. This will significantly aid structural analysis of the proteins and of protein interactions.

Subsequent studies will utilize stable proteolytic fragments derived from the replication proteins to further delineate the nature of any observed interactions. This approach should simplify the interpretation of complex interaction patterns and will be especially useful for those proteins, like O protein and dnaB protein, which appear to have multiple functions located on independent domains. Each replication protein will be digested with various proteases

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and, after specific times of digestion, each sample will be analyzed for the presence of stable proteolytic fragments by SDS-polyacrylamide gel electrophoresis. Potentially useful polypeptide fragments will be extracted from the gel and identified by using the gas phase sequenator to determine their amino-terminal amino acid sequences. Desirable fragments will be purified in a native form by conventional and HPLC chromatography and characterized for the retention of functional activities. To complete the unequivocal identification of each fragment, we will determine its carboxyl-terminal amino acid sequence. Once again HPLC chromatography will be necessary to identify and quantitate the released amino acid residues. Because of its speed and resolution, HPLC gel filtration will also be used to attempt to identify weakly stable protein interactions. The lifetimes of many such complexes may be too transient to be picked up by the much slower conventional chromatographic or sedimentation procedures.

We will also investigate the nature of the interaction of the λ O protein with the λ replication origin. This dimeric protein binds to four tandem 19 base pair repeating sequences present in the viral origin, binding to the inner two repeats at low protein concentrations and to the outer two repeats, as well, at higher concentrations. Interestingly, the three dimensional structure of the O protein-origin complex changes dramatically upon binding of O protein to the outer repeats. A specific nucleosome-like globular complex is formed. Using bifunctional protein-protein and protein-DNA cross-linking agents, we will attempt to probe the structure of this novel complex. Similar studies will be performed on origin DNA-protein complexes containing subsaturating levels of O protein and on free O protein dimers. As described above, both peptide sequencing instrumentation and HPLC apparatus will be essential for the completion of these studies.

Approximately 5% of the E. coli dnaK protein exists in vivo as a phosphorylated species. Very high levels of the dnaK protein are required in each of the two in vitro replication systems we developed, perhaps because only the phosphorylated form of the protein is active. We intend to investigate this possibility. Because it is very difficult on a preparative scale to separate the two forms cleanly, we will approach this problem by quantitating the level of the phosphorylated form that is present in various specific complexes formed by the dnaK protein with other replication proteins during the prepriming stage of the strand initiation reactions. It is anticipated that the tryptic peptide carrying the phosphorylated residue will be identified through peptide sequencing. Subsequently, the levels of the modified and nonphosphorylated forms of this tryptic peptide in protein complexes can be rapidly ascertained with the aid of HPLC methodology.

Once the general nature of a particular protein-protein interaction or a specific protein-DNA interaction is identified by these and related studies, we will focus our efforts at defining the precise nature of the interaction. One approach that should be fruitful is to chemically modify or replace specific amino acid or nucleotide residues that are suspected of playing a crucial role in the interaction (using the aforementioned studies as a guide) and then to measure changes in the physical and enzymatic properties of the interaction caused by the alteration. In the case of the λ P protein, a large number of

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mutant proteins that have altered interactions with the bacterial dnaB and/or dnaK proteins are available for such studies. However, because of the relative ease by which specific nucleotide and amino acid alterations can be made using modern recombinant DNA technology, our general approach to these studies will be to chemically and enzymatically construct specific alterations in critical regions of these replication proteins or their DNA target sites. The nucleotide sequence coding for the region of interest will initially be cloned into the single-stranded chromosome of phage M13. Next, a complementary primer DNA fragment, about 15 to 20 base pairs in length and containing the desired nucleotide change in its center, is chemically synthesized. The primer is annealed to the hybrid M13 chromosome and this DNA is converted to the duplex form through the action of DNA polymerase I (Klenow fragment) and DNA ligase. The duplex DNA product is transformed into E. coli and phage progeny carrying the modified sequence are isolated. Ultimately, the modified sequence will be enzymatically inserted into an amplification vector to replace the wild type sequence. Large quantities of the mutant protein or DNA will then be isolated for use in biochemical studies of the interaction.

Finally, it is anticipated that the peptide synthesizer will be useful for this investigation. Once some general idea is obtained about the region(s) of a protein that is involved in a particular macromolecular interaction, we intend to elicit specific antibodies directed against this region. Having an arsenal of specific antibodies that block various macromolecular interactions should both augment and complement the studies with site-specific mutations described above. The antibodies may be used in pairs or three-way combinations in the analysis of the complex multi-step pathways involved in the initiation of DNA synthesis. Our approach will be to chemically synthesize oligopeptides (generally containing 8 to 20 amino acid residues... identical to the wild type sequence in the region of interest) that can be linked to carrier proteins and injected into rabbits. Lerner and coworkers have found that antibodies elicited by such oligopeptides often recognize and bind to the native protein as well. Sufficient quantities of the oligopeptide for immunization can readily (and relatively cheaply) be prepared by the peptide synthesizer.

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f. Barry Shane

REGULATION OF FOLATE AND ONE CARBON METABOLISM

For the last few years, a major interest of my laboratory has been the enzymology and regulation of one carbon metabolism. Folate-dependent reactions are involved in the biosynthesis of thymidylate, purines, methionine, and glycine, and in the metabolism of serine, formate, and histidine. We have shown that the physiological substrates for these reactions are polyglutamyl folate derivatives, and that these forms of the vitamin are preferentially retained by tissues. We are currently studying the mechanism by which regulation of folate polyglutamate formation in bacteria and mammalian tissues controls one carbon metabolism. Because of the role of folates in thymidylate and purine biosynthesis, folate antagonists have found widespread use as chemotherapeutic agents. The studies on the regulation of folate metabolism are being extended to cultured normal and tumorigenic mammalian cells with the aim of designing antagonists that will potentiate differences in requirements for products of one carbon metabolism between these cells.

The major emphasis of our studies has been on the enzymology of folylpolyglutamate synthesis. The enzyme folylpolyglutamate synthetase has been purified to homogeneity from Lactobacillus casei, Corynebacterium and hog liver and the substrate specificities and kinetic properties of the enzymes have been characterized. The Corynebacterium protein has been shown to possess dihydrofolate synthetase activity and appears to be a bifunctional protein. Folylpolyglutamate synthetase is considered a promising target enzyme for anticancer and antimicrobial chemotherapy. In addition, some antifolate agents only exert their cytotoxic effects when metabolized to polyglutamate forms. We are currently synthesizing a variety of potential inhibitors of the enzyme.

A detailed physical characterization of folylpolyglutamate synthetase has been precluded by the small amounts of protein that are obtained after its purification. For example, about 50 ug of the hog liver enzyme is obtained from one kilogram of tissue and the purified protein is unstable. To overcome this problem, we have isolated the E. coli folylpolyglutamate synthetase gene and have cloned it into a high expression plasmid. Bacteria transformed with this plasmid overproduce the enzyme which has allowed us to obtain large quantities of the purified protein. Studies have also been initiated, in

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collaboration with Dr. Gregory Milman, on the cloning of the human folylpolyglutamate synthetase gene as a means of obtaining large amounts of the human enzyme.

The availability of large amounts of these proteins will allow us to (i) map the substrate binding sites with mono- and polyglutamate affinity labels and (ii) dissect the catalytic mechanism of the enzyme. This will allow us to determine which factors are involved in making a folate or antifolate a good substrate for this enzyme and will greatly aid in the rationale design of inhibitors of the enzyme and in the design of "pro-drugs", folate antagonists that exert their cytotoxic effects when metabolized to polyglutamate forms.

The procedures we use for the synthesis of the folate polyglutamate substrates used in our studies are very tedious and time consuming. These polypeptide derivatives are synthesized by the Merrifield solid phase method, but all operations are carried out manually. We have also used this procedure to synthesize modified folate derivatives with modified glutamate residues or with other amino acid substitutions to assess the parts of the molecule which are involved in binding to the enzyme. Because of the time and effort that is required to synthesize these compounds, we have only been able to synthesize mono- and diglutamate analogs. The use of an automated peptide synthesizer would greatly simplify these syntheses and would make practical the synthesis of analogs of longer chain length folylpolyglutamates. We would also require a peptide synthesizer for the synthesis of affinity labels for folylpolyglutamate synthetase and for the synthesis of peptides to produce antibodies to specific regions of the protein.

Affinity labels will be used to map the substrate binding sites of folylpolyglutamate synthetase and dihydrofolate synthetase, and residues involved in substrate binding and catalysis will be modified with chemical agents. These studies will require the use of a peptide sequenator for the identification of the amino acids involved. The availability of a microsequenator will also make possible studies on the mammalian folylpolyglutamate synthetase which is presently available only in microgram quantities. We expect the method we have developed for the purification of the hog liver should also be applicable for the purification of small amounts of the human enzyme. The availability of a sequencer will allow us to determine the amino acid sequence of different regions of the human protein, thus allowing us to prepare DNA probes to aid in our studies on the cloning of the human gene.

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(2) Other Major Users (all members of Biochemistry Department)

1. Gregory Milman/Associate Professor

Cloning and expression of foreign genes: herpes type 1 & 2 Ag, EB virus nuclear Ag, nematode parasite Ag and human folylpolyglutamate synthetase.

Need synthetic peptides to elicit monoclonal antibody for the proteins of interest.

Need sequencer to determine the cloned foreign gene products.

2. Sharon S. Krag/Associate Professor

Glycosylation of soluble and membrane associated proteins in cultured eukaryotic cells.

Need synthesizer to make peptides of specific sequences as reagents for immunology and as substrate for enzymatic reaction.

Need sequenator to determine sequences of proteins being studied.

3. John Scocca/Associate Professor

1. Sexually transmitted diseases
2. DNA interaction in bacterial transformation.

Need sequenator to examine DNA binding protein on membrane for transformation.

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(3) Representative relevant research projects by pre and postdoctoral fellows:

Alfano, C.	Protein-protein interactions involved in initiation of DNA replication
Andersen, R.	Fish metal binding proteins: electrochemical and affinity properties
Bongar, A.	Cloning and characterization of human folylpolyglutamate synthetase genes
Brown, D.	Cloning and expression of a dihydroorotase gene
Caron, P.	Mechanism of the synergistic actions of uvrA,B,C and D
Cismowski, M.	Mutated metallothionein gene product generated for NMR studies
Claussen, L.	Structural analysis of uvrA,B,C proteins
Cook, J.	Specificity of substrate binding site of folylpolyglutamate synthetase
Criscuolo, B.	Purification and characterization of a potential regulatory enzyme in the lipid-linked glycosylation pathway
Dickstein, R.	Expression of E. coli DNA repair protein in XP cell
Dinman, J.	The role of promoter and coding sequence in metallothionein gene inducibility
Goodman, S.	Sequence characterization of membrane associated DNA binding protein from Gonococcus. Structure and function of mobile prepriming complexes involved in DNA replication
Hauser, M.	Haemophilus DNA binding proteins associated with membrane, physical and biological properties
Huh, A.	Expression of uvrB,C from E. coli in XP cells
Lebowitz, J.	Mechanisms of DNA strand initiation during bacteriophage, lambda and *E. coli* DNA replication
Liberman, N.	Hypermutability of the variable regions of immunoglobulin heavy chain
Liu, S.	Expression of cloned metallothionein gene in a high efficiency expression vector utilizing lambda P_L promoter and CI857 ts repressor
Mazur, S.	Thermodynamic characterization of uvrA,B,C enzymatic mechanism
Morris, S.	Amplification and evolution of metallothionein gene sequences

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O'Melia, A.	Transcriptional control of metallothionein genes in CHO mutants
Oh, C.	Properties of uvrA,B,C DNA repair system
Pine, R.	Site specific and recombinational mutations in vitro in metallothionein coding sequences
Roberts, J.	Cloning and characterization of the lambda O protein, a protein essential for the initiation of viral DNA replication
Stoll, J.	Isolation of mutants of Chinese hamster ovary cells lacking oligosaccharide-protein transferase activity
Sussman, D.	Expression of human folylpolyglutamate synthetase in CHO cell mutants
Washabaugh, M.	E. coli dihydroorotase: purification, characterization and mechanism of action

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Estimated % Usage of the Instruments

Laboratory of:	Protein synthesizer/sequencer
Collins, K.*	10%
Gearhart, P.*	10
Grossman, L.*	15
Huang, P.C.*	20
Krag, S.	5
McMacken, R.*	15
Milman, G.	5
Scocca, J.	5
Shane, B.*	15
Total	100% usage

* Primary users

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C. Institutional plans

(1) Maintenance and operation

a. Oversight

While six major users joined to submit this application, three additional members in the department will also benefit by the availability of the instruments requested.

The use of the protein module consisting of the peptide synthesizer, sequencer, and HPLC will be reviewed by a service committee consisting of three members. Barry Shane, Kim Collins, and P.C. Huang. The overall coordination of the users will be the resposibility of Dr. Huang. Dr. Dennis Powers of the Biology department is in charge of a protein sequencer on the Homewood Campus and is experienced in the use of this equipment. He will be a consultant to this group. Drs. Shane and Collins have already begun work on polypeptide synthesis using manual procedures.

Members of the user group have a long standing record of collaboration. Most members are faculty of the Biochemistry department and their laborators are physically located on the same floor. The department operates on a communal basis sharing all instruments, which include a JCM100c electron microscope, ultracentrifuges, scintillation counters, polynucleotide synthesizer and fermentors. One faculty member is assigned responsibility for the oversight of each piece of equipment. The department as a whole shares a training grant and the faculty participates as a group in consultation work and donates the fees to support departmental graduate students. Five members (Lawrence Grossman, P.C. Huang, Roger McMacken, Gregory Milman, and Patricia Gearhart) collaborate in a program project. Other collaborative efforts include the cloning of the human folylpolyglutamate synthetase gene (Shane and Milman), the cloning of the *E. coli* dihydroorotase gene (Collins and McMacken), and sequencing the HP1C1 phage genome (Scocca and Huang). Thus, the sharing of information, techniques, and instrumentation is well established among the members of the proposed user group.

The consultant, Dr. Powers is not only a colleague of good standing, but is also a collaborator. Drs. Powers and Huang are jointly responsible for a course on "Nucleic Acid and Protein Sequencing" which was taught for four summers. Dr. Powers helped Dr. Collins in the analysis of protein samples. A continuous support by Dr. Powers can be expected. (see letter attached)

b. Policy for Usage

The equipment will be housed in a small laboratory (approx. 200 sq. ft.) in the Biochemistry department. Service investigators will have use of the modules as required but will inform the committee (see above) before using the machines. In cases of heavy usage of the modules, time will be apportioned equally between the different investigators by the committee.

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The modules will be operated by a full-time technical assistant who will be assisted by representatives of whichever laboratory is using the equipement. The technician will be paid 20% each by the principal investigator (P.C. Huang) B. Shane and L. Grossman. The remainder of the technician's salary plus supplies and maintenance costs, including service contracts after the first year, will be apportioned between the user group, depending on usage. These costs will be assessed by the committee.

We envision that some of the maintenance cost will be covered in the core component of a departmental program for which five of the user group already serve as investigators.

(2) NonFederal Cost Sharing

The major portion of cost sharing will be placed is the catagory of faculty salary, for which none is requested in this application.

The estimated usable life span of the requested instruments in ten years, assuming rigorous maintenance and replacement for wear and tear.

(3) Commitment Letters

(see attached pages marked:
a. From Chairman
b. From Consultant)

a. From Chairman

THE JOHNS HOPKINS UNIVERSITY
SCHOOL OF HYGIENE AND PUBLIC HEALTH
615 North Wolfe Street
Baltimore, Maryland 21205, U.S.A.

DEPARTMENT OF BIOCHEMISTRY
(301) 955-3671

DOE University Research Instrumentation Program
Energy Programs and Support Division
U.S. Department of Energy
Oak Ridge Operations
P.O. Box E
Oak Ridge, Tennessee 37831

To Whom It May Concern:

I'm writing to certify the administrative support of a grant proposal to you for Instumentation as part of a community Macromolecular Structure Laboratory for the Johns Hopkins Unversity School of Hygiene and Public Health. This facility will contain an already obtained DNA synthesizer and an amino acid analyzer system. It is the purpose of this grant request to supply the gas phase protein sequenator and a peptide synthesizer to complete the major equipment needs of such facility. Although there are some components available in the University their primary mission is in support of the work of others and we have been informed that only a limited amount of time on their machines could be made available to us.

The Department of Biochemistry will provide the space, maintenance, insurance and technical expertise to scientifically maintain this facility. It will be administered by Ms. Elsie Entwisle the departmental administrator, who has had a great deal of such experience in administering to the Fermentation and Electron Microscope facilities supported by the National Science Foundation. Procedures have, therefore, been established for inventory controls, routine maintenance procedures and for following the strict organizational policies of the department and the school.

Sincerely yours,

Lawrence Grossman,
E.V. McCollum Professor
and Chairman

1076382

b. From Consultant

THE JOHNS HOPKINS UNIVERSITY

BALTIMORE, MARYLAND 21218

ARTMENT OF BIOLOGY

February 10, 1984

Professor P.C. Huang
Department of Biochemistry
School of Hygiene and Public Health
The Johns Hopkins Medical Institutions

Dear P.C.:

I am happy to learn that your department plans to establish a protein sequencing facility. Such a facility is needed in your department and I would be happy to provide any expertise that you may find useful.

I wish you the best of luck in your effort.

Sincerely,

Dennis A. Powers
Chairman, Biology Department
Director, McCollum-Pratt Institute

1076383

(7) Estimated cost and budget

	Request from DOE	Subtotal
Applied Biosystems Gas-Phase Protein Sequencer (470-A)	108,000	108,000
Beckman Peptide Synthesizer Model 990 (350709)	89,600	
Preparative reaction vessel kit (350673)	3,200	92,800
Beckman 346 HPLC binary system with Model 165 detector	23,100	
Model 450 Data System	15,900	
Second channel for data system and 128k RAM	1,750	
Serial communications and software for above system	1,750	
Autosampler - Model 504	9,750	
Post column derivatization unit	9,000	
Column temperature controller	1,820	63,070
	Total	263,870

	Institutional (source)
Installation	
Operation and Maintenance (on annual basis with expected increase of 10% over a five year period)	$ 7,800 20,000 (Research grants)

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8. Faculty Resumes

Publications, (representative, selected)

1076385

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
P.C. Huang	Professor	

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
National Taiwan University	B.S.	1953	Genetics
Virginia Tech.	M.S.	1956	Genetics
Ohio State Univ.	Ph.D.	1960	Genetics/Biochem
California Inst. of Technology	Postdoc.	1960-65	Chemical Biology

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

BIOGRAPHICAL SKETCH

Principal Investigator - P.C. Huang, Ph.D.

Dr. Huang is Professor of Biochemistry and a preceptor in the Hopkins Human Genetics Program. His major research interest lies in gene organization and expression. In addition to his formal training at National Taiwan Univ. (B.S.), Virginia Tech. (M.S.), Ohio State (Ph.D.) and Caltech (PDF) in Genetics and Biochemistry, he attended the NATO Winter School for DNA sequencing in 1969 and spent a year (1972) of Sabbatical leave studying in Dr. Fred Sanger's laboratory at Cambridge. He has continuously and persistently contributed through publications in the area of nucleic acids and proteins research for the past 20 years. He teaches a course with Roger McMacken in Molecular Biology and his current research, supported mainly by NIH and NSF, employs recombinant DNA methodology. He will be in charge of the overall operation of the requested instruments.

Assisting Dr. Huang will be Dr. Barry Shane, Associate Professor of Biochemistry. Dr. Shane is trained as a biochemist, particularly in enzyme mechanism and protein chemistry. A recent recipient of the Mead Johnson and J. George Harlar Awards, as well as an RCDA awardee, Dr. Shane has been a productive investigator. His research is supported by NIH grants and will be greatly aided by the instruments requested. Dr. Shane has extensive experience in peptide synthesis techniques and in analytic methodologies including HPLC.

All major users are established investigators, active in research supported by grants and essentially all the projected use of the requested equipment will be in support of funded research projects.

See attached for individual biographical sketches arranged in alphabetical order.

107638b

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: _____

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Kim D. Collins	Assistant Professor	

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
MIT, Cambridge, Massachusetts	B.S.	1966	Molecular Biology
Stanford Medical School, Stanford, CA	Ph.D.	1971	Biochemistry
Harvard University, Cambridge MA	-	1971-73	Chemistry & Biochem

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience:

1976	Assistant Professor - Biochemistry - School of Hygiene & Public Health - The Johns Hopkins University
1974-1976	Research Associate - Yale Medical School
1973-1974	Assistant Professor - Biochemistry - Baylor College of Medicine
1972-1973	Research Fellow - Biochemistry & Molecular Biology - Harvard University Fellow of The Jane Coffin Childs Memorial Fund For Medical Research
1971-1972	Research Fellow - Chemistry Department - Harvard University Fellow of The Jane Coffin Childs Memorial Fund for Medical Research

HONORS

MIT: Phi Lambda Upsilon, Chemistry Honor Society
Stanford: Predoctoral Trainee, USPHS
Harvard: Fellow of the Jane Coffin Childs Memorial Fund for Medical Research

PUBLICATIONS (representative, selected)

Washabaugh, Michael W. and Collins, Kim D. Purification of Aqueous Ethylene Glycol. Analytical Biochemistry 134, 144-152 (1983).

Washabaugh, Michael W. and Collins, Kim D. Dihydroorotase from Escherichia coli, Purification and Characterization, J. Biol. Chem., 259, 3293-3298 (1984).

Blodgett, James K., Collins, Kim D., and Loudon, G. Marc, Cleavage of Peptides at Aspartyl Side Chain Hydroxamic Acid Groups. submitted to J. Amer. Chem. Soc.

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BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Patricia J. Gearhart	Assistant Professor	

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
University of Illinois, Champaign-Urbana	B.S.	1967	Microbiology
Cornell University, Ithaca, N.Y.	M.S.	1969	Microbiology
University of Pennsylvania, Philadelphia	Ph.D	1974	Immunology
The Johns Hopkins University, Baltimore	Post-doc	1975-78	Immunology

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1976-1978 Helen Hay Whitney Postdoctoral Fellow

1978-1982 Staff Associate, Department of Embryology, Carnegie Institution of Washington, Baltimore, Md.

1982-present Assistant Professor, Department of Biochemistry, The Johns Hopkins University

MEMBERSHIPS: Member, American Association of Immunologists

PUBLICATIONS: (representative, selected)

Gearhart, P.J., Johnson, N.D., Douglas, R. and Hood, L. 1981. IgG antibodies to phosphorylcholine exhibit more diversity that their IgM counterparts. Nature 291, 29-34.

Gearhart, P.J., and Cebra, J.J. 1981. Most B cells that have switched surface immunoglobulin isotypes generate clones of cells which do not secrete IgM. J. Immunol. 127, 1030-1034.

Pierce, S.K., Speck, N.A., Gleason, K., Gearhart, P.J., and Kohler, H. 1981. BALB/c T cells have the potential to recognize the T15 prototype antibody and its somatic variants. J. Exp. Med. 154, 1178-1187.

Rodwell, J.D., Gearhart, P.J., and Karush, F. 1983. Restriction in IgM expression. IV. Affinity analysis of monoclonal anti-phosphorylcholine antibodies. J. Immunol. 130, 313-316.

Gearhart, P.J., and Bogenhagen, D.F. 1983. Clusters of point mutations are found exclusively around rearranged antibody variable genes. Proc. Natl. Acad. Sci. 80, 3439-3443.

Johnson, N.D., Douglas, R.H., and Gearhart, P.J. 1981. Somatic mutation increases diversity in IgG and IgA but not IgM antibodies. In "Immunoglobulin Idiotypes", (C. Janeway, E. Sercarz, and H. Wigzell, eds.) p. 293-302. Academic Press, New York.

Gearhart, P.J. 1982. Generation of immunoglobulin variable gene diversity. Immunology Today 3, 107-112.

Gearhart, P.J. 1982. In "Idiotypes-Antigens on the Inside", (C. Berek, H. Etlinger, M. Julius, eds.) p. 16-26. Roche, Basle, Switzerland.

Gearhart, P.J. The effect of somatic mutation on antibody affinity. 1984. N.Y. Academy of Science, Immune Networks ed. C. Bona and H. Kohler, 418, 171-176.

Gearhart, P.J. The adaptable somatic repertoire. 1984. Ann. Immunol., (Inst. Pasteur) 135c, 137-142.

Perlmutter, R.M., Crews, S.T., Douglas, R., Sorensen, G., Johnson, N., Nivera, N., Gearhart, P.J., and Hood, L. 1984. The generation of diversity in phosphorylcholine-binding antibodies. Adv. Immunol. 35, 1-37.

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PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: _____

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Lawrence Grossman	E.V. McCollum Professor and Chairman	

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
College of the City of New York	-	-	Mathematics/ Engineering
Hofstra University, Hempstad, NY	B.A.	1949	Chemistry
University of Southern California, Los Angeles, CA	Ph.D.	1954	Biochemistry

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience

1975-present	E.V. McCollum Professor and Chairman-Biochemistry-The Johns Hopkins University, School of Hygiene and Public Health
1957-1975	Professor, Associate Professor and Assistant Professor of Biochemistry, Brandeis University, Waltham, MA
1956-1957	Scientist-National Institutes of Health, Bethesda, MD
1954-1956	Postdoctoral Fellow-The Johns Hopkins University, McCollum Pratt Institute

Honors

1951-1954	Predoctoral Fellow-National Institutes of Health
1954-1956	Postdoctoral Fellow-National Institutes of Health
1964-1974	Research Career Development Awardee-National Institutes of Health
1964-1965	Commonwealth Fund Fellow
1973-1974	Guggenheim Fellow

Editorial Boards

Critical Reviews of Biochemistry
Cancer Research-Associate Editor

Advisory Committee

1965-1968	American Cancer Society
1973-1977	Biochemistry Study Section-National Institutes of Health
1980-1984	American Cancer Society
1980-present	Argonne National Laboratory-Biology and Medicine-University of Chicago
1984-present	U.S. Delegate to the International Union of Biochemistry

PUBLICATIONS (representative, selected)

Grossman, L. Enzymes Involved in Repair of Damaged DNA. Arch. Biochem. and Biophys. 211, 511-522 (1981) (invited review).

Grafstrom, R.H., Shaper, N.L. and Grossman, L. Human Placental AP Endonuclease: Its Site and Mechanism of Action. J. Biol. Chem. 257, 13459-13464 (1982).

Grafstrom, R.H., Park, L. and Grossman, L. The Properties of the Dimer Specific Endonuclease from *Micrococcus Luteus*. J. Biol. Chem. 257, 13465-13474 (1982).

Yeung, A.T., Mattes, W.B., Oh, E.Y., Yoakum, G.H., and Grossman, L. Enzymatic Properties of the Purified *Escherichia coli* uvrABC Protein. Proc. Natl. Sci. 80, 6157-6161 (1983).

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR:

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
P.C. HUANG	Professor	

EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
National Taiwan University	B.S.	1953	Genetics
Virginia Tech.	M.S.	1956	Genetics
Ohio State University	Ph.D.	1960	Genetics/Biochemistry
California Institute of Technology	Postdoc	1960-65	Chemical Biology

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to recent representative publications, especially those most pertinent to this application. Do not exceed 2 pages.

1960-65 Research Fellow - Chemical Biology - Caltech
1965-69 Assistant Professor - Biochemistry - The Johns Hopkins University
1969-76 Associate Professor - Biochemistry - The Johns Hopkins University
1976-Pres. Professor - Biochemistry - The Johns Hopkins University

Other Professional Experiences

Drafted Military Service 1954-1955
Abstractor, Chemical Abstract Service, American Chemical Society, 1959-1969
Visiting Lecturer, Academia Sinica, National Tsin-Hua and Taiwan Univ., 1964 Summer
Lecturer, MBL, Woods Hole, 1973-76 Summers, Nucleic Acid Chemistry
Visiting Scholar, MRC Lab Molecular Biology, University Cambridge, Postgraduate Medical School (1972 Sabbatical - Laboratory of Fred Sanger)
Member, Cancer Special Program Advisory Committee, NCI, 1981-1984.

Honors
1948-52 National University Merit Scholar at N.T.U.
1961-64 U.S. Public Health Postdoctoral at Caltech
1966-71 NIH Research Career Development Award

PUBLICATIONS (representative, selected)

Danielson, K.G.**, Ohi, S.** and Huang, P.C. (1982). Immunochemical detection of metallothionein in specific epithelial cells. Proc. Natl. Acad. Sci. USA 79, 2301-2304.

Corrigan, A.** and Huang, P.C. (1982). A basic microcomputer program for plotting the secondary structure of proteins. Computer Program for Biomedicine 15, 163-168.

Benjamin, R.C.**, Fitzmaurice, W.P.*, Huang, P.C. and Scocca, J.J. (1984). Nucleotide sequence of cloned segments from the chromosome of haemophilus influenzae bacteriophage HP1c1. Gene 31, 183-195.

Fitzmaurice, W.P.*, Benjamin, R.P.**, Huang, P.C. and Scocca, J.J. (1984). Characterization of sites on DNA segments from bacteriophage HP1c1 which interact with the specific DNA recognition system of transformable Haemophilus influenzae Rd. Gene 31, 197-206.

Andersen, R.A.**, Frazier, J.M. and Huang, P.C. (1984). Transition metal binding proteins from three Chesapeake Bay fish species. Env. Health Prospective (in press).

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BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Krag, Sharon S.	Associate Professor	

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
Texas Lutheran College, Seguin, Texas	B.S.	1969	Chemistry,Biology
The Johns Hopkins University, Balto., MD	Ph.D.	1974	Biochemistry

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience

1982- Associate Professor - Biochemistry - School of Hygiene & Public Health - The Johns Hopkins University

1976-1982 Assistant Professor - Biochemistry - School of Hygiene & Public Health - The Johns Hopkins University

1974-1976 Postdoctoral Fellow - The Jane Coffin Childs Memorial Fund for Medical Research; Department of Biology, Center for Cancer Research, M.I.T.; Dr. Philips W. Robbins, mentor

1969-1974 Predoctoral Fellow - Department of Physiological Chemistry, The Johns Hopkins University, School of Medicine; Dr. William J. Lennarz, mentor

Awards and Fellowships:

1974-1975 Samuel Landfair Rosenberry Fellow of the Jane Coffin Childs Memorial Fund for Medical Research

1978-1980 Basil O'Connor Fellow of the March of Dimes Birth Defects Foundation

1980-1985 Research Career Development Awardee from the National Cancer Institute

Publication (representative, selected)

Krag, Sharon S. A Concanavalin A-Resistant Chinese Hamster Ovary Cell Line Lacks the Ability to Synthesize [^{3}H] Glucosyl Oligo saccharide-Lipid. J. Biol. Chem. 254, 9167-9177 (1976).

Stoll, J., Robbins, A.R., and Krag, S.S. A Mutant of Chinese Hamster Ovary Cells with Altered Mannose 6-Phosphate Receptor Activity is Unable to Synthesize Mannosylphosphoryl Dolichol. Proc. Natl. Acad. Sci. USA, 79, 2296-2300, (1982)

Criscuolo, B.A. and Krag, S.S. Selection of Tunicamycin-Resistant Chinese Hamster Ovary Cells With An Increased Enzymatic Activity. J. Cell Biol., 94, 586-591 (1982).

Krag, S.S. and Robbins, A.R. A Chinese Hamster Ovary Cell Mutant Deficient in Glucosylation of Lipid-Linked Oligosaccharide Synthesizes Lysosomal Enzymes of Altered Structure and Function. J. Biol. Chem. 257, 8424-8431 (1982).

Stoll, J., Rosenberg, L., Carson, D.D., Lennarz, W.J., and Krag, S.S. (1984) A Single Enzyme Catalyzes the Synthesis of the Mannosylphosphoryl Derivative of Dolichol and Retinol in Rat Liver and Chinese Hamster Ovary Cells. J. of Biol. Chem., in press.

Robbins, A.R., Oliver, C., Bateman, J.L., Krag, S.S., Galloway, C.J., and Mellman, I. (1984) A Single Mutation in Chinese Hamster Ovary Cells Impairs Both Golgi and Endosomal Functions. J. Cell Biol. 99, 1296-1308.

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BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
McMacken, Roger L.	Associate Professor	

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
University of Washington, Seattle	B.S.	1965	Chemistry
University of Wisconsin, Madison	Ph.D.	1970	Biochemistry
Yale University, New Haven, Ct.			Biochemistry
University of Florida, Gainesville			Biochemistry
Stanford University, Palo Alto, Ca.			Biochemistry

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1965-1970	Graduate research assistant with Dr. Hatch Echols. Research on the mechanism of λ gene regulation.
1970-1974	Postdoctoral fellow with Dr. Richard Boyce. Research on the genetics and biochemistry of phage λ DNA replication in vivo.
1974-1976	Postdoctoral fellow with Dr. Arthur Kornberg. Research on the enzymology of ϕX174 DNA replication in vivo.
1977-1982	Assistant Professor of Biochemistry, The Johns Hopkins University
1983-present	Associate Professor of Bicohemistry, The Johns Hopkins University
Mar. 1985	Session chairman, Bacteriophage DNA Replication, American Society of Microbiology Meeting in Las Vegas.
Aug. 1985	Invited speakers, Symposium on the Enzymology of DNA Replication. Thirteenth International Congress of Biochemistry, Amsterdam, The Netherlands.

Membership: Member (ad hoc) of USPHS Biomedical Sciences Review Group - 3.

Publications: (representative, selected)

Wold, M. S., Mallory, J. B., Roberts, J. D., LeBowitz, J. H., and McMacken, R. (1982) "Initiation of Bacteriophage λ DNA Replication in Vitro with Purified λ Replication Proteins". Proc. Natl. Acad. Sci. USA 79, 6176-6180.

Roberts, J. D. and McMacken, R. (1983) "The Bacteriophage λ O Replication Protein: Isolation and Characterization of the Amplified Initiator". Nucleic Acids Res. 11, 7435-7452.

McMacken, R., Wold, M. S., LeBowitz, J. H., Roberts, J. D., Mallory, J. B., Wilkinson, J. A. and Loehrlein, C. (1983) "Initiation of DNA Replication in Vitro Promoted by the Bacteriophage λ O and P Replication Proteins" in Mechanisms of DNA Replication and Recombination (Cozzarelli, N. R., ed.) pp. 819-848, A. R. Liss, New York.

LeBowitz, J. H. and McMacken, R. (1984) "The Bacteriophage λ O and P Protein Initiators Promote the Replication of Single-Stranded DNA". Nucleic Acids Res. 12, 3069-3088.

Echols, H., Dodson, M., Better, M., Roberts, J. D. and McMacken, R. (1984) "The Role of Specialized Nucleoprotein Structures in Site-Specific Recombination and Initiation of DNA Replication". Cold Spring Harbor Symp. Quant. Biol. XLIX, in press.

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BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
John J. Scocca	Associate Professor	

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
The Johns Hopkins University, Balto., MD	B.A.	1961	Biology
The Johns Hopkins University, Balto., MD	Ph.D.	1966	Biochemistry
The Johns Hopkins University, Balto., MD	Postdoc	1966-68	Biochemistry

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

NSF Undergraduate Research Participant, 1959-1961
NIH Predoctoral Fellow, 1961-1966
NIH Postdoctoral Fellow, McCollum-Pratt Institute, 1966-1968
Assistant Professor of Biochemistry, School of Hygiene and Public Health, 1968-1973
Associate Professor, 1973-present

PUBLICATIONS:

Sutrina, S.L. and Scocca, J.J. Haemophilus Influenzae Periplasmic Protein Which Binds DNA: Properties and Possible Participation in Genetic Transformation. J. Bacteriol. 139:1021-1027 (1979).

Mathis, L.S. and Scocca, J.J. Haemophilus Influenzae and Neisseria gonorrhoeae Recognize Different Specificity Determinants in the DNA Uptake Step of Genetic Transformation. J. Gen. Microbiol. 128:1159-61 (1982).

Sutrina, S.L. and Scocca, J.J. DNA Binding Proteins of Haemophilus Influenzae: Purification and Characterization of a Major Intracellular Binding Protein. J. Bacteriol. 155:246-253 (1983).

Fitzmaurice, W.P. and Scocca, J.J. Restriction Map and Location of Mutations on the genome of bacteriophage Hplcl of Haemophilus Influenzae Rd. Gene 24:29-35 (1983).

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
SHANE, Barry	Associate Professor	

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
Queen Elizabeth College, University of London	B.Sc.	1966	Biochemistry/Mathematic
Charing Cross Hospital Medical School, University of London	Ph.D.	1970	Biochemistry

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1971-1973 Postdoctoral research biochemist, Department of Biochemistry, University of California, Berkeley with Dr. Esmond E. Snell.

1973-1977 Postdoctoral research biochemist, Department of Nutritional Sciences, University of California, Berkeley with Dr. E. L. Robert Stokstad.

1977-1982 Assistant Professor, Department of Biochemistry, The Johns Hopkins University School of Hygiene and Public Health.

1982-date Associate Professor, Department of Biochemistry, The Johns Hopkins University School of Hygiene and Public Health.

Honours
1981 J. George Harrar Research Award (Nutrition Foundation)
1982 Research Career Development Award (NCI)
1984 Mead Johnson Award (American Institute of Nutrition)

Committees
1984- Nutrition study section (NIH)

Publications (representative, selected)

BOGNAR, A.L., CICHOWICZ, D.J. and SHANE, B. (1983). Purification and characterization of folylpolyglutamate synthetase from Lactobacillus casei and hog liver. In: Chemistry and Biology of Pteridines, Blair, J.A., ed., pp. 627-632, de Gruyter, Berlin

BOGNAR, A.L. and SHANE, B. (1983). Purification and properties of Lactobacillus casei folylpolyglutamate synthetase. J. Biol. Chem. 258: 12574-12581

BRODY, T., SHANE, B. and STOKSTAD, E.L.R. (1984). Folic acid. In: Handbook of the Vitamins, Machlin, L.J., ed., pp. 459-496, Marcel Dekker, New York

SHANE, B. and STOKSTAD, E.L.R. (1984). Folates in the synthesis and catabolism of histidine. In: Folates and Pterins, Blakley, R.L. and Benkovic, S.J., eds., vol. 1, pp. 433-455, Wiley, New York

SHANE, B. Pterins and folates in the nutrition of lower organisms. In: Folates and Pterins, Blakley, R.L. and Whitehead, M.V., eds., vol. 2, Wiley, New York (in press)

SHANE, B. Identification of folylpolyglutamate chain length by cleavage to and separation of p-aminobenzoylpolyglutamates. Meth. Enzymol. (in press)

BOGNAR, A.L. and SHANE, B. Bacterial folylpolyglutamate synthetase - dihydrofolate synthetase. Meth. Enzymol. (in press)

SHANE, B. and STOKSTAD, E.L.R. Vitamin B_{12} - folate interrelationships. Ann. Rev. Nutr. (in press)

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PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR:

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
MILMAN, Gregory	Associate Professor	

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
Harvey Mudd College, Claremont, CA	B.S.	1962	Physics
Harvard Univ., Cambridge, MA	Ph.D.	1968	Biophysics
N.I.H. - Dr. M. Nirenbeng	Postdoc.		Biochemistry

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional experience:

1962-1967 Graduate Student, Laboratory of Dr. Robert Langridge, Harvard Universi

1967-1970 Postdoctoral Fellow, Laboratory of Dr. Marshall Nirenberg, N.I.H.

1970-1976 Assistant Profesor, Department of Biochemistry, University of Californ at Berkeley

1976-present Associate Professor, Department of Biochemistry, The Johns Hopkins Uni

Honors and Professional Activities:

Fellowships: National Science Foundation Predoctoral Fellowship
National Institutes of Health Predoctoral Fellowship
Helen Hay Whitney Foundation Postdoctoral Fellowship

Societies: American Tissue Culture Association (1970)
American Society of Biological Chemists (1974)
New York Academy of Sciences (1979)
American Association for the Advancement of Science

Publications:

G. Milman, E. Lee, G.S. Ghangas, J.R. McLaughlin & M. George, Jr. (1976) "Analysis of HeLa hypoxanthine phosphoribosyltransferase mutants and revertants by two-dimensional polyacrylamide gel electrophoresis: Evidence for silent gene activation" Proc. Natl. Acad. Sci. USA 73:589-4593

G. Milman, S.W. Krauss & A.S. Olsen (1977) "Tryptic peptide analysis of normal and mutant forms of HeLa hypoxanthine phosphoribosyltransferase" Proc. Natl. Acad. Sci. USA 74:926-930

G.S. Ghangas & G. Milman (1977) "Two-dimensional gels of hypoxanthine phosphoribosyltransferase from normal and Lesch-Nyhan hemolysates" Science 196:1119-1120

A.S. Olsen & G. Milman (1977) "Human hypoxanthine phosphoribosyltransferase: Purification and properties" Biochem. 16:2501-2505

S.W. Krauss & G. Milman (1977) "Micromethod for characterization of cyanogen bromide peptides of human hypoxanthine phosphoribosyltransferase" Anal. Biochem. 82:38-45

G. Milman (1977) "Biochemical genetics of human hypoxanthine phosphoribosyltransferase" J. Supra. Mol. Struct. 1977(S1):54

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G. Milman (1978) "Chinese hamster purine nucleoside phosphorylase" Methods in Enzymology 52: 538-543

J. Leavitt & G. Milman (1979) "Multiple species of HPRT polypeptides in mouse L cells" Exp. Cell Res. 123: 402-406

J. Epstein, G.S. Ghangas, A. Leyva, G. Milman & J. Littlefield (1979) "Analysis of HGPRT- CRM+ human lymphoblast mutants" Som. Cell Gen. 5: 809-820

G. Milman & M. Herzberg (1981) "Efficient DNA transfection and rapid assay for thymidine kinase activity and viral antigenic determinants" Som. Cell Gen. 7: 161-170

L. Grossman, M. Herzberg, G. Milman, G. Yoakum & B. Goldfarb (1981) "Approaches to studying the expression of endogenus DNA in repair defective human cell lines" Fed. Proc. 40: 1558

N.B. Dinces & G. Milman (1982) "Encapsidation and expression of the Herpes thymidine kinase gene in Polyoma virus" J. Virol. 42: 691-699

N.B. Dinces & G. Milman (1982) "Viable polyoma virus with four Bam H1 decanucleotide linkers inserted at the 26 minute Hinc II site" J. Mol. Appl. Gen. 1: 395-404

C. Flexner, G.S. Hayward, G. Milman & P.S. Leitman (1982) "Selective uptake of 5-(^{125}I)iodo-2-deoxycytidine by cells biochemically and morphologically transformed with herpes simplex virus DNA" Curr. Chemother. Immunother., Proc. Int. Congr. Chemother., 12th. 2: 1074-5

S.W. Liu & G. Milman (1983) "Purification and characterization of E. coli guanine-xanthine phosphoribosyltransferase produced by a high efficiency expression plasmid utilyzing a lambda P_L promoter and cI857 temperature sensitive repressor" J. Biol. Chem. 258: 7469-7475

A.S. Waldman & G. Milman (1983) "Purification and characterization of herpes (type 1) thymidine kinase produced in E. coli by a high efficiency expression plasmid utilyzing a lambda P_L promoter and cI857 temperature sensitive repressor" J. Biol. Chem. 258: 11571-11575

D.J. Sussman & G. Milman (1983) "Short-term high efficiency expression of transfected DNA" Mol. Cell. Biol. 4:1641-1643.

A. Waldman & G. Milman (1983) "Transfer of herepes thymidine kinase synthesized in bacteria by a high expression plasmid to tissue culture cells by protoplast fusion" Mol. Cell. Biol. 4:1644-1646.

D. Sussman, A.L. Bognar, B. Shane & G. Milman (1984) "Expression of the human folylpolyglutamate synthetase gene in Chinese hamster ovary cell mutants (AUX B1) lacking folylpoyglutamate synthetase activity" (in preparation)

D. Sussman, A.L. Bognar, B. Shane & G. Milman (1984) " In situ autoradiographical detection of short-term expression of the transfected human folylpolyglutamate synthetase gene in Chinese hamster ovary cell mutants" (in preparation)

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9. Civil rights

U.S. DEPARTMENT OF ENERGY

ASSURANCE OF COMPLIANCE

Nondiscrimination in Federally Assisted Programs

(Hereinafter called the "Applicant")

(Name of Applicant)

HEREBY AGREES to comply with Title VI of the Civil Rights Act of 1964 (Pub. L. 88-352). Section 16 of the Federal Energy Adminstration Act of 1974 (Pub. L. 93-275), Section 401 of the Energy Reorganization Act of 1974 (Pub. L. 93-438), Title IX of the Education Amendments of 1972, as amended, (Pub. L. 92-318, Pub. L. 93-568, and Pub. L. 94-482), Section 504 of the Rehabilitation Act of 1973 (Pub. L. 93-112), the Age Discrimination Act of 1975 (Pub. L. 94-135), Title VIII of the Civil Rights Act of 1968 (Pub. L. 90-284), the Department of Energy Organization Act of 1977 (Pub. L. 95-91), and the Energy Conservation and Production Act of 1976, as amended, (Pub. L. 94-385). In accordance with the above laws and regulations issued pursuant thereto, the Applicant agrees to assure that no person in the United States shall, on the ground of race, color, national origin, sex, age, or handicap, be excluded from participation in, be denied the benefits of, or be otherwise subjected to discrimination under any program or activity in which the Applicant receives Federal assistance from the Department of Energy.

Applicability and Period of Obligation

In the case o: any service, financial aid, covered employment, equipment, property, or structure provided, leased, or improved with Federal assistance extended to the Applicant by the Department of Energy, this assurance obligates the Applicant for the period during which Federal assistance is extended. In the case of any transfer of such service, financial aid, equipment, property, or structure, this assurance obligates the transferee for the period during which Federal assistance is extended. If any personal property is so provided, this assurance obligates the Applicant for the period during which it retains ownership or possession of the property. In all other cases, this assurance obligates the Applicant for the period during which the Federal assistance is extended to the Applicant by the Department of Energy.

Employment Practices

Where a primary objective of the Federal assistance is to provide employment or where the Applicant's employment practices affect the delivery of services in programs or activities resulting from Federal assistance extended by the Department, the Applicant agrees not to discriminate on the ground of race, color, national origin, sex, age, or handicap, in its employment practices. Such employment practices . ay include, but are not limited to, recruitment, recruitment advertising, hiring, layoff or termination, promotion, demotion, transfer, rates of pay, training and participation in upward mobility programs, or other forms of compensation and use of facilities.

Subrecipient Assurance

The Applicant shall require any individual, organization, or other entity with whom it subcontracts, subgrants, or subleases for the purpose of providing any service, financial aid, equipment, property, or structure to comply with laws cited above. To this end, the subrecipient shall be required to sign a written assurance form, however, the obligation of both recipient and subrecipient to ensure compliance is not relieved by the collection or submission of written assurance forms.

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THE JOHNS HOPKINS UNIVERSITY
SCHOOL OF HYGIENE AND PUBLIC HEALTH
615 North Wolfe Street
Baltimore, Maryland 21205, U.S.A.

DEPARTMENT OF BIOCHEMISTRY
(301) 955-3671

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December 11, 1984

Mr. Walker K. Love
Contracting Officer
Contract Management Branch
Procurement and Contracts Division
Oak Ridge Operations
Oak Ridge, Tennessee 37831

Dear Mr. Love:

Enclosed please find copies of a request for support to purchase a protein-peptide synthesizer and sequencer. The equipment application is prepared under energy are "Health and Environmental Impacts." A similar request was submitted to NSF in October. Thank you for your attention.

Sincerely,

P.C. Huang, Ph.D.
Professor of Biochemistry

PCH:mmh

Enclosure

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