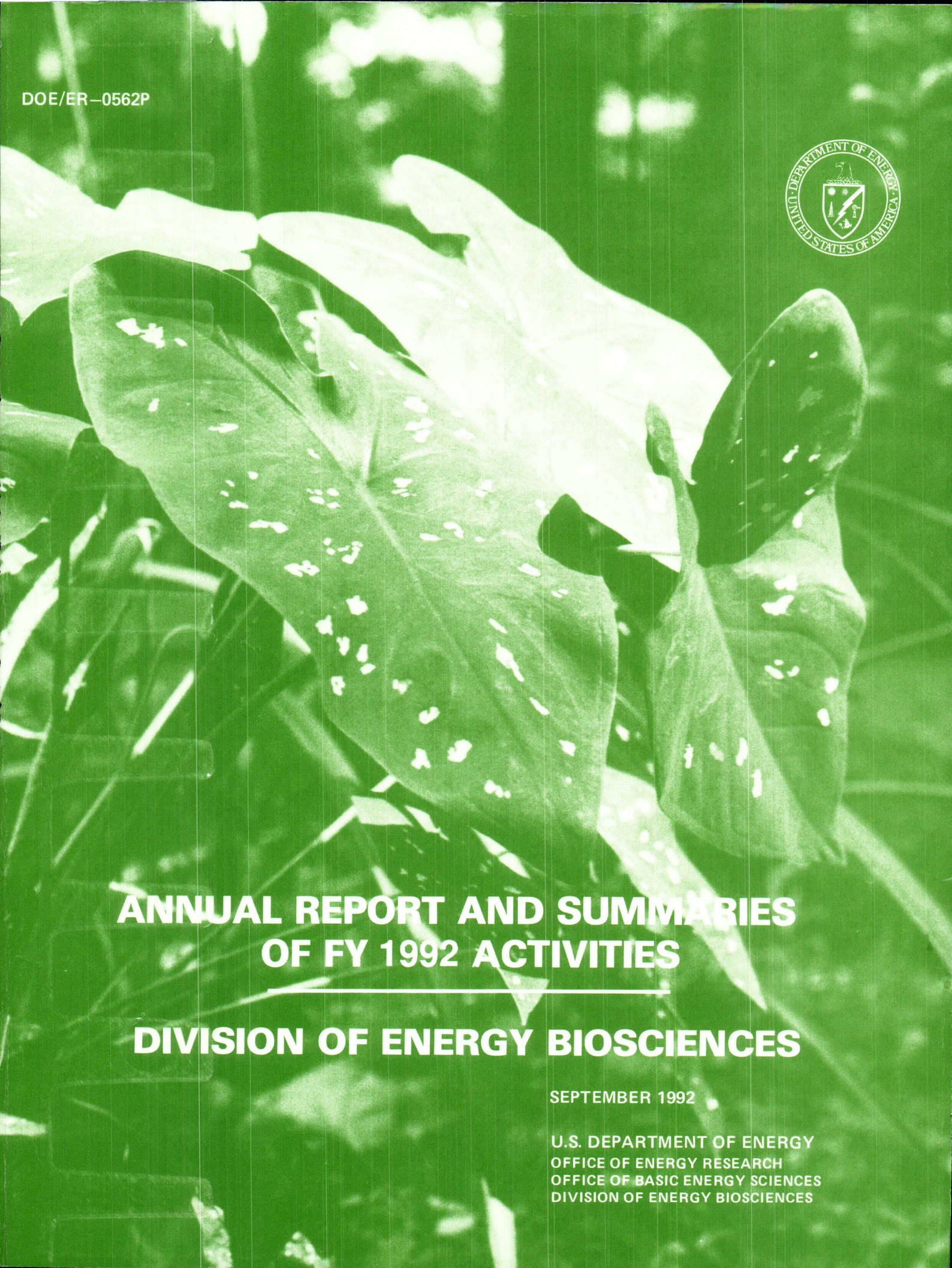
DOE/ER–0562P



ANNUAL REPORT AND SUMMARIES OF FY 1992 ACTIVITIES

DIVISION OF ENERGY BIOSCIENCES

SEPTEMBER 1992

U.S. DEPARTMENT OF ENERGY
OFFICE OF ENERGY RESEARCH
OFFICE OF BASIC ENERGY SCIENCES
DIVISION OF ENERGY BIOSCIENCES

Cover Picture: The leaf is the organ responsible for carrying out the critical biological energy conversion process, known as photosynthesis. Virtually all other life forms depend on plants for their existence. Plant studies therefore are of high priority. The pictured example is a tropical ornamental species of *Caladium*.
(Photo by R. Rabson)

DOE/ER–0562P

ANNUAL REPORT AND SUMMARIES OF FY 1992 ACTIVITIES

DIVISION OF ENERGY BIOSCIENCES

SEPTEMBER 1992

U.S. DEPARTMENT OF ENERGY
OFFICE OF ENERGY RESEARCH
OFFICE OF BASIC ENERGY SCIENCES
DIVISION OF ENERGY BIOSCIENCES
WASHINGTON, D.C. 20585

Program Overview of the Division of Energy Biosciences

As our modern society becomes more aware of the prospects of using plants and microorganisms for the production of various resource materials and for bioprocessing applications, the need for fundamental biological research dealing with such organisms becomes more urgent. In the U.S. today, such research is a critical and a wise investment for the future. Plants and microbes fit easily into the energy context by virtue of serving as renewable resources for fuel and other fossil resource substitutes, as vehicles to restore previously disrupted environmental sites, and for carrying out reactions that conserve energy compared to chemical conversions. The Energy Biosciences (EB) program addresses fundamental biological mechanisms in plants and microorganisms not studied in the health field. The mission is to build foundations of knowledge in order to provide bases for new and improved biotechnologies that relate to energy matters. It is clear that such information could well impact other fields and vice versa.

One of the major objectives of the EB program is to probe the enormous capabilities of these organisms to carry out biochemical conversions. For centuries, cultures around the world have depended on plants and microbes for their food, fiber, medicine, fuel, plus a wide variety of other uses. But the earlier usages were the result of empirical findings without the benefit of the powerful research approaches now becoming available. Approaches such as molecular biology, analytical chemistry, computational analysis and others have opened entirely new horizons and opportunities. The limitations are functions of the commitments being made in generating and merging the new knowledge to realize entirely new products and processes via biotechnology. One of the crucial ingredients to this effort is the study of the basic biology of organisms. Such knowledge will then afford the advantage of developing procedures to the benefit of people and their society.

The EB program is aimed at generating a sufficient breadth of understanding of basic biological mechanisms so that principles of organisms' growth, survival, maintenance, morphological and biochemical characteristics, and energy utilization will be understood. Ultimately, such knowledge will underpin new biotechnologies. This information is crucial to assure that the technologies that emerge not only serve the needs of society, but do so without unexpected risks to both the health of people and the environment in which they live.

It is quite clear that the expanse of any program is governed by a number of factors. The EB program is small but covers a fairly broad scope. Attention is given to key research areas and attempts are made to generate interest in critical research areas that are underpopulated. Investigators are encouraged to explore ideas in areas that may not fit within highly popular research efforts. The EB program does support activities falling within the popular flow of scientific interests. All of these types of efforts are essential in contributing to innovation and building the desired base for future applications.

The broad scope of the EB program is described below. Although the program is arbitrarily broken down into three categories, there is a significant amount of integration and exchange among the three categories reflecting the manner in which biological systems work--not in isolation but as an integrated entity.

I. Primary Biological Energy Conversion. This category comprises research on plant and microbial photosynthesis, the energy driven process central to the support of life on earth. This category includes the initial carbon dioxide fixing mechanisms as well as the associated water splitting and other component reactions. The program also supports research on the fundamental processes that ultimately govern the form and amount of biomass which a plant produces, such as the control of growth and development, and plant interactions with the natural environment (including stress reactions, as well as the interactions with biological agents such as pathogens). Constituents of this broad research category include biophysical, biochemical and physiological, and genetic investigations.

II. Bioconversion of Products. The utilization of the products of the primary energy conversion process is the nature of this second category. A prime component is studies on the diverse metabolic capabilities of plants and microorganisms in synthesizing materials that may ultimately be utilized for fuels and chemicals. These fundamental investigations focus on understanding pathways of metabolism and the genetic and biochemical regulatory mechanisms that determine the nature and amount of metabolic compounds converted. Of special interest are the synthesis, structure and function of plant cell walls, the predominant renewable biomass resource. Also of interest are the mechanisms by which lignocellulose of plant cell walls is degraded biologically into compounds of potential utility. Associated with this category is how plant-microbial interactions occur in symbiotic energy exchanges. A substantial amount of this category covers fundamental microbial conversions, such as methanogenesis and other diverse fermentative pathways.

III. Infrastructure. Underpinning the development of future biotechnologies is a key objective of the EB program. In this category are efforts to better understand genetic mechanisms involving the transfer of genetic information and its expression in plant and microbial systems, which oftentimes have not been studied very extensively. Also included in this portion of the program are such activities as the development of critical data bases, specific techniques and instrumentation. A training component for nurturing areas that are important but underpopulated is also encompassed.

Please refer to pages 115-135 for additional breakdown of the program into categories.

Illustrative of the some of the advances made in the past year that portray the nature of the EB program include:

Transfer of biosynthetic capabilities into higher plants

Using the substantial background technology which has been developed for the transfer and expression of genetic information, Dr. Chris Somerville of the Plant Research Laboratory of Michigan State University and several colleagues have succeeded in demonstrating that it is feasible to install the capability for the biosynthesis of polyhydroxybutyrate into a higher plant, in this case *Arabidopsis*. The origin of the genetic complex encoding the biosynthetic pathway was the bacterium, *Acaligenes eutrophus*. The work demonstrated that it was possible not only to perform a genetic transfer of such a complex, but also have an expression of the genes in the host in a manner that was not easily distinguishable from the original in viewing micrographs of the gene products in the form of polymer bodies.

The relationship of this work to energy matters pertains to the future prospects of using plants as renewable resources as replacement for petroleum products. In this case, the possibility of using plants as a source of a biodegradable plastic would have distinct advantages not only in displacing petroleum, but also in providing a product that can be disposed of easily by virtue of its susceptibility to ready breakdown by microbes. Industry has a growing appreciation about the future opportunities of using plants as a resource for new products. The research sets a pattern for future efforts in devising new products from plants. Much remains to be done in furthering the concept of using plants in this manner in respect to developing the system in larger plants with appropriate concentrating mechanisms to make the products cost effective. The results have been incorporated into at least one commercial firm's activities.

Microbial Mechanism of Glucose Uptake

The fermentative bacterium, *Zymomonas mobilis*, very efficiently converts glucose to ethanol. The reason for this is that very little energy is acquired by the cell for each molecule of glucose metabolized. The organism must process a large quantity of substrate to sustain growth. Studies on the metabolic control of carbon flow through *Z. mobilis* are currently underway in the laboratory of Dr. Tyrrell Conway of the University of Nebraska. Recent results have shown that a type of non-energy requiring uptake system, not previously described in bacteria, is found in this organism. This system, a passive glucose facilitator, instead of the more typical energy requiring active transport system, appears to be intimately associated with the overall control of the glucose to ethanol pathway. The glucose facilitator is likely an adaptation to the low energy yield realized from glucose metabolism. Such information may ultimately be useful in enhancing the production of ethanol.

Discerning Genetic Control and Differentiation

One of the most complex biological processes is differentiation of tissues. While the recent years have witnessed considerable advances in understanding the processes in organisms such as *Drosophila*, plants are only now beginning to be better understood in this context. One such example is in studies of floral differentiation. In recent studies, Dr. Elliot Meyerowitz of the California Institute of Technology and colleagues have gained insights into genetic interactions associated with transitions from vegetative to floral growth. Mutants in *Arabidopsis* have been used for these studies. As these differentiation processes become better understood, it is quite possible that such knowledge may be applied in the future in influencing the overall productivity of plants (renewable resources).

A perusal of the abstracts in the body of this document will indicate the broad diversity of the program.

In the course of a number of research fields supported by the EB program, a number of patents have been acquired by the investigators involved and their institutions. In virtually every case where a patent was acquired, the information has been transferred to an industrial firm for future applications development.

The breakdown of how the resources available to EB were distributed in FY 1992 is indicated in the following table.

	Number of Projects	FY 92 funding (in thousands)	Percent of total funds
University and Non-Profit Institutions	175	$ 16,684	68%
Michigan State University Plant Research Laboratory	13	2,824	11%
Three-Agency Plant Science Collaboration Activities-- Universities	4	1,550	6%
National Laboratories Brookhaven National Lab Lawrence Berkeley Lab Los Alamos National Lab National Renewable Energy Lab	12	2,483	10%
Small Business Innovation Research (SBIR) contribution, and Miscellaneous		727	3%
Conferences, Educational Activities	8	382	1.5%
Databases (joint funding)	1	50	0.5%
	213	$ 24,700	

Some words of explanation are appropriate about the funding figures indicated with abstracts. Most projects are funded for three years, oftentimes receiving two years of funding at a time with a third year to follow. The apparently large figures for the National Laboratory projects (Brookhaven and Lawrence Berkeley Laboratory) include full costs of virtually all salaries and other costs, with no institutional sharing. Likewise, the figures for the projects at the Plant Research Laboratory at Michigan State University include full costs for the infrastructure operations, making the figures appear higher than average.

The Energy Biosciences program in Fiscal Year 1992 was the recipient of 212 new research applications following the screening of numerous preapplications. Of the new applications received, some 26 new projects were funded (ca.12%). A fraction of the funding for new projects derives from the turnover of previously supported research. The strong competitiveness illustrated by these figures prevails in plant science programs of other agencies as well. A similar situation also exists in regard to coverage of certain microbiological topics. All of this adds up to a situation where a considerable number of investigators with quality projects are not receiving support from Federal agencies.

Partial funding for scientific meetings/courses:

1. International Symposium on Topics in Microbial Diversity, Metabolism, and Physiology, University of Illinois, Urbana, May 21-23, 1992.

2. Plant Biochemistry Course, University of California, San Diego--La Jolla, California, June 28-July 19, 1992.

3. Summer Investigations into the Metabolic Diversity of the Microbial World, Marine Biological Laboratory, Woods Hole, MA, Summer 1992.

4. 6th International Symposium on Molecular Plant-Microbe Interactions, University of Washington, Seattle, July 11-17, 1992.

5. Ninth International Workshop on Plant Membrane Biology, Monterey, California, July 19-24, 1992.

6. Keystone Conference on Extracellular Matrix of Plants: Molecular, Cellular and Developmental Biology, Santa Fe, New Mexico, January 9-15, 1993.

7. Funds had also been provided earlier for the partial support of a satellite meeting to the Ninth International Congress on Photosynthesis. The title of that meeting was "Photosynthetic Responses to the Environment" which was convened in Hawaii in late August 1992, prior to the Congress which was held in Japan afterwards.

Once again, three post doctoral fellowships were awarded through the competitive fellowship program of the Life Sciences Research Foundation. The three awardees this year were:

Dr. Scott A. Ensign (Ph.D., University of Wisconsin)
Dr. Cynthia A. Lincoln (Ph.D., Indiana University)
Dr. Erica J. Pascal (Ph.D., University of California-Berkeley)

As part of the Energy Biosciences program, a research directions workshop is convened every three years to examine the content of the program and make suggestions about potential future directions to pursue. The participants include a diverse group, none of whom have current affiliation with the program. Attendees are asked to offer suggestions and criticisms about the operation of the program. The sessions are informal with considerable encouragement for candid suggestions. One of the strong points brought out in the January session was the distinctive need for mechanisms for recruiting and training young persons in the areas relating to basic microbiology (physiology, diversity, etc.). The program anticipates pursuing this need in the near future. Details will likely be announced in the coming months.

An activity that emerged during the past year was an extension of the three agency (DOE,NSF,USDA) collaborative activities in the plant sciences which had begun in 1987 with the funding of four plant science centers at the University of Georgia, Cornell University, Arizona State University and Michigan State University, each covering different topic areas. The continuation of the collaborative effort has shifted away from the support of centers to the support of efforts in training young people in multidisciplinary research and also in establishing networks in certain research areas. The networks are designed to encourage greater activity and broader approaches, along with increased interactions with greater exchanges of ideas, materials, and investigators. A competition for the available funds was held in the Spring of 1992 and resulted in funding six multidisciplinary research training programs and seven network projects. The three agencies look forward to results from these efforts that will enhance plant science activities significantly. A key aspect has been the closeness with which the three agencies with common interests have been able to work together as though a single agency.

Another noteworthy achievement during the past year has been the building of the Complex Carbohydrate Structure Database (CCSD), and CarbBank, the computer program that operates the CCSD. With the cooperation of Chemical Abstracts Service and others, the content of the database grew during the past year from about 8000 structures to over 20,000. The entries now cover almost all of the previously reported structures, thus making the database an evermore useful tool with general availability. The person most responsible for CarbBank activities is Dr. Scott Doubet of the University of Georgia Complex Carbohydrate Research Center. Support for this effort has been a cooperative one with four U.S. agencies besides DOE contributing (National Library of Medicine, National Institute of General Medical Sciences, National Science Foundation and the Agricultural Research Service). Support has also come from the European Community through projects in Denmark, Germany and the Netherlands. This joint effort illustrates what can be achieved in a cooperative manner when the driving force is a common scientific goal. There are other examples as well, such as the *Arabidopsis* cooperative effort in which EB participates.

In addition to the review of new applications, all EB program on-going projects are reviewed regularly every third year, with a few extending to four and five years. The varying review processes involve mail reviews, site visits and panels, where in each case the evaluators are asked to provide individual criticisms and suggestions about each

specific project, as well as their estimation of the importance and quality of the science and other factors that could affect the success of the research.

Once again, the staff of the Energy Biosciences program wishes to express its deep appreciation to the numerous scientists in this country and abroad who have contributed their time and effort so generously in providing technical evaluations of research applications and projects. This program and all other research programs would be seriously impaired without these contributions, called peer review, which provide the basis for selection of quality projects.

Should the reader of this report wish additional information about the EB program, the communication contacts are as follows:

Telephone number: 301-903-2873
Fax number: 301-903-6067
E-mail: Dilworth@OERV01.ER.DOE.GOV
Rabson@OERV01.ER.DOE.GOV

The staff members of the Energy Biosciences program are :

Dr. Robert Rabson
Dr. Gregory L. Dilworth
Ms. Patricia Snyder
Ms. Mary Jo Martin

Division of Energy Biosciences
Office of Basic Energy Sciences
ER-17, GTN
U.S. Department of Energy
Washington, DC 20585

U.S. Department of Agriculture
Madison, WI 53705-2398

1. Molecular Organization in the Native State of Woody Tissue: Studies of Tertiary Structure Using the Raman Microprobe, Solid State ^{13}C NMR and Biomimetic Tertiary Aggregates

R.H. Atalla, Forest Products Laboratory *$120,000*

The studies of wood cell wall structure continue significant focus on the range of variation of molecular composition and organization within the walls, but they are expanded to include questions of structure formation. In addition to Raman spectroscopy and the Raman microprobe, and solid state ^{13}C NMR, a number of complementary instrumental methods are used to explore the states of aggregation and their relationship to cell wall geometry and morphology. The complementary methods include liquid state NMR, UV-visible absorption spectroscopy, measurements of photoconductivity, theoretical modeling of intermolecular interactions, and traditional chemical analytical procedures. The effort to understand the intermolecular interactions which dominate cell wall states of organization has been expanded to include biomimetic aggregates incorporating cellulose, hemicelluloses, and lignin precursors polymerized within the polysaccharide matrices. Preparation and characterization of these aggregates will allow assessment of the effects of the tertiary structure of the polysaccharide environment on the primary structure of the lignins formed within them. Key observations which underlie the program include evidence for: (1) organization of lignin relative to cell wall morphology; (2) a strong influence of cell wall matrix polysaccharides and lignin precursors on the aggregation of cellulose during biogenesis; (3) strong associative interactions between lignin precursors or models and cell wall polysaccharides; (4) photoconductivity in woody tissue suggesting unanticipated pathways for charge transport within the cell wall matrix.

U.S. Department of Agriculture
Raleigh, NC 27695-7631

2. Control of Sucrose Biosynthesis in Plants by Protein Phosphorylation

S.C. Huber, USDA/ARS and Departments of Crop Science and Botany, NCSU
$120,000 (FY 91 Funds/2 years)

Sucrose-phosphate synthase (SPS) is an important control point in the sucrose formation pathway in leaves, and the activity of the enzyme is known to be controlled by protein phosphorylation. When spinach or maize leaves are illuminated, SPS is activated/dephosphorylated by a protein phosphatase, and in the dark, the enzyme is inactivated/phosphorylated by a protein kinase. We have partially purified and characterized the interconverting enzymes that act on SPS in an effort to identify the signals that mediate the light modulation of this cytoplasmic enzyme. We have tentatively concluded that rapid activation of SPS by light involves reduction in cytosolic Pi, an inhibitor of SPS-protein phosphatase (SPS-PP), an increase in cytosolic Glc-6-P, an inhibitor of SPS-kinase, and a light activation of SPS-PP, by a novel mechanism that involves (either directly or indirectly) protein synthesis. Thus, pretreatment of leaves with cycloheximide in the dark strongly reduces the rate of SPS activation/dephosphorylation *in situ*, apparently by preventing the activation of SPS-PP. Therefore, the signal transduction pathway mediating the light activation of SPS involves elements of 'fine' and 'coarse' control. We have also obtained preliminary evidence that spinach leaf NADH:nitrate reductase (NR) is phosphorylated on seryl residue(s) *in vivo* in response to light/dark signals in a

fashion that is analogous to SPS; i.e., both enzymes are phosphorylated and inactivated in darkness. Collectively, these results suggest that protein phosphorylation is an important mechanism for the control of carbohydrate metabolism (sucrose synthesis) and nitrogen assimilation (nitrate reduction) in the cytoplasm. Both processes are dependent upon photosynthesis, and it is possible that a major (and previously unrecognized) mechanism for the coordination among the pathways involves regulation of the protein kinases and protein phosphatases that act on SPS and NR.

Arizona State University
Tempe, AZ 85287-1604

3. Center for the Study of Early Events in Photosynthesis*

R.E. Blankenship, J.P. Allen, W.D. Frasch, J.D. Gust, J.K. Hoober, A.L. Moore, T.A. Moore, G.R. Seely, W.F.J. Vermaas, A.N. Webber and N.W. Woodbury

$1,295,882 (FY 91 Funds/30 months)

A USDA/DOE/NSF Plant Science Center for the Study of Early Events in Photosynthesis was established at Arizona State University in 1988. The Center serves as an infrastructure supporting individual ASU scientists who study photosynthesis using a wide variety of methods and approaches, ranging from molecular biology and biochemistry to organic chemistry, ultrafast laser spectroscopy, X-ray crystallography and theoretical chemistry. The Center is structured to foster multidisciplinary cooperative research projects. Graduate and postdoctoral training programs are central components of the activities of the Center. In addition, the Center brings visiting scientists to ASU.

The ultimate objective of the Center research is to elucidate the basic principles that govern the biochemical and biophysical processes of photosynthetic energy storage. This goal is being pursued via investigations of the early events of photosynthesis, including light absorption and excitation transfer in photosynthetic antennas; the mechanism of primary photochemistry in plant and bacterial systems; secondary electron transfer processes; structure and assembly of photosynthetic antennas, reaction centers and electron transfer proteins; pigment-protein interactions; artificial and biomimetic photosynthetic systems; and the mechanisms of biological electron transfer reactions.

*(A unit of the USDA-DOE-NSF Plant Science Centers program.)

Arizona State University
Tempe, AZ 85287-1604

4. Antenna Organization in Green Photosynthetic Bacteria

R.E. Blankenship, Department of Chemistry *$219,054 (FY 91 Funds/2 years)*

All photosynthetic organisms contain chlorophyll pigments that function as an antenna, absorbing light and transferring excitations to a photochemical reaction center where energy storage takes place by a series of chemical reactions. The green photosynthetic bacteria are characterized by large antenna complexes known as chlorosomes. The overall objective of this project is to determine the molecular organization and mechanism of excitation transfer in chlorosome antennas. The chlorosome pigments are organized *in vivo* into pigment oligomers in which direct pigment-pigment interactions are of

dominant importance. Model compounds are being used to gain more information about the structure of the pigment oligomers found in chlorosomes. Time-resolved spectroscopy has elucidated the pathway and kinetics of excitation flow from the peripheral region of the chlorosome to the reaction center. Recent work has identified a redox-activated control of energy transfer efficiency in green sulfur bacteria. At high redox potential, most excitations are quenched within the chlorosome, while at low redox potentials almost all are transferred to the reaction center. The quenching effect may be a control mechanism that protects the cell from damage during conditions where light and oxygen are present simultaneously. A multidisciplinary approach is being taken to understand the mechanism of this redox-activated control effect, utilizing protein chemistry, spectroscopy and molecular biology.

Arizona State University
Tempe, AZ 85287-1601

5. The Chlorophyll-Binding Protein CP47 in Photosystem II
W.F.J. Vermaas, Department of Botany *$178,010 (2 years)*

Chlorophyll-binding proteins in photosynthetic systems provide the proper environment for efficient transfer of light energy from antenna chlorophylls to the reaction center, where the absorbed energy is used to fuel biological processes. Our research project focuses on the question where in chlorophyll-binding proteins the pigment molecules are bound, and what the effect of binding of individual chlorophylls is on energy transfer efficiency in the antenna system and on stability of chlorophyll-binding proteins. We utilize CP47, a major chlorophyll-binding protein of photosystem II in thylakoids, as a representative system to address these questions. A number of mutations have been introduced in various conserved His residues in hydrophobic regions of CP47 from a transformable cyanobacterium; these His residues are possibly sites of chlorophyll ligation, and we observe that mutation to residues that cannot provide a ligand to the central Mg in the chlorophyll molecule appears to lead to decreased light-harvesting efficiency. However, a detailed spectroscopic *in vivo* comparison of mutant and wild type is difficult because of a major contribution of photosystem I-associated chlorophylls and phycobilins to the spectra. To alleviate this problem, we have cloned the *apc*E and *psa*AB genes, coding for the phycobilisome linker and photosystem I reaction center proteins, respectively, and are in the process of interrupting or partially deleting these genes from the cyanobacterial genome. The resulting mutants are anticipated to have photosystem II fluorescence properties that are well-resolved and are relatively straightforward to analyze.

University of Arizona
Tucson, AZ 85721

6. Engineering the Production of Sugar Alcohols in Transgenic Plants: Extending the Limits of Photosynthesis?
H.J. Bohnert and R.G. Jensen, Department of Biochemistry *$202,000 (2 years)*

Several factors limit photosynthetic carbon assimilation. These include the intrinsic limitations of the photosystems, of the ribulose-1,5-bisphosphate carboxylase/oxygenase enzyme, of sugar biosynthesis, translocation and sugar storage. While strategies to circumvent each of those bottlenecks are currently being examined, we suggest to explore a different approach. We have diverted carbon from sugar production into the production of sugar alcohols and will investigate the effect on photosynthesis

and biomass production. Genes from different organisms have been introduced into model plants which lead to the production of a single polyol or several polyols which are novel to the targeted plant. Once sugar alcohols have been produced they tend, even in plants that normally synthesize these compounds. They accumulate as carbon compounds novel to the transgenic plant. We are working to exploit this concept and, at present, analyze the expression of genes that lead to the accumulation of mannitol. We will increase the diversion of carbon into mannitol to approach or exceed amounts that are significant with respect to total carbon allocation.

At present, we are working to increase the amount of enzyme(s) that produce the novel sugar alcohols in different crop species and to target different cell types to accumulate ten percent of primary carbon assimilation into these products. Experiments are underway by which mannitol, or other transgenically produced polyols, can be translocated or stored in compartments other than the compartment in which they were synthesized.

University of Arizona
Tucson, AZ 85721

7. Role of Pectolytic Enzymes in the Programmed Release of Cells from the Root Cap of Higher Plants

M.C. Hawes, Departments of Plant Pathology and Molecular and Cellular Biology

$170,500 (FY 91 Funds/2 years)

Many plant species release thousands of healthy root "border" cells from their root tips daily. This controlled release of living somatic cells into the environment is unique among higher organisms, and its function is unknown. The purpose of this project is to elucidate the biochemical and molecular mechanism(s) of border cell separation in *Pisum sativum*. Border cell separation is regulated during development, and varies in response to environmental signals. Border cell separation is correlated with the activity of at least two types of pectolytic enzymes, polygalacturonase (PG) and pectinmethylesterase (PME). The goals of the project are to characterize pectolytic enzymes whose activity is correlated with border cell separation; to clone the genes encoding the enzymes; and to test the role of the genes in cell separation. Both pectolytic enzymes are bound to the cell wall of the root cap, and neither is detectable in border cells. Optimum PG activity is detectable at pH 6.0. This enzyme is unstable upon purification. PME activity in root caps is high; at pH 7.0, up to 70 units of PME can be detected in a single root cap. The root cap PME is stable at room temperature, but an inhibitor of PME activity is present in root extracts. Hairy roots induced by inoculation of pea seedlings with *Agrobacterium rhizogenes* will be used to study expression of genes encoding pectolytic enzymes, and to analyze their role in border cell separation.

University of Arizona
Tucson, AZ 85721

8. Role of Zein Proteins in Structure and Assembly of Protein Bodies and Endosperm Texture

B. Larkins, Department of Plant Sciences *$186,001 (2 years)*

In maize endosperm, protein bodies containing the four structurally distinct types of zein proteins form within the lumen of the rough endoplasmic reticulum (RER). Two of these, beta- and gamma-zeins, are cysteine-rich proteins that appear to initiate the process of protein body formation. However, the mechanisms by which these and the other zein proteins interact and are retained within the lumen of the RER are poorly understood. Mutations that alter the types and quantities of several of these proteins result in a soft, starchy endosperm in the mature kernel, suggesting that zeins play an important role in the formation of hard, vitreous endosperm. We are using two experimental approaches to study the mechanisms through which zeins interact and form vitreous endosperm. We are characterizing opaque mutants that result from the reduced synthesis of the sulfur-containing zein proteins. These mutants are being analyzed biochemically and ultrastructurally for their effects on protein body formation. We are also characterizing the synthesis and processing of individual zein proteins and their interactions within the endomembrane system. For these experiments we have constructed hybrid genes containing the coding sequence of each type of zein and a functional endosperm-specific promoter from rice seed storage protein genes. These sequences have been transferred to tobacco and rice plants with which we are characterizing the synthesis and transport of each type of zein protein through the endomembrane system. Transgenic plants producing large amounts of individual zeins will subsequently be crossed and their seeds analyzed for interactions between different types of zeins.

University of Arizona
Tucson, AZ 85721

9. Phytoalexin Detoxifying Enzymes in the Plant Pathogenic Fungus Nectria haematococca

H.D. VanEtten, Department of Plant Pathology *$154,501 (FY 91 Funds/2 years)*

The production of antimicrobial compounds (phytoalexins) by plants is believed to function as an active mechanism for disease resistance. Our research has indicated one way that successful pathogens can overcome this resistance mechanism is by detoxifying their hosts' phytoalexins. Our primary model system has been the disease caused by the fungus *Nectria haematococca* (*Fusarium solani*) on pea (*Pisum sativum*). Our results have indicated that the pathogenicity of this fungus on pea requires pisatin demethylase (pda), a substrate-inducible cytochrome P-450 that detoxifies the pea phytoalexin pisatin. A gene encoding this cytochrome P-450 has been isolated and used, in conduction with conventional genetic analyses, to demonstrate that there is a family of *Pda* genes in *N. haematococca*. Furthermore, a survey of other fungal pea pathogens has demonstrated the common occurrence of pda activity, suggesting that pisatin detoxification may be a common requirement for pathogenicity on pea. Studies during this past year have revealed that only one other fungal pathogen of pea, *Fusarium oxysporium*, containing DNA with significant homology to the *Pda* genes of *N. haematococca*. Biochemical tests of other pea pathogens having pda activity indicates that, as in *N. haematococca*, both the induction of pda and the substrate preference of the enzyme are specific for pisatin. If pisatin detoxification is a general requirement for pathogenicity on pea, then these results suggest that the *Pda* genes evolved independently in the different fungal pathogens.

Boston College
Chestnut Hill, MA 02167

10. Osmoregulation in Methanogens
M.F. Roberts, Department of Chemistry *$166,000 (FY 91 Funds/2 years)*

Nuclear Magnetic Resonance techniques are used to investigate osmoregulation in methanogenic archaebacteria. These experiments fall into three basic groups: (i) *in vivo* NMR studies of methanogens using soluble ^{13}C or ^{15}N labeled substrates and monitoring how sudden changes in external osmotic strength affect intracellular levels of osmolytes; (ii) elucidation of osmolyte biosynthetic pathways using ^{13}C and ^{15}N labeling studies; and (iii) identification of organic osmolytes in other methanogens. Non-NMR experiments are centered on the purification and characterization of a lysine 2,3-aminomutase activity from *Methanococcus thermolithotrophicus*. The osmolyte produced by this enzyme (N_{Σ}-acetyl-ß-lysine) accumulates only at high external NaCl and appears to be carefully regulated. The ultimate goal of these studies is to understand on a molecular level how these anaerobic organisms deal with osmotic stress and to use these insights for increasing the salt tolerance of other cells.

Boyce Thompson Institute for Plant Research, Inc.
Ithaca, NY 14853-1801

11. Differential Regulation of Plastid mRNA Stability
D.B. Stern *$76,610*

The expression of photosynthetic proteins requires a cooperative interaction between the nuclear and plastid genomes, and the development of molecular tools to manipulate plants depends in part on understanding how these genes are regulated. Our research focuses on the control of mRNA stability in a model system, spinach chloroplasts, since mRNA accumulation is an important regulatory mechanism during plastid differentiation in this plant. Plastid mRNA stability is controlled by three interactive components: RNA secondary structure, RNA-binding proteins, and ribonuclease activities. We have exploited an *in vitro* system to study each of these factors. We have shown that hairpin structures located at mRNA 3' ends are required for RNA stability, and that they are also required for correct mRNA processing. We have characterized and partially purified several RNA-binding proteins from spinach chloroplasts. At least two of these proteins bind to the 3' hairpin, and appear to be involved in the formation of multicomponent RNA:protein complexes. One RNA-binding protein of 55 kd co-purifies with a site-specific endoribonuclease, and we are determining whether the same polypeptide is responsible for both functions. We have characterized two types of ribonucleases. The exoribonucleases are non-specific enzymes involved in RNA processing and also in the bulk turnover of RNA. We have also identified endonucleases that cleave within or near the hairpin and are potential rate-limiting enzymes for mRNA decay. We have obtained preliminary evidence that one of these enzymes is active *in vivo*. Using a recently developed chloroplast transformation system, we are beginning to confirm our *in vitro* results in transformed tobacco chloroplasts.

Brandeis University
Waltham, MA 02254

12. Carbon and Hydrogen Metabolism of Green Algae in Light and Dark
M. Gibbs, Department of Biology *$153,814 (FY 91 Funds/2 years)*

We have characterized for the first time the oxyhydrogen reaction (the reduction of O_2 to water by H_2) coupled to the reduction of CO_2 in the darkened isolated *Chlamydomonas reinhardtii* chloroplast by monitoring the rate of $^{14}CO_2$ incorporation into acid-stable products. The endogenous rate of CO_2 uptake (50 to 150 nmole/mg chlorophyll/hour) was increased three-fold by externally supplied ATP and additionally when combined with glucose. The rate was diminished when H_2 was replaced by N_2 or by air. Diminution of CO_2 incorporation by electron transport inhibitors was attributed to an inhibition of the oxyhydrogen reaction resulting in an elevated NADPH/NADP ratio. Our data was consistent with the proposal of Gaffron (1942) that CO_2 fixation in the dark under these conditions is dependent upon extrachloroplastic (mitochondrial) metabolism. The role of an aerobic electron transport chain in the darkened *C. reinhardtii* chloroplast coupled to the evolution of CO_2 from glucose was characterized by the addition of conventional inhibitors. The results were interpreted as evidence for a linear electron transport pathway. Chloroplast respiration was estimated to account for at least 10% of the total respiratory capacity (endogenous release of CO_2) of the algal cell.

Brookhaven National Laboratory
Upton, NY 11973

13. Plant Molecular Genetics
B. Burr and F.A. Burr, Biology Department *$315,000*

We have developed a means of rapidly mapping genes in maize using families of recombinant inbreds. They have been used to compile a detailed molecular genetic map, and have also been used to integrate three maize RFLP maps. These populations are ideal for mapping quantitative trait loci - those genes having metric rather than qualitative effects upon the traits they control. Precise mapping of these genetic factors benefits from a densely populated map, and the use of replicated progeny improves the estimation of the genetic, compared with the environmental, component of variation.

We approach this problem with the expectation that loci affecting quantitative traits are essentially wild-type genes that vary at some level in their expression. Our goal has been to pick a model system where enough genes are known to see if genetic factors affecting the trait in a quantitative fashion map to these previously identified loci.

One model system is anthocyanin biosynthesis. Approximately 70% of the variation in one population can be accounted for by six loci. For three of these, messenger RNA levels are two regulatory genes and one structural gene are correlated with allelic variation associated with pigment synthesis. We are looking for further evidence of allelic variation in gene expression at these loci and for indications of interactions between them.

Telomeres, the chromosomal termini, have lengths that vary more than 25 fold among maize inbreds surveyed. These lengths segregate reproducibly in a recombinant inbred family where 50% of the variation can be accounted for by three loci. In the course of studying this character we have been able to map eight maize telomeres and have learned that the dynamic control of telomere length acts very rapidly to arrive at new lengths in F_1 hybrids.

Brookhaven National Laboratory
Upton, NY 11973

14. Molecular Bases of Photoadaptation in Unicellular, Eucaryotic Algae
P.G. Falkowski and J. LaRoche, Dept. of Applied Science *$105,000*

The photosynthetic apparatus in eucaryotic algae is remarkably plastic. The cellular level of light harvesting pigment protein complexes (LHCP's) can vary by five-fold or more solely in response to changes in growth irradiance. Such large changes, which occur within a few hours, optimize light harvesting at low irradiance levels while reducing photodamage at higher irradiance levels. In algae, photoadaptation is not spectrally dependent and is not mediated by phytochrome. The goal of this research effort is to elucidate the molecular signal transduction pathways which lead to the irradiance-induced changes in the abundance and composition of photosynthetic proteins. Using two species of eucaryotic algae as model organisms, we have isolated, characterized and sequenced the cDNA clones encoding the LHCP's. Northern and western blot techniques have revealed large increases in LHCP mRNA within 2 h upon reduction in irradiance levels, followed by accumulation of the proteins. Hybridization with other photosynthetic gene probes indicates that this response is specific to LHCPs in chlorophyte algae (which contain chlorophylls a and b), but not in chromophytes (which contain chlorophylls a and c). Several genomic clones are being characterized to identify upstream control sequences and DNA-binding proteins. Sequence comparison and reconstitution studies have been initiated to identify how specific pigment protein complexes are regulated and the consequences of the differential expression on the pigment composition and optical absorption cross section of the photosynthetic apparatus.

Brookhaven National Laboratory
Upton, NY 11973

15. Regulation of Energy Conversion in Photosynthesis
G. Hind, Biology Department *$355,000*

Photosynthetic electron transport, energy transformation, and regulatory processes are studied by non-destructive biophysical techniques coupled with the isolation and characterization of membrane-associated enzymes and complexes. The pigment-protein complexes of thylakoids are organized into chiral macrodomains that presumably influence long range energy migration. Much, but not all of the organizational force resides in the light-harvesting chlorophyll a/b complexes (LHC-II). Circular dichroism spectroscopy of mutant and wild-type thylakoids is used to monitor the reversible effects on macrodomain size of electrostatic screening, osmotic pressure of the medium, and energization of the membrane.

Slow structure adaptations known as state transitions also originate in LHC-II. They fine-tune the apportioning of excitation energy between the photosystems and are effected through activity of a membrane-bound kinase and a phosphatase. We have cloned and sequenced the gene for the protein kinase and have purified the phosphatase. The mechanism through which kinase activity is controlled by ambient redox poise is unknown and is studied using biochemical and genetic approaches. We are specially interested in low potential carriers, loosely associated with the stromal

aspect of the thylakoid, that return electrons from photosystem I to plastoquinone. These include ferredoxin, NADPH:ferredoxin oxidoreductase and presumably a plastoquinol:ferredoxin oxidoreductase whose identity we seek.

This work will provide knowledge of mechanisms that optimize photosynthetic efficiency while possibly also protecting the thylakoid against photoinhibition and photodestruction under stress.

Brookhaven National Laboratory
Upton, NY 11973

16. The Biochemistry and Molecular Biology of the Cyanobacteria
H.W. Siegelman, Biology Department *$250,000*

Ten strains of LPP-1 and LPP-2 of cyanobacterial phage were cloned and purified by CsC1 gradient centrifugation. They all have icosohedral morphology, a short tail, and linear double-stranded DNA of about 40 Kbp. Restriction fragment mapping of the ten phage DNAs separated them into two distinct groups identical to their prior serological classification. A physical map of the LPP-1 DNA was constructed, and it showed that all the LPP-1 phage have a discontinuity or nick in their DNAs, while the LPP-2 DNAs are continuous. Complete sequencing of the genomic DNA of the LPP-1 phage is underway by directed walking high-throughput techniques. The photosynthetic light collection system of cyanobacteria is based on phycobiliproteins. The structural and physical properties of allophycocyanin are being examined by neutron scattering and fluorescence lifetime measurements.

Brookhaven National Laboratory
Upton, NY 11973

17. Characterization of Stearoyl-ACP Desaturase
J. Shanklin, Biology Department *$100,000*

The process of fatty acid desaturation is poorly understood despite its central role in lipid metabolism. The plant stearoyl-ACP desaturase represents the best model system to understand this process because: (1) It is the only soluble desaturase identified in any system. (2) It can be expressed in bacteria, is easily purified, and yields a stable functional protein. (3) Desaturases possess common biochemical features, indicating that they probably share a common mechanism of catalysis. Areas of research will be as follows: (A) Characterization of the active site components using various spectroscopic techniques and using site directed mutagenesis to test resulting predictions. (B) Producing large quantities of protein for X-ray crystallographic studies. (C) Exploring the gene regulation in the model plant system, *Arabidopsis thaliana*.

Brown University
Providence, RI 02912

18. δ-Aminolevulinate Biosynthesis in Oxygenic Prokaryotes
S. Beale, Division of Biology and Medicine *$191,000 (FY 91 Funds/2 years)*

Tetrapyrrole pigments function as essential components in the processes of respiration and photosynthesis, and also exist as cofactors in other important enzyme reactions. The tetrapyrroles arise from a branched biosynthetic pathway having δ-aminolevulinic acid (ALA) as its first committed member. ALA is known to be formed by two distinct routes: by condensation of glycine and succinyl-CoA in animal, fungal, and some bacterial cells, and by transformation of the intact carbon skeleton of glutamate in plants, algae, and other bacterial cells. We are characterizing the reaction components for ALA biosynthesis derived from oxygenic prokaryotes, comparing them to their counterparts in plants, and studying the regulation of their activity in response to light and nutritional status. The potential of the prokaryotes for molecular genetic studies is being exploited by generating ALA auxotrophs, and identifying the enzymatic lesions by *in vitro* reaction complementation with purified, identified reaction components obtained from wild-type cells. Genetic complementation of the auxotrophic cells will be carried out by plasmid transformation with genomic libraries obtained from wild-type cells and carried in *E. coli*. The genes coding for the macromolecular reaction components will be isolated, identified, and made available for use as probes for studying the regulation of their expression during adaptation of the cells to light and nutritional status. The probes will also be evaluated for use in measuring expression of analogous genes in eukaryotic algae and higher plants.

California Institute of Technology
Pasadena, CA 91125

19. Genetics in Methylotrophic Bacteria
M.E. Lidstrom, Environmental Engineering Science *$94,142*

The purpose of this project is to use genetic techniques to study the transcriptional regulation of C-1 specific functions in methylotrophic bacteria. The approach involves analyzing C-1 specific genes in a facultative methanol utilizer, *Methylobacterium extorquens* AM1 and using this organism as a host to study genes encoding similar function in methane-utilizers. We have focused on methanol oxidation genes, and have cloned several of these from both methanol and methane utilizing bacteria. We are now analyzing the promoter regions from some of these genes. We have cloned a fragment upstream of *moxF* from *M. extorquens* AM1 and from a Type I methanotroph, *Methylomonas albus* BG8 into a broad host range promoter cloning vehicle that uses *lacZ* as the reporter gene, and fragments show orientation-specific ß-galactosidase activity, suggesting they contain promoter sequences. However, the promoter-containing fragment from *M. extorquens* AM1 is constitutively expressed. We have shown that this improper regulation is an artifact of the plasmid construction, and chromosomal constructions are regulated properly. We have used this fusion in the chromosome to assay transcription of *moxF* in a series of Mox mutants that exhibit pleiotropic phenotypes. Three of these, MoxB, MoxQ and MoxE exhibit only background transcription from this promoter, suggesting that they encode transcriptional regulators. Current work involves testing other putative regulatory mutants for defects in transcription, as well as defining the functions deficient in each. A working model has been developed for regulation of the Mox system in this bacterium.

California Institute of Technology
Pasadena, CA 91125

20. Molecular and Genetic Analysis of LEAFY, a Gene Controlling Floral Induction and Flower Development in *Arabidopsis thaliana*

E. Meyerowitz, Division of Biology *$232,940 (FY 91 Funds/2 years)*

The first step in flower development is the generation of a floral meristem, or floral primordium, by an inflorescence meristem. We are studying the genetic control of this process in the flowering plant *Arabidopsis thaliana*, and have made progress in determining the molecular mechanism by which cells that derive from the inflorescence meristem are instructed to form a flower. We have shown that the production of flowers requires the activity of two regulatory genes, LEAFY and APETALA1, which act together to specify flowers. When the activity of these two genes is eliminated, no flowers are formed, and the plant remains vegetative. When each gene is singly mutated, the resulting phenotype is a partial conversion of flowers to stems. We have cloned the LEAFY gene, and, consistent with the mutant phenotype, we find that LFY RNA is expressed strongly in young flower primordia. LEAFY expression precedes expression of the homeotic genes AGAMOUS and APETALA3, which specify organ identity within the flower. The molecular activity of the protein coded by the LEAFY gene is still unknown, but we have demonstrated that LEAFY is the *Arabidopsis* homologue of the FLORICAULA gene of the distantly related plant, *Antirrhinum majus*.

University of California
Berkeley, CA 94720

21. Genetic Analysis of Adh1 Regulation

M. Freeling, Department of Plant Biology *$182,000 (FY 91 Funds/2 years)*

The pattern of maize *Alcohol dehydrogenase1* (*Adh1*) enzyme expression shows specificity with respect to organ, tissue, developmental stage, and environmental conditions. We are investigating how such expression patterns are established by identifying cis-acting sequences and trans-acting regulatory factors that interact to insure controlled expression of the gene.

We are searching for mutants which exhibit an altered *Adh1* expression pattern. Genetic and molecular biology experiments allow us to distinguish cis-acting from trans-acting mutants. We were able to isolate three potential trans-acting mutants which are most likely transposon-induced and the genes are therefore clonable. We identified two genes encoding the transcription factor TFIID which interacts with the TATA box region and plays an important role in initiation of transcription. Several new cis-acting mutants are under investigation to determine their altered *Adh1* expression pattern. Two transposon-induced, cis-acting, organ-specific mutants have been isolated earlier and detailed information is available. In mutant *r17* the TATA box region is deleted which leads to organ-specific selection of surrogate TATA function and transcription initiation. Its effect on translation is under investigation. In mutant *r53* ADH1 enzyme activity is barely detectable in roots but reaches 50% of wild type activity in other organs. Hypoxic inducibility is not affected. The very low level of enzyme activity in roots appears to be the result of normal expression in only some "specialized" root cells. Cloning of the *r53* allele is in progress and DNA sequence alterations will be indicative for important *Adh1* sequences involved in root cell specific expression.

University of California
Berkeley, CA 94720

22. Regulation of Tomato Fruit Growth by MVA and GTP-Binding Proteins
W. Gruissem, Department of Plant Biology *$214,500 (2 years)*

Cell division, cell growth and differentiation are strictly controlled in growing parts of the plant, but there is little information on the regulatory proteins and molecular mechanisms coordinating plant growth control. Recent discoveries in animals and yeast have established mevalonic acid (MVA) synthesis and prenylation of growth-related signal transduction proteins as critical factors for progression through the cell cycle, and normal cell growth and differentiation. Prenylation of signal transduction proteins therefore establishes a connection between the sterol biosynthesis pathway and mechanisms for coordinating cell division and cell growth, although most of the molecular details are still unknown. Experimental evidence in tomato and *Arabidopsis* suggests that MVA synthesis is critical for normal growth and development, but it is unknown how the sterol biosynthesis pathway integrates with control of cell division and cell growth. The research project will approach this problem from different directions, using tomato fruit growth as a model system. In one approach, different experimental strategies are being used to identify and isolate growth-related proteins from developing tomato fruit and to determine if they must be prenylated to attach to membranes and for biological activity. In a parallel approach, efforts are underway to clone the genes for prenyltransferases from tomato. In a third approach, transgenic techniques are being developed to selectively disrupt the expression of the *hmg1* gene, which is the only one of the four tomato HMGR genes expressed during fruit growth. Together, the experiments will establish the role MVA synthesis has in cell growth control during fruit development.

University of California
Berkeley, CA 94720

23. Determination of Genes and Phenotypes of Bacteria Necessary for Epiphytic Colonization and Survival on Plants
S.E. Lindow, Department of Plant Pathology *$71,965 (FY 91 Funds/2 years)*

Bacteria that live as epiphytes on the surface of healthy plants are important as plant pathogens, in causing plant frost injury by catalyzing ice formation, and in other processes. The objectives of this study are to determine the traits of these epiphytic bacteria which allow them to grow and/or survive in the hostile leaf surface environment. The genes and phenotypes of a strain of *Pseudomonas syringae* that are necessary for epiphytic fitness on bean leaves are being determined by an evaluation of the fitness and phenotypes of 5,300 individual Tn5-induced insertion mutants. Attention is being placed on several mutants which exhibit no known phenotypic alteration, but which while growing normally on moist leaf surfaces succumb to the stresses associated with dry leaf surfaces, unlike the parental strain. Two genes from stress-sensitive epiphytic fitness mutants that have individually large effects on fitness have been identified in a cosmid library of the parental *P. syringae* strain by sequence homology to Tn5-containing fragments cloned from mutant strains. A 4.0 kb region subcloned from one cosmid restores the ability of mutant strains to survive drying stress on leaves. Fusions of these regions with a promoterless *lnaZ* gene have been made to determine the regulation of transcription of these regions *in situ* by measuring the ice nuclei produced in merodiploid strains containing these fusion genes. An Ice$^-$ derivative of the parental *P. syringae* strain has been made by marker-exchange mutagenesis to allow measurements of ice nuclei *in situ* in these merodiploids exposed to different conditions on leaves.

University of California
Berkeley, CA 94720

24. Phytochrome from Green Plants: Assay, Purification and Characterization
P.H. Quail, Department of Plant Biology *$194,000 (2 years)*

The demonstration that phytochrome is encoded by a small family of divergent *phy* genes (*phyA, phyB, phyC*, etc.) has focused attention on the possibility that individual phytochromes may have discrete biological functions. To begin to approach this question we (a) have examined *Arabidopsis* long hypocotyl (*hy*) mutants, defective in light-responsiveness, for phytochrome deficiency; and (b) have overexpressed a "type 2" or "green-tissue" phytochrome in transgenic *Arabidopsis*. Using monoclonal antibodies, developed against individual recombinant phytochromes, we have shown that phytochromes B and C are light-stable and are together the predominant species in green tissue. These two photoreceptor species are thus "type 2" phytochromes. We have also shown that the *hy3 Arabidopsis* mutant is severely deficient in phytochrome B but not in other phytochromes. Because this mutant is defective in the shade-avoidance response, the data indicate that phytochrome B is specifically involved in directing this particular response. Overexpression of either rice or *Arabidopsis* phytochrome B in transgenic *Arabidopsis* results in a light-dependent short-hypocotyl phenotype. The overexpressed phytochrome is spectrally active, undergoes red/far-red-light dependent conformational changes, is synthesized in its inactive Pr form and is stable in the light. This system thus provides a means for performing structure-function analysis on a "green-tissue" phytochrome using site-directed mutagenesis.

University of California
Berkeley, CA 94720

25. Cloning and Characterization of Genes Determining Disease Resistance in Arabidopsis-Pseudomonas Interactions
B.J. Staskawicz, Department of Plant Pathology *$193,000 (FY 91 Funds/2 years)*

We are currently employing a genetic approach to identify and characterize genes from both the host and the pathogen that specify disease resistance. We have employed both natural and induced variation in *Arabidopsis* to study the genetic inheritance of resistance. Our results suggest that there are two loci for resistance in crosses between the resistance ecotype Col-0 and susceptible ecotype Po-1. In addition, we have also developed a screen to detect *Arabidopsis* mutants that are altered in their ability to express resistance to strains of *Pseudomonas syringae* pv. tomato carrying the bacterial avirulence gene, avrRpt2. To date, 6,000 M2 plants derived from diepoxybutane (DEB) mutagenized seed of ecotype Col-0 have been screened for susceptibility. Three fully susceptible mutants and one partially susceptible mutant have been isolated and are in the process of being further characterized.

One fully susceptible *Arabidopsis* mutant, D203, has been genetically characterized in detail and our results demonstrate that this phenotype is controlled by a single recessive locus. This mutant is altered specifically in its ability to recognize bacteria expressing the avrRpt2 gene, as it retains resistance to bacteria containing other avr genes. We have designated this locus, Rpt2, since it controls the specific recognition of strains of *Pseudomonas syringae* pv. tomato that contain the avrRpt2 avirulence gene. We are in the process of mapping this mutant and our preliminary results suggest that this locus resides on chromosome 4. We are currently constructing a fine structure RFLP map of this region and will initiate experiments to clone this locus by chromosome walking.

University of California
Berkeley, CA 94720

26. Analysis of Genes Essential for Floral Development in Arabidopsis
P. Zambryski, Department of Plant Biology *$85,000*

We have begun to characterize a novel mutation, *tousled* (*tsl*), that results in abnormal flower development in *Arabidopsis*. Plants homozygous for *tsl* show relatively normal vegetative growth, except for a slight ruffling at the leaf margins. The flowers, however, show a dramatically altered morphology. The number of floral organs in each flower varies and fewer than wild-type numbers of organs are formed. The floral organs that are formed are rumpled and deformed in appearance. No homeotic conversion of organs is observed. Scanning electron microscope analysis of early *tsl* floral meristems reveals that the absence of floral organs results for a failure to initiate organ primordia. The *tsl* mutant line was found segregating in a population of T-DNA transformed plants (from Dr. K. Feldmann, University of Arizona). The T-DNA insert allowed the cloning of the region disrupted in the mutant line. The nucleotide sequence of a cDNA clone overlapping the insertion site reveals an open reading frame with significant homology to protein kinases. All of the key residues conserved in the catalytic domain of both serine-threonine and tyrosine type protein kinases are found in the *tsl* sequence. Protein kinases play important roles in regulatory pathways, such as control of cell cycle, and control of signal transduction pathways activated by extracellular factors. As such, protein kinases have been shown to be involved in specifying cell fate during development in animals. The *tsl* mutant represents the first example of a defective protein kinase that affects plant development.

University of California
Davis, CA 95616

27. Cellulose Binding Proteins of Clostridium cellulovorans Cellulase
R.H. Doi, Department of Biochemistry and Biophysics *$212,000 (2 years)*

The overall purpose of this investigation is to understand the structure and function of the complex *C. cellulovorans* cellulase which is capable of degrading crystalline cellulose. The specific purpose of this proposal is to characterize the cellulose binding protein (CbpA), one of the subunits of this multisubunit complex consisting of at least 10 different subunits, most of which show endoglucanase activity. The CbpA is a major component of the cellulase complex and is a large protein with a mass of 190 kDA. The protein binds tenaciously to crystalline cellulose and is only removed from cellulose by extensive washing with distilled water. The subunit is required for the degradation of crystalline cellulose, but not for soluble forms of cellulose, e.g. carboxymethylcellulose. No enzymatic activity has been demonstrated for CbpA. The gene for CbpA (*cbpA*) has been cloned and sequenced. An analysis of the sequence structure indicates that it contains information for a signal peptide, two types of putative hydrophilic cellulose binding domains (CBD), and 8 highly conserved and repeated hydrophobic sequences. We will determine the cellulose, endoglucanase, and intermolecular binding sites present on CbpA by dissecting and expressing parts of the gene and analyzing the various functional domains *in vitro*. In this manner we will demonstrate how CbpA interacts with the substrate, the other enzymatic subunits of the cellulase complex, and with CbpA subunits to form the active complex.

University of California
Davis, CA 95616

28. Modifying K^+/Na^+ Discrimination in Salt-Stressed Wheat Containing Chromosomes of a Salt-Tolerant Lophopyrum

E. Epstein and J. Dvorak, Departments of Land, Air and Water Resources, and Agronomy and Range Science *$188,000 (2 years)*

The capture of solar energy by terrestrial green plants dwarfs all other sources of energy utilized by mankind for the production of useful materials -- food, fiber, fuel, building materials, specialty chemicals and medicinals. Over large areas of land, however, salinity of soils and water diminishes this process by damaging plants, particularly crops. The aim of this project is to tap into the salt-tolerance competence possessed by many wild plants, first, to understand the genetic and physiological mechanisms that make for salt tolerance, and second, to use that knowledge to introgress that competence from wild species possessing it into crop species that do not. The focus is on the discrimination between potassium, a major plant nutrient, and sodium, which preponderates in most saline environments. As excess sodium may swamp the mechanisms for potassium absorption and utilization by plants, plants in saline environments must discriminate sharply between these two elements. We have experimented with salt-sensitive wheat, *Triticum aestivum*, and salt-tolerant tall wheatgrass, *Lophopyrum elongatum*, as well as wheat containing *Lophopyrum* chromosomes. The wheat/*Lophopyrum* amphiploid with all seven *Lophopyrum* chromosomes is much more salt tolerant than wheat, both in the field and in salinized solution cultures. Potassium/sodium discrimination goes hand in hand with salt tolerance in field experiments. Evidence, as yet tentative, shows this trait to be expressed gradually, as the plant develops.

University of California
Davis, CA 95616

29. Transposon Tagging of Disease Resistance Genes

R.W. Michelmore, Department of Vegetable Crops *$45,000*

We are developing a transposon mutagenesis system for lettuce to clone genes for resistance to the fungal pathogen, *Bremia lactucae*. Our recent efforts have been focused on the heterologous transposon, *Ac*, from corn. We have detected no transposition of wildtype *Ac* in whole lettuce plants. Transposition could be detected in callus culture using selection for excision from a drug resistance gene. Most of the past year has been devoted to demonstrating transposition in whole plants and generating seed stocks to screen for inactivation of resistance genes (*Dm*). A two element system (*Ds* and chimeric transposase; courtesy of J. Jones) was tested by dual or sequential cocultivation of lettuce explants. This confirmed that both components were functional in lettuce and that trans-activation occurred in regenerated lettuce plants as visualized by excision from a spectinomycin resistance gene, PCR, and Southerns. We then introduced each component separately into a genotype containing 4 *Dm* genes. Over 100 *Ds*-containing lines and 20 transposase-containing lines were generated. These two genotypes were intercrossed and the *Ds*-containing lines were also

crossed to line without any *Dm* genes. 1100 F_1s were tested by PCR for presence of transposase and *Ds*. 150 positives were selected and are being grown to produce selfed seed. This F_2 seed will be tested this summer for inactivation of the *Dm* genes by simultaneously screening for susceptibility to one of four isolates, each detecting one *Dm* gene. F_1s between the *Ds*-containing and susceptible lines are being selfed; these F_2 progeny will be screened to map the *Ds* relative to the targeted *Dm* genes.

University of California
Davis, CA 95616

30. Vacuole Biogenesis in Differentiating Plant Cells
T.A. Wilkins, Department of Agronomy and Range Science *$236,000 (2 years)*

Plant vacuoles are dynamic multifunctional organelles that function in important cellular processes such as homeostasis and cell turgor. Dramatic morphological and biochemical changes associated with the vacuolar compartment in rapidly elongating seed trichomes from cotton offer an excellent system to investigate vacuole biogenesis. Stewart's group (Joshi *et al.*, 1988) showed that significant levels of ATPase activity are associated with the tonoplast and endoplasmic reticulum only in rapidly elongating seed trichomes (fibers). Our efforts are directed towards investigating the regulation of vacuolar H^+-ATPases (VATPase) in elongating cotton trichomes as a focal point to studying vacuolar biogenesis during the differentiation and growth of plant cells. Two related cDNA clones encoding the 69 kD catalytic VATPase subunit (subunit A) have been isolated from developing ovules. Northern blot analysis and *in situ* hybridizations revealed that the catalytic subunit is developmentally regulated in elongating cotton trichomes. The catalytic subunit mRNAs increase significantly to peak levels at *two* stages of development corresponding to the onset of elongation at anthesis and again at 10 days-post-anthesis. Efforts are continuing to explore the tissue-, cell- and organelle-specificity of VATPases in wild-type and genetic fiber mutants in order to assess the role of VATPases during plant cell differentiation.

University of California
Irvine, CA 92717

31. Membrane Bioenergetics of Salt Tolerant Organisms
J.K. Lanyi, Department of Physiology and Biophysics *$150,000*

Energy-generating and utilizing membrane systems are elements of a unique salt tolerant physiology in extremely halophilic archaebacteria. The proton and chloride transporting bacterial rhodopsins and the proton transporting ATPase are described. Studies of the former two systems, bacteriorhodopsin and halorhodopsin respectively, concentrate on the thermodynamics of the reaction cycles, the configuration of the immediate environment of the retinal Schiff base, and on single residues identified or assumed to play roles in the ion transfer reactions. Site-specific mutagenesis with gene expression in *Halobacterium halobium* and optical multichannel spectroscopy are special methods developed for this work. Studies of the latter system, the membrane ATPase of *Halobacterium saccharovorum*, are in the direction of cloning and the sequencing the structural gene, and of describing the catalytic ATP binding sites, the nature and causes of non-linear hydrolytic kinetics, and the shared features with eubacterial and eukaryotic ATPases.

University of California
La Jolla, CA 92093-0116

32. Structure, Biosynthesis and Role of Complex Protein-bound Glycans
M.J. Chrispeels, Department of Biology *$90,000*

Plant glycoproteins contain two types of asparagine-linked oligosaccharide sidechains (glycans). Both types originate as high-mannose glycans in the endoplasmic reticulum when the proteins are first synthesized. Then, as the proteins pass through the Golgi complex, some glycans are modified by enzymes in the Golgi. We are studying the biosynthesis of these glycans, as well as the function of specific glycoproteins that carry such glycans. We recently isolated a mutant of *Arabidopsis thaliana* that is blocked in the pathway of glycan modification. It appears that all glycans accumulate in the $Man_5GlcNAc_2$ form, suggesting that the mutant is deficient in GlcNAc-transferase I. Further studies on this mutant plant will include the characterization of the biochemical lesion and complementation with mammalian GlcNAc transferase I. The glycoproteins that are being studied are extracellular invertase and arcelin/α-amylase inhibitor. We have cloned the gene for cell wall invertase and our efforts will be directed at expressing this gene with a root-specific promoter to study carbohydrate unloading in roots. We have cloned the gene for α-amylase inhibitor and introduced it into tobacco and pea. The purpose of introducing it in pea is to determine if the gene can indeed be used for genetic engineering of legume seeds for resistance to bruchids. We have excellent expression in pea seeds and experiments with bruchids will start in the near future. We are also cloning arcelin genes (arcelin 4 and 5) and hope to understand how phytohemagglutinin, arcelin and α-amylase inhibitor genes (and glycoproteins) are related.

University of California
Los Angeles, CA 90024-1569

33. Energy Capture and Use in Plants and Bacteria
P.D. Boyer, Molecular Biology Institute *$71,312*

The research focuses of how ATP is made by the membrane-bound ATP synthase. One aspect makes use of the exchange of phosphate oxygens with water oxygens as ATP is hydrolyzed or made. This can reveal how substrate concentrations modulate catalytic events and whether all catalytic sites on the enzyme have similar catalytic pathways. For example, our unpublished studies show that when one catalytic site is derivatized with fluorosulfonylbenzoyladenosine, the other two sites retain weak activity but with different characteristics. One catalytic pathway resembles that for native enzyme but the other shows extensive oxygen exchange without substrate modulation. How these and related results with other modifying agents relate to the proposed binding change mechanism and rotational catalysis is being explored. Another study concerns the relationship of nucleotide binding at noncatalytic sites to catalysis and control. In another approach we are attempting to determine whether two or three catalytic sites must be filled with nucleotides for rapid photophosphorylation to occur.

University of California
Los Angeles, CA 90024

34. Molecular Biology and Genetics of the Acetate-Utilizing Methanogenic Bacteria
R.P. Gunsalus, Department of Microbiology and Molecular Genetics
$227,000 (2 years)

Methane formation from acetate by the *Methanosarcina* spp. is often a rate limiting step during anaerobic biodecomposition. Using recently developed methods to grow these methanogens as single cells, and to extract high molecular weight DNA and RNA without shearing, we are examining the molecular and genetic basis for acetate conversion to methane and CO_2. The genes for an acetate inducible carbon monoxide dehydrogenase have been cloned from *Methanosarcina thermophila* TM-1 and are being characterized at the transcriptional and translational levels to understand how they are regulated in response to acetate availability. The molecular and physiological basis of another highly regulated process in these organisms, the ability to osmoregulate in response to changes in salinity is also under study. We are examining how two ß-amino acids, N-acetyl-ß-lysine and ß-glutamine that are produced in response to increased external salt are synthesized and how their levels are controlled during changes in salinity. Each project offers a useful model to elucidate regulatory mechanisms in the acetotrophic methanogens. The *M. thermophila* studies should aid in our understanding of how methanogens are able to sense environmental change and adapt accordingly for growth in varying anaerobic acetotrophic habitats.

University of California
Los Angeles, CA 90024

35. The Gibberellin A_{20} 3ß-hydroxylase: Isolation of the Enzyme and Its Molecular Biology
B.O. Phinney and J. MacMillan, Department of Biology *$69,000*

The long-term objective of this program is to study the biochemical properties of the enzymes that control specific steps in the GA-biosynthetic pathway in maize. The immediate goal of this project is to: (1) locate specific tissue regions in maize shoots that carry out the metabolic step, GA_{20} to GA_1; and (2) explore methods for isolating the 3ß-hydroxylase that catalyzes this step. We measure the release of tritiated water following incubation of tissue preparations with [1ß,2ß,3ß-$^3H_3$]GA_{20} in the presence of buffer and cofactors. The assay measures production of both GA_1 and GA_{29}. Using [17-^{13}C,^3H]GA_{20} as substrate, we have found by GC-MS studies that the metabolic products from our assays are GA_1 and GA_{29}; the highest activities are from nodal tissues with yields of 0.4% and 4.4% respectively. In ancillary studies with an Ac-induced GA mutant, allelic to *dwarf-1* (in cooperation with S.L. Dellaporta, Yale University), we have shown by Southern blots that a 2.8kb Eco RI restriction fragment cosegregates with the *dwarf-1* phenotype. This mutant is useful since it controls 3ß-hydroxylase activity in maize. We are now using this system to obtain the 3ß-hydroxylase in quantities for future biochemical studies.

University of California
Los Angeles, CA 90024-1606

36. Sensory Transduction of the CO_2 Response of Guard Cells
E. Zeiger, Department of Biology *$182,000 (2 years)*

Carbon dioxide is a key regulator of stomatal movements and a coupling signal between stomata and the photosynthesizing mesophyll. Metabolism of carbon dioxide in the guard cells can result in the accumulation of two distinct osmoregulatory products: organic acids, primarily malate, and sugars, primarily sucrose, glucose and fructose. Characterization of the organic acid and sugar content of isolated guard cells by HPLC analysis is making it possible to determine which osmoregulatory pathway(s) is modulated when stomatal apertures change in response to CO_2 signals. Stomatal apertures from *Vicia faba* leaves grown in a growth chamber under a 12 h light/12 h dark cycle, and constant temperature and humidity, are tightly coupled to the intercellular CO_2 concentrations in the chamber (r^2=0.98; aperture ranges: 6-15 um). We are currently characterizing the sugar and organic acid content of guard cells sampled at different points of the daily cycle, and following CO_2 enrichment in the chamber. These measurements will be complemented with radiotracer experiments aimed at the characterization of the initial reactions leading to the osmoregulatory changes.

University of California
Santa Cruz, CA 95064

37. Tonoplast Transport and Salt Tolerance in Plants
L. Taiz, Biology Department *$90,000*

The vacuolar ATPase pumps protons across the tonoplast, generating a pH and electrical gradient which can be used for the transport of various solutes into the vacuole. We have shown that this enzyme is present on a number of organellar membranes, including tonoplasts, Golgi and coated vesicles. We have cloned and partially sequenced the genes for three putative isoforms of the carrot A subunit of the V-ATPase, which may correspond to two major spots observed on western blots of 2-D gels of microsomal membranes. In contrast, purified tonoplasts yield only one spot. T1 plants in which expression of an A subunit gene has been blocked by antisense DNA to the A subunit all have altered leaf morphologies and reduced rates of cell expansion, as well as reduced tonoplast ATPase activities. Interestingly, Golgi ATPase activity is normal, consistent with the observation that only the tonoplast spot is missing from 2-D gels of antisense microsomal membranes. This suggests V-ATPase isoforms are organelle-specific. Progeny of the T1 plants (T2 generation) segregated into phenotypes ranging from wild type to mutant. The presence of the antisense gene was correlated with the mutant phenotype. This provides the first direct evidence for the role of the V-ATPase in cell expansion. Experiments are underway to compare the salt tolerance of the control and antisense mutants.

University of Chicago
Chicago, IL 60637

38. Organization and Regulation of the Genes for Nitrogen Fixation in Rhodobacter capsulatus

R. Haselkorn, Department of Molecular Genetics and Cell Biology *$89,000*

Our goal is still an understanding of the control circuits that regulate expression of the genes for nitrogen fixation in *Rhodobacter capsulatus*. In previous work, we identified four genes and W. Klipp found a fifth whose products regulate transcription of the *nif* genes. *Nif*R1 and *nif*R2 are homologues of *ntr*B and *ntr*C of *Klebsiella*, genes producing a two-component regulatory system responding to the nitrogen status of the cell. The *nif*R4 gene encodes a σ factor required for transcription of the *nif* gene promoters. The *nif*A gene encodes a DNA-binding factor that activates transcription from those promoters. Finally, *nif*R5 encodes PII, a regulator of adenylyl transferase, in which mutations render the cell constitutive with respect to ammonia. In addition, the *nif* genes are transcribed only under anaerobic conditions. Inhibitors of DNA gyrase specifically prevent transcription of the *nif* genes. To improve our understanding of these multiple interactions, we have attempted to purify RNA polymerase, to study the effect of oxygen on DNA supercoiling, and to characterize both polymerase and gyrase genes in *Rhodobacter*. The first two classes of experiment have been uninformative so far. The *rpo*BC operon has been cloned and its sequence is being completed. The *rpo*B gene is very similar to its *E. coli* counterpart. The *gyr*B gene, encoding the B subunit of DNA gyrase, has also been cloned and sequenced. It too is very similar to its *E. coli* counterpart, including the location of several DNA replication genes in the immediate neighborhood. A gene we thought was a second copy of *gyr*B turned out to be *par*E, a closely related gene whose product functions in the partition of chromosomes after the completion of replication.

A physical map of the chromosome of *R. capsulatus* was constructed by overlapping the large restriction fragments generated by endonucleases *Ase*I and *Xba*I. Single fragments were hybridized with the fragments blotted from pulsed-field gels and by grouping cosmids of a genomic library into contigs, corresponding to the restriction fragments, and further grouping of the contigs. Hybridization with cloned genes was needed to close the physical map. In all, 41 restriction sites were mapped on the 3.6-Mb genome and 22 genes were positioned at 26 loci of the circular map. The cosmid clones are being ordered and will be available as a hybridization panel for the rapid mapping of new genes as soon as they are cloned.

University of Chicago
Chicago, IL 60637

39. Signal Transduction in Plant Development: Chemical and Biochemical Approaches to Receptor Identification

D.G. Lynn, Department of Chemistry *$176,000 (FY 91 Funds/2 years)*

Striga asiatica is a small chlorophyll-containing angiosperm that has developed the remarkable ability to establish a vascular connection with another plant. The development of the attachment organ, the haustorium, is one of the most rapid organogenesis events known. Since this parasitic ability is widespread among the plant families, it has generally been assumed that the development of the attachment organ relies on ubiquitous plant developmental programs. It was therefore quite surprising

when it was discovered that relatively simple host-derived phenolic compounds were sufficient to induce haustorial development. The subsequent discovery that simple phenolic compounds had evolved as the primary signal initiating *vir* expression in Agrobacterium and *nod* expression in Rhizobium and that some of these same compounds were important in plant growth and development have raised fundamentally new questions about the role of these compounds in plants.

Subsequent work on the phenolic signals in *Striga* has provided evidence that the compounds are detected via a chemical reaction, again quite distinct from our current models of hormone/growth factor detection by membrane localized binding proteins. Evidence is presented that the recognition mechanism is a redox reaction most likely controlled by plasma membrane localized oxidoreductases. While the existence of these redox systems have been demonstrated in both plants and animals, only recently has convincing evidence connecting e^- transport with plant development emerged.

Striga therefore can teach us not only about the development of host-parasite interactions but also represents a rapid and well defined developmental transition where the chemistry of the inducing signal can be exploited to provide insights into the developmental process. Work over the past year has allowed us to reproduce the activity in a cell free system and to demonstrate the unusual nature of the chemistry that it performs. We propose to use this chemistry both to provide information about the initial detectors controlling haustorial development and to help in the exploitation of the molecular biology of *Striga*.

Clemson University
Clemson, SC 29634-1903

40. The Magnesium Chelation Step in Chlorophyll Biosynthesis
J.D. Weinstein, Department of Biological Sciences *$85,000*

In photosynthetic organisms, the biogenesis of energy generating membranes requires the coordinate synthesis of prosthetic groups, proteins, and various lipids. Chlorophyll and heme are two of the major prosthetic groups and, share a common biosynthetic pathway that diverges at the point of metal insertion into protoporphyrin IX (Proto). Insertion of iron leads to the formation of hemes, while insertion of magnesium is the first step unique to chlorophyll formation. This project is directed toward identifying the enzyme(s) responsible for magnesium insertion and elucidating the mechanism which regulates the flux of precursors through the branch point enzymes. We have now developed an organelle-free preparation from the chloroplasts of pea seedlings which is capable of high rates (25-40% of intact chloroplasts) of magnesium insertion into Proto [Walker, C.J. & Weinstein, J.D. (1991) *PNAS*, 88:5789-5793]. The activity requires MgATP and two protein fractions, one completely soluble and one loosely associated with the membranes. Using a newly developed continuous assay, we have shown that there is an initial lag before maximal activity is attained; preincubation with MgATP shortens this lag, suggesting that an ATP-dependent activation step may be involved in the enzyme mechanism [Walker, C.J., Hupp, L.R. & Weinstein, J.D. (1992) *Plant Physiol. Biochem.* 30(3): In press]. Work is continuing on characterization of the enzymology and regulation of this system.

Cold Spring Harbor Laboratory
Cold Spring Harbor, NY 11724

41. The Suppression of Mutations Generated by Mu Transposons in Maize
R. Martienssen and V. Sundaresan *$144,000 (FY 91 Funds/2 years)*

Transposable elements of the Robertson's *Mutator* family are highly active in the maize genome, and are widely used for gene isolation by "transposon tagging". However, in some cases phenotypic expression of *Mu* induced mutants depends on the activity of the *Mutator* system. In the case of the non-photosynthetic seedling lethal mutant *hcf106*, a *Mu1* transposon has inserted near the site of transcript initiation. When *Mu* is in an active phase, transcripts from the mutant allele fail to accumulate. When plants lose *Mu* activity, transcripts initiate from a novel promoter formed from the junction between the *Mu1* element and the gene. This results in suppression of the mutant phenotype as the resulting message can encode a functional gene product. DNA methylation of *Mu* elements is also correlated with phenotypic suppression, and may be involved in establishing or maintaining altered transcriptional states. There are three other examples of *Mu*-suppressible mutations at cloned loci in maize, and in each one there is a *Mu1* insertion close to the start of transcription. We are developing a simple PCR-based method for cloning *Mu*-suppressible mutations based on this observation, and we are using it to clone a number of suppressible mutants that have so far proved inaccessible to molecular analysis.

Plants carrying the *hcf106* mutation can lose *Mu* activity somatically, resulting in sectors of phenotypically suppressed tissue. These sectors progressively increase in size in successive leaves, and have methylated *Mu* transposons, including the recently discovered autonomous transposon *MuR/MuA2*. Similar sectors can be observed in the ear, and methylated elements in ear sectors are inherited through meiosis. The progressive somatic loss of *Mu* activity thus accounts for differences in the inheritance of *Mu* through male and female gametes. Factors controlling sectoring, which might include cycling autonomous elements, are being characterized genetically. We are also investigating the developmental basis for sectoring by *in situ* hybridization, and by making double mutants with a variety of mutations in maize that affect the pattern of normal development. This research will help us to understand interactions between transposable elements and their host genome, and may lead to more efficient schemes for transposon-tagging and gene isolation.

Cold Spring Harbor Laboratory
Cold Spring Harbor, NY 11724

42. Organ-Specific Gene Expression in Maize: The P-wr Allele
T. Peterson *$95,000*

The ultimate aim of this project is to determine mechanism(s) of organ-specific expression of the maize *P-wr* gene. The *P* gene is required for production of a red flavonoid pigment in certain floral organs, including the pericarp and cob glumes. The *P-wr* allele gives a distinctive organ-specific expression pattern of colorless pericarp and red cob glumes. Preliminary data suggests that *P-wr* transcripts are present in the colorless pericarp as well as in the pigmented cob glumes. Northern analysis will be used to determine which, if any, of the structural genes for flavonoid biosynthesis are expressed in *P-wr* pericarps. The Northern experiments will be complemented by *in situ* hybridization of sections of immature maize ears. *P*-specific antibodies will be used to determine whether the *P-wr* messages are

translated into proteins and also test whether they are post-translationally modified in a pericarp- or cob-specific manner. cDNA clones will be isolated from *P-wr* pericarp and cob glumes to detect any differences in the expressed message in these two organs, possibly arising from alternative splicing. Transient expression experiments using the particle gun will test the functionality of each cDNA in the pericarp and the cob glumes. Finally, several *P-ww* mutants derived from *P-wr* will be analyzed to identify sequences necessary for cob pigmentation. Our results should help to elucidate general mechanisms of organ-specific gene expression in plants.

Cornell University
Ithaca, NY 14853

43. Anaerobic Metabolism of Aromatic Compounds by Phototrophic Bacteria: Biochemical Aspects

J. Gibson, Biochemistry Section, Division of Biological Sciences
$67,910 (FY 91 Funds/21 months)

Compounds containing aromatic nuclei are widespread in natural products, particularly those from the plant kingdom. Increasingly, industrial wastes containing aromatic constituents are also being released into the environment. Many of these products are potentially toxic to humans, and there is concern about their possible contamination of water supplies. Much material containing aromatic residues accumulates in anoxic environments, where it can be attacked only by a limited range of microbial species. Metabolism is thought to proceed by a reductive pathway that is known only in outline, and involves enzymatic processes that are hardly defined at all. This project is using the versatile phototrophic bacterium *Rhodopseudomonas palustris* for studies of the metabolism of two model aromatic acids - benzoate and 4-hydroxybenzoate, that appear to be central intermediates in the anaerobic breakdown of lignin monomers. Further breakdown of these compounds requires formation of coenzyme A thioesters, and two distinct ligases with restricted substrate specificities have been purified; cloning of their genes is under way. Small quantities of two cyclohexadienecarboxylCoA derivatives have been unequivocally identified in the intracellular pools of cells photometabolizing benzoate, suggesting sequential two-equivalent reductive steps in benzenoid ring saturation. The further metabolism of these potential intermediates is being investigated using extracts of wild-type cells and mutants that have restricted growth substrate utilization patterns. The initial reduction of benzoylCoA appears to be rate-limiting *in vivo*; understanding the molecular and biochemical characteristics of the enzymes involved here and later in the reaction sequence offers the possibility of overcoming this limitation, and achieving greater rates of removal of aromatic compounds from anaerobic environments.

Cornell University
Ithaca, NY 14853-2703

44. Molecular and Physiological Analysis of Cytoplasmic Male Sterility

M.R. Hanson, Division of Biological Sciences *$186,000 (2 years)*

The ultimate aims of the project are to understand the molecular mechanism of the disruption in pollen development which occurs in cytoplasmic male sterile plants and to understand the control of respiratory energy flow in the higher plant cell. A mitochondrial locus termed S-*pcf* segregates with sterility and with an alteration in respiration in *Petunia*. This cloned locus contains three genes, an abnormal fused gene termed *pcf*, a gene for a subunit of an NADH dehydrogenase complex, and a small ribosomal subunit protein gene. The *pcf* gene is comprised of partial sequences of ATPase

subunit 9, cytochrome oxidase subunit II, and an unidentified reading frame. Components of the S-*pcf* locus have been introduced into the nucleus of a fertile genotype under the control of several different promoters and mitochondrial transit sequences. Transgenic plants will be analyzed with respect to protein and RNA-level expression and targeting of the chimeric gene product; male fertility and respiratory function will be evaluated. This information may reveal how mitochondrial DNA affects pollen development in the large number of plant species which exhibit the agronomically important trait of male sterility.

Cornell University
Ithaca, NY 14853-1902

45. Mechanisms and Genetic Control of Interspecific Crossing Barriers in Lycopersicon

M.A. Mutschler, Department of Plant Breeding and Biometry
S. McCormick, USDA Gene Expression Lab, Albany, CA

$142,000 (FY 91 Funds/2 years)

Several mechanisms limit cross fertilization among species. Interspecific crossing barriers can result in poor pollen tube growth, lack of fertilization, reduced embryo survival or seed viability in the interspecific cross of its F_1 or F_2, and in aberrant segregation ratios in interspecific F_2's. The interspecific barriers affects the exchange of genes between species, and impedes the transfer of desirable multigenic traits from unadapted and wild germplasm to crop species. Since many of the traits needed for crop improvement are multigenic in nature, it is imperative to understand the nature and genetic control of the interspecific barriers. We are studying the interspecific barriers in the genus *Lycopersicon* using a combination of cytological, genetic and molecular techniques. We have studied the functional basis of unilateral incongruity, determining its timing within the reproductive process and the tissues and genomes involved. Our goals are to determine: 1) the developmental step(s) interrupted and the genetic control of UI, 2) the functional basis of hybrid breakdown (HB), the chromosomal regions associated with HB, and whether the effects of cytoplasm on non-fecundity are direct or indirect, and 3) the functional basis of interspecific aberrant ratio syndrome (IARS) including the stage of reproductive development affected, the tissues and genomes involved, and the basis of cytoplasmic effects of IARS. The long term goals of this project are to study mechanisms controlling interspecific reproductive barriers and to use the information and materials produced to facilitate the transfer of desired quantitative traits.

Cornell University
Ithaca, NY 14853-5908

46. Characterization of a Putative S-locus Encoded Receptor Protein Kinase and its Role in Self-Incompatibility

J.B. Nasrallah and M.E. Nasrallah, Section of Plant Biology, Division of Biological Sciences

$176,000 (FY 91 Funds/2 years)

Self-pollination in *Brassica* species is prevented by the action of genes that reside at the highly polymorphic self-incompatibility (*S*) locus. The basis for self/non-self recognition is attributed to the activity of identical *S* alleles in pollen and stigma. Incompatible pollen fails to germinate or to invade the walls of the papillae, the surface cells of the stigma which capture pollen. Our molecular genetic studies point to a complex *S* locus consisting of at least two genes that are specifically expressed in

reproductive tissues. Of these two genes, the *SLG* gene encodes a secreted glycoprotein, while the *SRK* gene encodes a transmembrane protein kinase. Our research is aimed at understanding the role of the *SRK* gene in the pollen-stigma interaction of self-incompatibility. We have characterized the structure of the *SRK* gene in different self-incompatibility genotypes of *Brassica*. This analysis has shown that within one self-incompatibility genotype, the extracellular domain of *SRK* shares extensive sequence similarity with the *SLG* gene, suggesting that the two genes have evolved in concert. In addition, the expression of the *SRK* gene in pistils and anthers has been demonstrated by RNA blot analysis of endogenous *SRK* transcripts and in transgenic *Brassica* plants. These results suggest a model of cell-cell recognition based on the functional interaction of the secreted glycoprotein and the transmembrane receptor. Future experiments will be directed at deciphering the role of the *SRK* gene product, and include the immunochemical identification of the SRK protein, its subcellular localization, and the characterization of its protein kinase activity.

Cornell University
Ithaca, NY 14853

47. Mechanisms of Inhibition of Viral Replication in Plants
P. Palukaitis, Department of Plant Pathology *$168,000 (2 years)*

Viruses are a major class of plant pathogens that are responsible for crop losses and reductions in plant biomass. In some cases, natural resistance genes are known that can obviate the effects of such pathogens although none of these resistance genes have been isolated. Many of these resistance genes function by blocking the cell-to-cell movement of plant viruses, by unknown mechanisms. To elucidate the mechanisms of inhibition of plant virus movement, we have constructed transgenic tobacco and tomato plants expressing the movement genes of cucumber mosaic virus (CMV) and tobacco mosaic virus (TMV), respectively. These transgenic plants, in combination with viruses either affected by resistance gene products or capable of breaking natural resistances to their cell-to-cell movement, are being used to dissect elements of the interaction between virus and plants leading to either resistance or systemic infection. These plants also provide a system to study the cell biology of cell-to-cell movement. In addition, the movement proteins of TMV and CMV are being overexpressed in a eukarotic system to facilitate the characterization of those proteins, the extent to which they interact or compete with each other, and as probes for the host factors involved in establishing natural resistance to viral cell-to-cell movement.

Cornell University
Ithaca, NY 14853

48. Effects of Freezing and Cold Acclimation on the Plasma Membrane of Isolated Cereal Protoplasts
P.L. Steponkus, Department of Soil, Crop and Atmospheric Sciences *$100,000*

The current focus of this project is on the plasma membrane lesion that limits the maximum freezing tolerance of cereals. Freeze fracture electron microscopy studies reveal that the maximum freezing tolerance of both winter rye and spring oat, which represent the extremes in freezing tolerance of cereals, is associated with the occurrence of localized deviations in the fracture plane of the plasma membrane: the fracture plane 'jumps' from the plasma membrane to aparticulate regions of various endomembranes (chloroplast envelope, endoplasmic reticulum, and tonoplast) that are in close

apposition with the plasma membrane. In rye, this form of injury, which is referred to a the 'fracture-jump lesion', occurs at temperatures below -20°C; in oat, it occurs at -10°C. The lipid composition of the plasma membrane of oat leaves is vastly different from that of rye leaves, with the plasma membrane of oat leaves containing substantially higher proportions of acylated sterylglucosides and cerebrosides and lower proportions of free sterols and phospholipids. Studies are in progress to determine (i) if the fracture-jump lesion is the result of (a) the formation of interlamellar attachments and localized fusion of the plasma membrane and subtending lamellae or (b) interdigitation of lipids that have undergone an L_{α}-L_{β} phase transition in localized domains of the plasma membrane and (ii) to establish a causal relationship between the differences in the plasma membrane lipid composition of oat and rye and the differential in temperature at which the fracture-jump lesion occurs.

Cornell University
Ithaca, NY 14853-8101

49. Genetic Control of Nitrate Assimilation in Klebsiella pneumoniae
V.J. Stewart, Section of Microbiology, Division of Biological Sciences
$153,012 (FY 91 Funds/2 years)

Klebsiella pneumoniae is an enteric bacterium closely related to *Escherichia coli.* Nitrate and nitrite are important alternate nitrogen sources not only for *K. pneumoniae*, but also for many other microorganisms and for most plants. In the absence of ammonium, nitrate (or nitrite) induces the synthesis of assimilatory nitrate reductase and assimilatory nitrite reductase, which act in sequence to convert nitrate to ammonium. Our work focuses on understanding the physiological and molecular mechanisms by which ammonium and nitrate regulate the synthesis of nitrate assimilation enzymes. We have identified and cloned two genes, *nasA* and *nasB*, that encode assimilatory nitrate and nitrite reductases, respectively. Transcription of *nasBA* is induced by nitrate or nitrite in the absence of ammonium. We have determined the DNA sequence for most of the *nas* region, revealing a probable operon of *nasBA.* The predicted *nasB* gene product shares similarity with assimilatory nitrate reductase of *Aspergillus nidulans*, and the predicted *nasA* gene product shares similarly with a variety of nitrate reductases. *In vivo* protein expression experiments have identified the NasB and NasA polypeptides. Surprisingly, the *nas* region is immediately adjacent to the genes for respiratory nitrite reductase (*nar*), even though their regulation and physiological roles are completely different. Our continuing work will focus on understanding the genetic basis for both general nitrogen regulation as well as for nitrate and nitrite induction of *nas* gene expression. Together, these studies will lead to a more detailed view of how nitrogen metabolism is coordinated and regulated.

Cornell University
Ithaca, NY 14853

50. Studies of the Genetic Regulation of the Thermomonospora Cellulase Complex
D.B. Wilson, Department of Biochemistry, Molecular and Cell Biology *$74,000*

The goals of this project are to determine the molecular mechanisms regulating cellulase synthesis in the soil bacterium *Thermomonospora fusca* and to determine the molecular mechanism by which *T. fusca* cellulases degrade crystalline cellulase. We have determined a 2.6Å structure for the *T. fusca* E_2 catalytic subunit (E_2-30) by x-ray crystallography. This structure is quite similar to that of *T. reesei* CBHII but there are a number of differences. One is that the E_2 active site is in a cleft while that of

CBHII is in a tunnel. This is an expected result since E_2 is an endocellulase. Large amounts of homogenous E_5 catalytic subunit have been prepared and attempts to crystallize it are underway. Crystals of E_2-30 were soaked in cellobiose and modified crystals diffracted well, however difference Fourier analysis showed many changes, so that we could not localize cellobiose in the 3-D structure of E_2-30. This implies that binding of cellobiose causes a significant change in the structure of E_2-30. The stereochemistry of the cleavage catalyzed by E_1, E_2 and E_5 was determined in collaboration with Dr. Stephen Withers and E_1 and E_2 inverted the glycoside linkage while E_5 does not. The entire E_1 and E_4 genes have been introduced into *Streptomyces lividans* where they are expressed at a high level and the E_1 and E_4 are completely secreted into the medium. Studies on the synergism between the exocellulase E_3 and the endocellulases E_2 or E_5 show that both exo and endocellulase activities are stimulated when they are assayed together.

Cornell University
Ithaca, NY 14853

51. Conversion of Acetic Acid to Methane by Thermophiles
S.H. Zinder, Department of Microbiology *$99,000*

The objective of this project is to provide an understanding of thermophilic anaerobic microorganisms capable of breaking down acetic acid, the precursor of two-thirds of the methane produced by anaerobic bioreactors. Recent results include: 1) the isolation of *Methanothrix* strain CALS-1, which grows much more rapidly than mesophilic strains; 2) the demonstration that thermophilic cultures of *Methanosarcina* and *Methanothrix* show minimum thresholds for acetate utilization of 1-2.5 mM and 10-20μM respectively, in agreement with ecological data indicating that *Methanothrix* is favored by low acetate concentration; 3) the demonstration of high levels of thermostable acetyl-coA synthetase and carbon monoxide dehydrogenase in cell-free extracts of *Methanothrix* strain CALS-1; 4) the demonstration of methanogenesis from acetate and ATP in cell free extracts of strain CALS-1. Methanogenesis occurred at a high rate (100-300 nmol min^{-1} [mg protein]$^{-1}$); 5) the demonstration that methanogenesis from acetate required 2 ATP/methane, and in contrast to *Methanosarcina*, was independent of hydrogen and other electron donors; 6) the finding that entropy effects must be considered when predicting the level of hydrogen in thermophilic syntrophic cultures. This prediction has been verified by others and has a considerable impact on the modeling of electron transfer in thermophilic anaerobic bioreactors; 7) the isolation and characterization of the *Desulfotomaculum thermoacetoxidans*. Current research is centered on factors which allow thermophilic *Methanothrix* to compete with *Methanosarcina*.

Dartmouth College
Hanover, NH 03755

52. Regulation of Gene Expression in the Bradyrhizobium japonicum/soybean symbiosis
M.L. Guerinot, Department of Biological Sciences *$89,000*

The importance of iron as a virulence factor in animal/pathogen interactions is well established; the role of this metal in plant/bacterial associations is just beginning to be explored. Our studies are directed at understanding the role of iron in regulating the symbiosis between *Bradyrhizobium japonicum* and soybeans. Iron may be an important regulatory signal *in planta* as the bacteria must

acquire iron from their plant hosts and iron-containing proteins figure prominently in all the nitrogen-fixing symbioses. For example,the bacterial partner is believed to synthesize the heme moiety of leghemoglobin which may represent as much as 25-30% of the total soluble protein in an infected plant cell. For this reason, we have focused our attention on the regulation by iron of the first step in the bacterial heme biosynthetic pathway. The enzyme which catalyzes this step, Δ-aminolevulinic acid synthase, is encoded by the *hemA* gene, which we had previously cloned and sequenced. We have now documented a three to ten-fold induction in the activity of a *B. japonicum hemA-lacZ* fusion in response to iron. We have also shown a 7 to 11 fold induction in the activity of a *R. meliloti hemA-lacZ* fusion in response to iron. A series of deletion constructs are currently being assayed to determine which 5' sequences confer iron regulation on the *hemA* gene. We will also identify genes which encode trans-acting factors which regulate *hemA* expression using a variety of screening strategies. Finally, we are identifying other genes which are transcriptionally responsive to changes in iron availability. These studies will shed light on how heme synthesis is regulated in nodules and will contribute to an overall understanding of the regulation of gene expression by metals.

University of Delaware
Lewes, DE 19958

53. Metabolic Mechanisms of Plant Growth at Low Water Potentials
J.S. Boyer, College of Marine Studies *$90,000*

In higher plants, growth is more frequently limited by water than by any other environmental resource. The project objective is to identify the underlying molecular mechanisms causing growth limitations by focusing on the process of cell enlargement. Studies so far have shown that, in localized growing regions in germinating soybean seedlings, turgor in most of the cells was completely maintained when water potentials were low enough to inhibit growth. However, gradients in water potential decreased between the vascular tissue and the enlarging cells. A few h later, the extensibility of the cell walls decreased enough to be inhibitory and a 28kD protein accumulated in the walls. The protein did not accumulate in the mature tissue of soybean stems, nor in the roots where growth continued unabated. Antibodies to this protein were used to select the cDNA for the 28kD protein and a related 31kD protein. The 28kD protein was found mostly in the epidermal cells, which are likely to control the rate of organ growth. The mRNA for the 28kD protein increased in cytoplasm in the shoot tissues where water potentials inhibited growth, but no increase occurred in mature stem tissue or roots. In contrast, the mRNA for the 31kD protein accumulated in the roots but not in the shoots. Experiments are underway to determine whether the protein has an enzymatic role that is related to cell growth.

Duke University
Durham, NC 27706

54. Stable Isotope Fractionation in Photosynthesis: Analysis of Autotrophic Competence Following Transformation of the Chloroplast Genome of Chlamydomonas
J.E. Boynton, N.W. Gillham and C.B. Osmond, Departments of Botany and Zoology *$127,580*

Using *in vitro* mutagenesis and chloroplast transformation, several mutations were introduced into the *Chlamydomonas reinhardtii psbA* gene encoding the photosystem II reaction center D1 protein to study relationship between the D1 synthesis/repair cycle to photosynthetic performance under varying light

conditions. We are characterizing the biochemical and physiological consequences of alterations in the D1 processing site (Ser_{345}->stop); in the Q_B/herbicide domain (Ser_{264}->Ala, Arg_{238}->Lys) and in a putative phosphorylation site (Thr_2->Ala/Ser). Transformants carrying point mutations in the 16S rRNA gene conferring spectinomycin and streptomycin resistance were also shown to have a reduced photosynthetic performance. This appears to result from a decreased synthesis of D1 compared to wild type under high light which slows down the replacement of light damaged nonfunctional reaction centers.

Stable isotope analysis can be used to gain further insights into the reduced photosynthetic performance of certain mutant strains. In addition to the establishment of the analytical system for carbon isotope fractionation, we have developed novel methods to determine the hydrogen and oxygen isotope composition of water in the physiological compartment in which photosynthetic metabolism occurs. One method measures the $\delta^{18}O$ value of the metabolic water of leaves in the CO_2 released, and hence distinguishes it from that in the vacuole and that released by transpiration. The second method involves on-line measurement of $\delta^{18}O$ during photosynthetic O_2 evolution. This reveals precisely the $\delta^{18}O$ of the water oxidized in photosynthesis since no fractionation is observed.

Duke University
Durham, NC 27706

55. Molecular Studies of Functional Aspects of Higher Plant Mitochondria
J.N. Siedow, Department of Botany *$78,000*

Mitochondria isolated from *cms*-T lines of maize are sensitive to toxins (T-toxins) derived from the fungus *Bipolaris maydis*, race T (and related fungi). T-toxin sensitivity is associated with a mitochondrially-encoded, 13 kDa protein, URF13, that interacts with T-toxin to produce pores in the inner mitochondrial membrane. The goal of this research (carried out in collaboration with C.S. Levings, North Carolina State University) is to characterize the mechanism by which URF13 and T-toxin interact to permeabilize biological membranes. Computer modeling has been used to develop a model whereby URF13 is localized in the membrane as an oligomeric complex, with each monomeric URF13 containing three membrane-spanning alpha-helices, two of which are amphipathic in nature and are associated with membrane pore formation. The expression of URF13 in *Escherichia coli* confers T-toxin sensitivity on the resulting bacterial cells and binding studies using radiolabeled T-toxin have established that the toxin binds to *E. coli* expressing URF13 in a specific and cooperative manner. An URF13 fusion protein containing specific epitopes at the N- and C-termini have been used to show the topological orientation of URF13 in the membrane is consistent with the three helix model. Cross-linking studies have confirmed that the oligomeric nature of URF13 in the membrane are being used to establish the role of specific amino acid residues in oligomer formation and T-toxin binding. Attempts are also being made to purify URF13 with the goal of reconstituting URF13 into liposomes to develop a more well-defined system for studying URF13/T-toxin interactions.

Florida State University
Tallahassee, FL 32306

56. Plant, Cell and Molecular Mechanisms of Abscisic Acid Regulation of Stomatal Apertures

W.H. Outlaw, Jr., Department of Biological Science *$190,000 (2 years)*

Stomatal aperture size is regulated to effect a compromise between the opposing priorities of avoiding water loss and of admitting CO_2. Pore enlargement is brought about by swelling of the subtending guard cell pair, a result of accumulation of solutes from the apoplast and synthesis of low MW substances. The specialized metabolism that these cells have evolved to fulfill their crucial role in the plant's physiology extends to more subtle aspects. Our current work has been in the following areas. First, following up on DOE-supported findings that different leaf cells differentially accumulate ABA after stress imposition, we are attempting to determine which leaf cells synthesize ABA. Finding results incongruous with the literature, we have changed our tack. Now, we seek to impair ABA biosynthesis: three inhibiting conditions are used: anoxia (because a mixed-function oxidase is the synthetic pathway), fluoridone (an inhibitor of carotenoid biogenesis) and CHI (a protein synthesis inhibitor that prevents ABA accumulation in response to water stress). Second, we have elaborated a bioassay for ABA; the goal was to conjugate ABA to various-sized peptides to identify the cellular site of the ABA receptor that mediates fast responses. Unfortunately, modifications of the ABA molecule rendered it ineffective, but since our failure, this approach has worked on another plant growth regulator. Several other projects are in preliminary stages (e.g., quantification of calmodulin in guard cells, purification of guard-cell phosphoenolpyruvate carboxylase, ABA- and CA^{2+}-responsive gene expression in guard cells).

University of Florida
Gainesville, FL 32611

57. Ethanologenic Enzymes of Zymomonas mobilis

L.O. Ingram, Department of Microbiology and Cell Science
$188,364 (FY 91 Funds/2 years)

Zymomonas mobilis is a Gram-negative bacterium, grouped taxonomically with the purple bacteria from which endosymbionts of mitochondria are proposed to have evolved. It is the only organism known which is both obligately fermentative and contains an Entner-Doudoroff pathway for glycolysis. A single mole of ATP is produced per mole of sugar fermented with ethanol and carbon dioxide representing 98% of the fermentation products. The enzymes of glycolysis and fermentation together comprise the ethanologenic pathway. High levels of these enzymes are required to generate ATP, comprising 50% of cytoplasmic protein. Little allosteric control exists in this organism provided ATP is consumed. Overall flux through the ethanologenic pathway results in the production of over 1 μM of ethanol (and carbon dioxide) per min per mg cell protein. The high levels of ethanologenic enzymes in *Z. mobilis* must be coordinated to provide the near stoichiometric activities required for efficient glycolysis. A series of regulatory levels are envisioned and investigation of these in *Z. mobilis* is the subject of our ongoing research. These studies focus on the lower enzymes of the pathway which are common to the Embden-Meyerhoff pathway. We are investigating the basis for high level expression, the mechanisms which ensure stoichiometric levels of individual enzymes, the contribution of each

enzyme to flux control, and the assembly of enzymes into macromolecular complexes. Extremely stable mRNA appears to be a primary feature responsible for high level expression. We have developed regulated expression vectors and used these to increase the activity of selected glycolytic enzymes. Doubling the activity of one of these has led to a 15% increase in flux. Immunocytochemical studies have been used to investigate enzyme complex formation within cells. GAP, PGK, and ADHI were co-localized and appear to form a functional complex. Other lines of research are also being pursued to investigate this and other *Z. mobilis* glycolytic complexes. The overall goal for our research is to develop a molecular understanding of glycolysis and ethanol production in *Z. mobilis*.

University of Florida
Gainesville, FL 32611

58. Gene-enzyme Relationships of Aromatic Amino Acid Biosynthesis in Higher Plants
R.A. Jensen, Department of Microbiology and Cell Science
$207,00 (FY 91 Funds/2 years)

The biosynthesis of aromatic amino acids in higher plants is of great significance, not only because of the role of aromatic amino acids in protein synthesis, but because they are precursors of a vast array of compounds of biotechnological and medical interest. Enzyme levels in the pathway have been shown to be regulated in response to physiological stage of growth and to stress induced by mechanical wounding. We have elucidated the enzyme steps of biosynthesis and allosteric patterns of control for the pathway located in the chloroplast compartment. A separate enzyme network in the cytosol has been partially identified and comprehensive enzymological characterizations will be carried out. The post-chorismate portion of the two pathways will be analyzed at the molecular-genetic level. Polyclonal antibodies raised against enzymes purified to homogeneity will be used to clone and obtain the nucleotide sequences of full-length cDNAs. Regulation of aromatic biosynthesis will be characterized at both the levels of enzyme expression and mRNA transcript formation during the growth cycle of suspension cell cultures of *Nicotiana silvestris*, in specialized tissues of organismal plants, and in response to environmental cues such as light/dark treatment, mechanical wounding, or UV illumination. Regulatory mutants capable of amino acid overproduction will be obtained following mutagenesis of haploid protoplasts. Data obtained will facilitate interpretations of the physical and evolutionary relationships between gene pairs encoding chloroplastic and cytoplasmic isoenzymes.

Georgia State University
Atlanta, GA 30303

59. Effect of Growth Temperature on Enzyme Folding
A.T. Abdelal, Department of Biology *$168,000 (FY 91 Funds/2 years)*

Carbamoylphosphate synthetase from *Salmonella typhimurium* consists of two unequal subunits that are encoded by the carAB operon. We have reported the unexpected finding that the growth temperature influences the kinetic and calorimetric properties of this enzyme. This effect could be a consequence of temperature-modulated folding of the enzyme. This hypothesis was tested recently by examining the effect of temperature on refolding of denatured enzyme. The results showed that

the effect of the refolding temperature on the kinetic properties of the enzyme was similar to that of the growth
temperature.

We have previously shown that the cold-sensitive phenotype that characterizes certain mutations in the carAB operon is the result of defective enzyme folding. The site of one such mutation was determined by DNA sequencing. This mutation resulted in a substitution of aspartate for glycine in a conserved polypeptide segment of the small subunit of the enzyme. Determination of amino acid substitutions in cold-sensitive mutants is expected to provide information on the specialized sequences that may direct the folding pathway of carbamoylphosphate synthetase.

Numerous reports indicate that thermal stability of enzymes synthesized by thermophiles is a function of the growth temperature. This effect has been interpreted in terms of expression of duplicate sets of genes at different growth temperatures. We have extended our studies to *Bacillus stearothermophilus* to test the alternative hypothesis that the observed effect of growth temperature might be a consequence of a mechanism for thermal adaptation that exploits the effect of temperature on enzyme folding.

University of Georgia
Athens, GA 30602

60. The Metabolism of Hydrogen by Extremely Thermophilic Bacteria
M.W.W. Adams, Department of Biochemistry and Center for Metalloenzyme Studies
$173,000 (FY 91 Funds/2 years)

Extremely thermophilic bacteria are a unique group of microorganisms that have the remarkable property of growing optimally near and above 100°C. They have been isolated mainly from marine volcanic environments, including deep sea hydrothermal vents. We grow some of these organisms in large scale culture (500 liters) and are studying their pathways of hydrogen (H_2) metabolism. The archaebacteria, *Pyrococcus furiosus* (T_{max} 105°C), *Thermococcus litoralis* (T_{max} 98°C) and "ES-4" (T_{max} 110°C) produce H_2 by the fermentation of carbohydrates and/or peptides and their growth is stimulated by tungsten (W), an element seldom used in biology. From each organism we have purified a nickel-containing hydrogenase, ferredoxin, rubredoxin, pyruvate ferredoxin oxidoreductase, and two different types of tungsten-containing enzyme that both function as aldehyde ferredoxin oxidoreductases. One type is thought to couple substrate oxidation to H_2 production in a new glycolytic pathway, while the other type appears to be involved in peptide catabolism. All of the enzymes have optimum temperatures for catalysis above 95°C, and the redox proteins are stable at 95°C for at least 12 hours. Recombinant DNA and crystallographic techniques are currently being used to investigate mechanisms of extreme thermostability. In addition, from the most thermophilic eubacterium currently known, *Thermotoga maritima* (T_{max} 90°C), we have purified a new type of iron-containing hydrogenase, which lacks the catalytic iron-sulfur cluster of mesophilic hydrogenases. Since molecular H_2 plays a central role in the commercial production of many chemicals, a long term objective of this research is to assess the utility of extremely thermophilic hydrogenases in industrial energy conversions.

University of Georgia
Athens, GA 30602-4712

61. CarbBank - A Structural and Bibliographic Database for Complex Carbohydrates
P. Albersheim, Complex Carbohydrate Research Center *$50,000*

The Complex Carbohydrate Structure Database (CCSD), an IBM PC-compatible computerized database, and CarbBank, the database management system, were created to provide a structure and citation information system to meet the needs of persons interested in complex carbohydrates. CCSD and CarbBank, which were initiated by an international body of carbohydrate scientists, have been in development since 1986 and have been commercially available since 1989. We propose to continue developing CarbBank-associated software, to continue building the CCSD, and to make the software available as part of larger database efforts administered by the National Center for Biotechnology Information (NCBI) and by Chemical Abstracts Service (CAS). We intend to create a completely open format for the CCSD and portable, well-documented program modules that will ultimately be available to academic and commercial groups interested in creating computer programs that access CCSD data. The CCSD and CarbBank will also be made compatible with several additional computer environments. The CCSD is expected to grow from its present 3,888 records to over 20,000 records. It is our goal to ensure that CarbBank plays a key role in managing carbohydrate structure information in the global biotechnology arena.

University of Georgia
Athens, GA 30602-4712

62. The University of Georgia Complex Carbohydrate Research Center (CCRC)*
P. Albersheim and A. Darvill, Complex Carbohydrate Research Center
$1,000,000

The CCRC, with its multidisciplinary faculty and staff, was formed to serve as a national resource for basic research in complex carbohydrates. The program of this center consists of research, training, and service activities. The CCRC assists scientists from other laboratories in defining the structures and studying the biological functions of plant and microbial carbohydrates. The research of the CCRC focuses on various aspects of carbohydrate science, including methods development, structural characterization, and function elucidation. Educational activities involve the training of graduate students, postdoctoral research associates, and visiting scientists in the analytical methods used for studying carbohydrate structures. Two week-long laboratory training courses are held annually for scientists from institutions and industries located throughout the United States. The services offered involve conducting routine analyses of carbohydrate samples provided by scientists from other institutions. These analyses include determination of glycosyl-residue and glycosyl-linkage compositions, and acquisition and interpretation of one-dimensional NMR and FAB-MS spectra. The CCRC also forms collaborations with scientists on more extensive research projects. The CCRC side of fourteen collaborations with scientists from other institutions are currently supported by this grant. These services and collaborative investigations are limited to non-proprietary research.

*(A unit of the USDA-DOE-NSF Plant Science Centers Program.)

University of Georgia
Athens, GA 30602-4712

63. The Structures and Functions of Oligosaccharins
P. Albersheim, Complex Carbohydrate Research Center *$168,389*

This project is concerned with the isolation and characterization of oligosaccharins, naturally occurring complex carbohydrates that possess biological regulatory activities. We have evidence that oligosaccharins, when released from the complex carbohydrates of cell walls, regulate various biological functions within plants. We are studying the following oligosaccharins. [1] Oligosaccharins isolated from plant cell walls that elicit phytoalexin (antibiotic) accumulation in plant tissues. Research in this area is emphasizing the involvement of fungal *endo*polygalacturonases (EPGs) and a plant-derived inhibitor (PGIP) of the fungal EPGs in the release of elicitor-active oligogalacturonides (DP=10-14) from plant cell walls. We are particularly interested in elucidating the mechanism by which EPGs cause necrosis in some plants. [2] An oligosaccharin that may trigger the hypersensitive resistance response in plants. We have purified to homogeneity an *endo*xylanase and an arabinosidase secreted by *Magnaporthe grisea* that are involved in the release of an oligosaccharin from isolated plant cell walls that kills plant cells. We are cloning the *endo*xylanase with the goal of determining genetically whether it is required for eliciting the hypersensitive response to this pathogen. We are also purifying and characterizing the bioactive oligosaccharide(s). The same or other wall fragments that kill plant cells are released by autolysis from walls isolated under conditions where the plant's own enzymes are not denatured. We are attempting to identify these fragments. [3] An oligosaccharin derived from xyloglucan that inhibits auxin-induced growth. The first oligosaccharin in this series contains nine sugars. Recently, a more active 11-sugar oligosaccharin has been identified. We have also purified to homogeneity and are cloning an α-fucosidase of plant cell wall origin that destroys the biological activity of these oligosaccharins. [4] An oligosaccharin that is able to induce the formation of flowers and vegetative shoots and inhibit the formation of roots in tobacco epidermal explants. Our studies with tobacco and soybean are emphasizing studies of α-1,4-linked oligogalacturonides with DPs of 12-14 that, when released from plant cell walls by *endo*-α-1,4-polygalacturonase, stimulate floral development and inhibit root development. Oligogalacturonides with DPs of 12-14 also elicit defense responses in plants. To elucidate the molecular mechanisms by which oligogalacturonides function, we are attempting to isolate and characterize a physiological receptor for the oligogalacturonides and are investigating the effects of the oligosaccharins on ion transport and membrane polarity of plant cells, which we have some reason to believe are an early step in the signal transduction pathway to the physiological effects of the oligogalacturonides. We are also studying endogenous plant endopolygalacturonase(s) to determine their role in the release of biologically active α-1,4-oligogalacturonides.

University of Georgia
Athens, GA 30602-4712

64. Structural Studies of Complex Carbohydrates of Plant Cell Walls
A. Darvill, Complex Carbohydrate Research Center *$392,589*

The cell walls of a plant determine the plant's structure and morphology and act as a barrier to pests. Cell walls are also a source of complex carbohydrates with biological regulatory properties (oligosaccharins). This project involves the isolation and structural characterization of the complex

carbohydrates that constitute approximately 90% of the primary cell walls of plants. These structural studies emphasize detailed analyses of the two pectic polysaccharides, rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II), and the hemicellulosic polysaccharide xyloglucan. We are chemically and enzymatically cleaving RG-I to produce side chain oligosaccharides, which will be purified and structurally characterized. We are continuing the characterization of the cell wall pectic polysaccharide RG-II by using a combination of chemical and enzymatic methods to determine the order and positions of attachment of the side chains to the α-1,4-galacturonosyl backbone with the aim of elucidating the entire glycosyl sequence of this polysaccharide. We are continuing the structural characterization of cell wall xyloglucan by characterizing *endo*glucanase-released xyloglucan oligosaccharides that contain 15-20 glycosyl residues. We are generating and characterizing monoclonal antibodies to specific cell wall epitopes for use in localizing polysaccharides and polysaccharide substructures in plant cell walls. The localization studies are a collaboration with Dr. L. A. Staehelin of the University of Colorado and also with Dr. K. Roberts of the John Innes Institute. We are beginning a study on characterizing the structures of RG-1, RG-II and xyloglucan from cell walls isolated from different plant tissues and organs. The research supported in this project will increase our knowledge of the primary structures of cell wall polysaccharides and of their locations in the cell walls and will enable us to build a more complete model of the primary cell wall of plants.

University of Georgia
Athens, GA 30602

65. Molecular Biology of Lea Genes of Higher Plants
L. Dure, Department of Biochemistry *$84,999*

The Lea proteins of higher plants are *l*ate *e*mbryogenesis *a*bundant. They appear to be universal in occurrence. Certain Lea proteins have been shown to be inducible in non-seed tissue by water stress and/or ABA treatment. Others appear to be seed specific and may function in preventing vivipary, in the acquisition of desiccation tolerance or in other facets of seed formation and survival. Several of the Lea proteins have amino acid sequences that strongly suggest secondary and tertiary structures. Further, these structures in turn suggest functions that would seem important in surviving extreme desiccation, e.g., the mature seed. We wish to describe tolerance to desiccation in terms of the properties of two families of Lea proteins: D-7 and D-29.

This involves biophysical characterization of protein structure by determining molecular weight, Stokes radii, CD spectra, NMR analyses and equilibrium dialysis against specific ions. This requires considerable pure protein. D-7 protein is currently being produced and purified from transformed *E. coli*. We plan to accumulate D-29 via bacterial expression also. Further, we plan the bacterial synthesis of a 39-mer artificial peptide that contains three 11-mer repeating units of amino acid sequence that typifies the D-7 and D-29 proteins and their homologs from other species. The biophysical measurements given above will be obtained for this model peptide. We anticipate that all these measurements will demonstrate a coiled-coil dimer with a very high affinity for K+/Na+ and PO_4^- ions.

Coincident with these measurements, the most likely 3D structure of the 11-mer repeating unit will be obtained by computer modeling (program SYBYL) and energy minimization (program AMBER) of a 23-mer abstract peptide containing two 11-mer repeats. From these empirical data and computer derived predictions, we hope to test the idea that the D-7, D-29 families of Lea proteins function as ion carriers in dehydrated cells.

University of Georgia
Athens, GA 30602

66. Mechanisms of Lignin Biosynthesis During Xylogenesis in Zinnia elegans
K-E.L. Eriksson and J.F.D. Dean, Department of Biochemistry $204,500 (2 years)

Lignin, an aromatic polymer that constitutes a significant proportion of the dry weight of plant cell walls, plays important roles in plant structure, defense and water conduction. We seek to identify the extracellular enzymes controlling the deposition of lignin, particularly in secondary cell walls. *Zinnia elegans* will be used as the biological system because techniques exist for this plant to coax relatively undifferentiated leaf mesophyll cells to differentiate reproducibly and synchronously into tracheid-like cells surrounded with secondary cell walls *in vitro*. In particular, starting with stems harvested from intact plants, we will purify, characterize, and raise antibodies against peroxidases and hydrogen peroxide-generating enzymes. As part of another research program looking at lignification of wood, we have shown that laccases may play a role in lignin deposition; thus, we will also look for laccases in *Zinnia*. Immunolocalization of the characterized enzymes with fluorescent probe-labeled monoclonal antibodies will enable us to assess expression of the enzymes in various plant tissues and subcellular locales, as well as during differentiation of mesophyll cells in culture. Simultaneous immunolocalization in these tissues of the enzymes and lignin, using our lignin-specific antibodies, will provide spatial, as well as temporal, correlations between specific enzymes and lignin deposition. The research has the potential to yield an integrated enzymatic model that accounts for the differences in lignin condensation and composition found in different plant tissues, as well as provide a foundation for attempts to manipulate lignin structure and composition for specific purposes.

University of Georgia
Athens, GA 30602

67. Environmental Stress-Mediated Changes in Transcriptional and Translational Regulation of Protein Synthesis in Crop Plants
J.L. Key and R.T. Nagao, Department of Botany $159,000 (2 years)

The influence of high temperature stress (commonly referenced as heat shock or HS) on mRNA and protein synthesis and on plant growth is the major focus of this research project. Stress agents which mimic somewhat the HS response are used as experimental tools in an attempt to enhance the understanding of regulatory mechanisms operative in the "HS response."

Amino acid analogs were used to study the soybean HS response. Although the treatment of amino acid analogs induced the transcription and translation of most hsp families, the data suggest that the synthesis of functional hsps is required for the normal regulation of HS gene expression and the acquisition of thermotolerance.

As a continuing effort to understand the complexity of HS gene families of soybean, the genes coding 110 kD hsps is under study. The hsp110 family has different kinetics of synthesis than the other hsp families. The yeast hsp104 gene is being used as a heterologous probe to screen the soybean genomic library, and a cDNA library constructed by enriched HMW HS-induced mRNAs is being screened as approaches to identify clones to this unique family of hsps.

The physiological role that LMW hsps play in HS and recovery from HS conditions has been approached by identifying and characterizing cDNAs which encode proteins targeted for the endomembrane system. The specific localization and potential function of these proteins are under

study. Over- and underexpression of these proteins driven by a strong HS promoter (at least 10 to 20 fold better than their normal promoters) and any physiological consequences on membrane-associated activities represent an initial approach to assess function.

University of Georgia
Athens, GA 30602

68. Regulation of Polyamine Synthesis in Plants
R.L. Malmberg, Botany Department *$188,000 (FY 91 Funds/2 years)*

Polyamines are small positively charged compounds that have been hypothesized to be involved in a wide variety of plant physiological and developmental functions. The regulation of the polyamine synthesis pathway is uniquely interesting because of the existence of two pathways to putrescine synthesis, and the consequent questions of how these two pathways are compartmentalized and how they interact with each other. The specific directions our research is taking are: (1) A characterization of arginine decarboxylase regulation in oats and in *Arabidopsis* using cDNA and genomic DNA clones and a set of antibodies we have developed. This includes analysis of protein processing as part of enzyme activation, cellular localizations, and the regulation of arginine decarboxylase by polyamines, and the construction of transformed plants containing an introduced arginine decarboxylase gene as a means of perturbing the pathway. (2) A similar characterization of the regulation of ornithine decarboxylase. (3) We are using the clones and antibodies to characterize the polyamine mutant collection we have developed. Our intent is to understand arginine decarboxylase structure and regulation in detail, and then to further explore regulatory differences between ornithine and arginine decarboxylases.

University of Georgia
Athens, GA 30602

69. Soybean Ribulose Bisphosphate Carboxylase Small Subunit: Mechanisms and Determinants of RNA Turnover in Higher Plants
R.B. Meagher, Department of Genetics *$182,000 (FY 91 Funds/2 years)*

Our research focuses on understanding the mechanisms and determinants controlling RNA turnover in higher plants. During the last few years we have characterized the *in vivo* degradation products of ribulose-1,5-bisphosphate carboxylase small subunit (rbcS) mRNA from soybean. The most stable products represent nested constellation of fragments, which are shortened from their 3' ends, and have intact 5' ends. We showed that these products are associated with polysomes and released by EDTA (Thompson et al., 1992, *Plant Cell*). Based on these data, we had success in developing a polysome based plant *in vitro* RNA degradation system, which generates the same rbcS RNA products from endogenous rbcS RNA as are observed in total RNA. In order to prove the precursor product relationship in the system, *in vitro* synthesized rbcS RNA was added to the system. The exogenous RNA degrades into a similar constellation of products. The kinetics of appearance of these exogenous products suggests degradation proceeds in an overall 3' to 5' direction. An exciting preliminary result suggests that changes in the sequence of the exogenously added rbcS RNA, which significantly alter RNA secondary structure, drastically transform the constellation of products. The next year's research will focus on further characterization of this *in vitro* system. Initial efforts to clone and express *Arabidopsis* poly(A) binding protein (pabp) genes and cDNAs have been successful. In the near future we hope to use the *in vitro* system to assay pabps and other potential determinants of plant RNA turnover.

University of Georgia
Athens, Georgia 30602

70. Microbiology and Physiology of Anaerobic Fermentations of Cellulose

H.D. Peck, Jr., L.G. Ljungdahl. L.E. Mortenson and J.K.W. Wiegel; Departments of Biochemistry and Microbiology *$350,000*

The conversion of polymers such as cellulose and hemicellulose to methane, acetic acid, ethanol or similar low molecular carbon compounds occurs in anaerobic environments and is catalyzed by consortia of anaerobic microorganisms. These consortia are complex and consist of several types of bacteria and fungi. Present work includes cellulolytic clostridia, acetogens, ethanologens, sulfate reducing bacteria, methanogens, and anaerobic fungi; the latter from rumen of cows. Prominent in the interactions of the microorganisms and in the transfer or generation of energy are hydrogen, CO, CO_2, formate, acetate and other C-2 compounds. Thus, major parts of our work deal with hydrogenases, formate dehydrogenases, and CO dehydrogenases, all of which have multiple metal centers. In addition most of the bacteria produce several forms of each enzyme. A special study involves the generation of energy in the acetogens *Clostridium thermoaceticum* and *C. thermoautotrophicum* during autotrophic grow. Acetogens previously believed to be heterotrophs only have now been found to have very diverse autotrophic metabolism that include electron acceptors such as sulfite, dimethylsulfoxide, CO, CO_2, aldehydes and even carboxylic acids. Membranes of them contain menaquinones, cytochromes, flavoproteins and ATPase systems. The electron transport system, ATP generation, and interspecies H_2-transfer and H_2-cycling is being studied on the molecular level. Several hydrogenases from *C. pasteurianum, C. thermoaceticum. A. woodii, and D. vulgaris* have been characterized in detail. However, the genetics of these bacteria are not well understood. Consequently the genetic system of *E. coli* which contains 3 different hydrogenases have been investigated. With this system nickel binding site and iron centers are being mapped. Genes needed for hydrogenase manufacture and control are also being investigated. One hydrogenase (the 20 Fe from *Clostridium pasteurianum*) has been crystallized and preliminary maps are being obtained in cooperation with Dr. Bolin at Purdue. The formate dehydrogenases have characteristics in common with the hydrogenase systems: Multiple enzyme species with different metal redox centers; MoSeFe, MoFe, WSeFe together with pterin; cytoplasmic and peroplasmic localizations, and involvement in the generation of a proton gradient by vectorial electron transfer. The structures of the metal clusters and their role in the metabolism of formate are being investigated with the goal of understanding the function of formate in the total synthesis of acetate from CO_2 and its role in the bioenergetics of these microorganisms. CO dehydrogenase, a key enzyme in the new anaerobic autotrophic CO_2 fixation pathway contains Ni plus non-heme iron and the structure of its metal redox centers will continue to be investigated. Cellulose and hemicellulose hydrolysis is being investigated with enzymes from *C. thermocellum* and the anaerobic fungi *Neocallimastix* and *Orpinomyces.* Most of this work is directed toward the elucidation of the structure of the multipolypeptide complex, called cellulosome. The architecture of this complex is being determined through partial dissociation to map the interaction between the about 25 different polypeptides of the complex. The fungi in contrast to *C. thermocellum* are efficiently breaking down hemicellulose. Several phenolic esterases have been recognized to participate in this breakdown. The esterases are being characterized as well as xylanases.

University of Georgia
Athens, GA 30602

71. Nitrogen Control of Chloroplast Development and Differentiation
G.W. Schmidt, Department of Botany *$77,000*

The development of the photosynthetic apparatus in plants and algae is directed by environmental stimuli and factors that mediate cellular differentiation. Whereas the most intensely studied environmental effector has been that of the photoregulation, our studies concern the role of nitrogen availability and the effects of this nutrient on chloroplast differentiation. Plants and photosynthetic microorganisms are commonly restricted in their growth and development by the availability of nitrogen and develop the classical symptom of chlorophyll deficiency. Toward understanding the molecular basis and physiological consequences of adaptation to nitrogen-deficiency, we study biosynthetic and gene expression processes in the unicellular green alga *Chlamydomonas reinhardtii* which is grown in an ammonium-limited continuous culture system. We have shown that mRNAs encoding apoproteins of light-harvesting complexes are among the nuclear gene products that are strongly regulated by nitrogen availability. Chloroplast gene expression is affected at the posttranscriptional level. Many other nuclear genes that are differentially expressed in response to the nutrient have also been cloned and are being characterized to elucidate potentially regulatory elements and the functions of the protein products. These should include enzymes for synthesis of starch and storage lipids that are accumulated in massive amounts in nitrogen-deficient chloroplasts. Also, thylakoids of nitrogen-deficient cells possess greatly enhanced chlororespiratory electron transport activity whose components are being characterized at the biochemical and molecular levels.

University of Georgia
Athens, GA 30602

72. Molecular Characterization of a Maize Regulatory Gene
S.R. Wessler, Botany Department *$92,000*

The *R* gene family controls the spatial and temporal distribution of anthocyanin deposition in the maize plant. Prior genetic and molecular analysis indicated that each family member encodes functional equivalent proteins with features of a transcriptional activator including acidic and basic domains; the latter containing a helix-loop-helix motif presumed to be involved in DNA binding and dimerization.

To understand how expression of this gene family results in the observed variety of pigmentation patterns characteristic of different maize strains, we have focused on both the expression of individual *R* genes and the function of the R protein. Previous studies revealed that alterations in the *R* promoter led to new patterns of pigmentation thus implicating transcription initiation as an important control point in *R* gene expression. Recently we have determined that the *R* gene *Lc* is also regulated at the level of translation. Specifically, we have used particle bombardment to introduce *in vitro* mutagenized constructs into maize tissues to quantify the effects of mutations in an upstream ORF. Our data indicates that translation of this short ORF (38 amino acids) is required for repression of downstream *R (Lc)* expression. Furthermore, the amino acid sequence of this putative 38 amino acid polypeptide influences the magnitude of repression of *R* expression leading to our hypothesis that this peptide is translated *in vivo* and represses translation of the *R* gene. Experiments to test this hypothesis will initiate this year. Interestingly, the composition of the untranslated leader region of other *R* genes

differ, some have this ORF, others have upstream AUGs while others lack all upstream AUGs. The evolution of translational control among *R* genes and the possible role of this level of gene control in tissue specific expression is also under examination.

Structure-function studies of the R protein are centered around the analysis of a large collection of *Ds*-induced mutations (isolated by J. Kermicle, Wisconsin) that display graded effects on pigmentation levels. Unlike *in vitro* mutagenized *R* genes that are assayed via particle bombardment, strains containing the *Ds* derivatives are isogenic, differing from each other by only a few amino acids at the site of *Ds* excision. Preliminary examination of these mutations has already provided different results from those obtained using the particle bombardment assay.

University of Georgia
Athens, GA 30602

73. Biochemistry and Genetics of Autotrophy in Methanococcus
W.B. Whitman, Department of Microbiology *$142,000 (FY 91 Funds/2 years)*

Even though methanogenic bacteria catalyze the terminal step in the anaerobic oxidation of organic matter, these bacteria have a very limited catabolic capability. To understand the apparent lack of catabolic activity toward complex substrates, two key biosynthetic anabolic enzymes systems, the carbon monoxide dehydrogenase (CODH) system and the pyruvate oxidoreductase/synthase (POR) system, will be characterized. Using enrichment methods developed in this laboratory, eight acetate auxotrophs of *Methanococcus maripaludis* have been isolated and shown to be defective in the CODH system. The proposed research will further characterize the CODH system from these auxotrophs and the wild type to identify the biochemical basis for these mutations. Because some of the auxotrophs are also defective in POR, the apparent coupling of the CODH system and POR will be explored. Preliminary studies have also demonstrated that the methanococci are unable to oxidize pyruvate in the presence of H_2. However, in the absence of H_2 resting cells will utilize pyruvate as an electron donor for methanogenesis. Genetic and biochemical studies will be performed to further examine the unidirectional nature of this enzyme system under H_2 and pyruvate oxidation *in vivo*. To facilitate genetic studies in the methanogens, a shuttle vector has been constructed from the methanococcal plasmid pURB500, the *Escherichia coli* cloning vehicle pUC19, and the puromycin-resistance cassette provided by Drs. Klein and Possot. Studies on the transformability of methanococci are also being performed with this vector and other markers. To determine the pathway of glycogen catabolism, enzymatic assays of possible pathways have been performed. These studies have identified the Embden-Meyerhof pathway in *Methanococcus maripaludis*.

University of Georgia
Athens, GA 30602

74. Hemicellulases from Anaerobic Thermophiles
J. Wiegel, Center for Biological Resources Recovery and Dept. of Microbiology
$80,000

The goal of this research effort is to obtain an anaerobic thermophilic bacterium that efficiently converts various hemicellulose-containing biomass to ethanol over a broad pH range. The strategy is to modify the outfit and regulation of the rate-limiting hemicellulase(s) in the ethanologenic,

anaerobic thermophile *Thermoanaerobacter ethanolicus*, isolated previously by us and which grows between pH 4.5 and 9.5. This requires knowledge of the involved enzymes. Thus, we have started to characterize the hemicellulolytic enzymes from this and other anaerobic thermophiles. The xylanase activity in *T. ethanolicus* is barely measurable, although it utilizes xylans. The results from this research will extend the presently limited knowledge of hemicellulases in anaerobic bacteria. We have isolated and partially characterized xylosidases/arabinosidases from three thermophiles and an acetyl (xylan) esterase, partially purified an O-methyl glucuronidase and xylanases from a slightly acidophilic, neutrophilic and slightly alkalophilic thermophile covering together a range from below pH 3.0 up to pH 10. We study presently regulatory properties of these enzymes including induction of the various enzymes involved. This year we will continue the characterization of the enzymes and start to clone the appropriate enzymes first into *E. coli* using probes made accordingly to our N-terminal sequences and then develop a proper shuttle vector for cloning into *T. ethanolicus*.

University of Georgia
Tifton, GA 31793

75. Development of Innovative Techniques That May be Used as Models to Improve Plant Performance

W.W. Hanna and G.W. Burton, Department of Agronomy *$45,000*

The objectives of this project are to: (1) establish the cytoplasmic diversity of germplasm in the weedy subspecies of the primary gene pool and demonstrate its value, (2) identify mechanism(s) for transfer of germplasm from the secondary gene pool to the cultivated species and evaluate the plant breeding potential of this germplasm, and (3) transfer gene(s) controlling apomixis from the tertiary gene pool to cultivated pearl millet for the purpose of producing true-breeding hybrids. Species within the genus *Pennisetum* are being used as test organisms. The approach uses plants of wild species with different genetic and cytoplasmic backgrounds, ploidy levels, and chromosome numbers crossed and backcrossed with different genotypes of diploid and tetraploid pearl millet. Significant cytoplasmic and/or cytoplasmic-genic effects due to *Pennisetum glaucum* subsp. *monodii* and *P. sweinfurthii* cytoplasms were found for forage yield, plant height, inflorescence length, days to flowering, seed set and seed weight. A cytoplasmic-genic male sterile (with disease resistance from *monodii*) and a pollinator with genes from *P. purpureum* were released to produce the first released pearl millet grain hybrid in the U.S. Apomictic BC_4 plants (closely resembling pearl millet) with 27, 28+ fragment, or 29 chromosomes have been produced.

University of Hawaii
Honolulu, HI 96822

76. Violaxanthin De-Epoxidase: Biogenesis and Structure

H.Y. Yamamoto, Department of Plant Molecular Physiology *$95,297*

Violaxanthin de-epoxidase (VDE) is a lumen enzyme which converts violaxanthin in the thylakoid to zeaxanthin. Enzyme activity requires low lumen pH and presence of reducing potential (ascorbate). *In vivo*, zeaxanthin forms under conditions of excess light relative to CO_2 fixation. VDE is of interest because zeaxanthin increases the rate of non-radiative (heat) dissipation energy, thereby apparently protects plants against potential damage from the excess light. VDE has been partially purified and characterized previously but nothing is known yet about its biogenesis and structure. In this project,

VDE will be purified from chloroplasts using standard methods and a new lipid-affinity purification step developed in preliminary studies. After N-terminal and internal amino-acid sequencing of the protein, nucleotide probes will be generated and VDE sequences amplified by polymerase chain reaction. cDNA libraries from *Lactuca sativa*, cv. Romaine will be probed and positive clones sequenced by standard methods. The complete cDNA sequence will yield insights about structure. Translation and transport studies will answer biogenesis questions. Cloning of VDE may make it possible to ask further questions about the role of zeaxanthin in evolutionary adaptation to light stress, the possible importance of the xanthophyll cycle in the ability of higher plants to withstand global climate changes and the effects of inactivating the de-epoxidase on plant protection and productivity.

University of Illinois
Chicago, IL 60680

77. Heavy Metal-lux Sensor Fusions and Gene Regulation
S. Silver, Department of Microbiology and Immunology
$204,000 (FY 91 Funds/2 years)

The project is studying gene regulation of the heavy metal resistance determinants from large plasmids of *Alcaligenes eutrophus* and other Gram negative and Gram positive bacteria, using primarily gene fusions to reporter genes *in vivo* and direct transcript mapping (both *in vivo* and *in vitro*). The *Alcaligenes* resistance determinants being studied include: (a) *czc*, cadmium, zinc and cobalt resistance; (b) *cop*, copper resistance; (c) *cnr*, cobalt and nickel resistance; (d) *chr*, chromate resistance; and (e) *mer*, mercury resistance. In addition, arsenic resistance (*ars*) determinants from other plasmids are being studied. Each of the systems is inducible. Most have been studied biochemically and have been cloned and sequenced. Using gene fusions to reporter genes, mostly *lux* (luciferase), or less often to *lacZ* (ß-galactosidase) or *bla* (ß-lactamase), we are characterizing the trans-acting regulatory genes ("*metalR*") and the range and specificity of the metal response. The gene fusions (especially with luciferase) obtained for laboratory studies will be subsequently useful as biosensors that can sensitively and specifically detect toxic metal ions in environmental settings and thus function for field measurements of "bio-available" toxic metal levels.

University of Illinois
Urbana, IL 61801

78. Genetics of Solvent-Producing Clostridia
H.P. Blaschek, Department of Food Science *$203,000 (FY 91 Funds/2 years)*

Genetic systems and methods for the manipulation of the acetone-butanol-ethanol (ABE) fermentation microorganism, *Clostridium acetobutylicum* are being developed and used to engineer isolates with enhanced extracellular cellulolytic activity. A plasmid designated pCAK1 was constructed by ligating the 5 kpb pAK102 *Escherichia coli* plasmid and the 6.6 kpb replicative form (RF) of the CAK1 viruslike particle from *C. acetobutylicum* NCIB 6444. pCAK1 was transformed into *E. coli* and *C. acetobutylicum* ATCC 824. The pCak1 ssDNA generating construct is the basis for the development of a versatile M13-like genetic system for *C. acetobutylicum*. A *C. cellulovorans* phage lambda library in lambdaZap II was screened for cellulase components. A cellulase positive plaque was isolated and *in vivo* excision of the pBluescript plasmid from the lambdaZap II vector resulted in the plasmid pBAW401 which contains by CMCase encoding insert. Restriction enzyme mapping, hybridization and

sequence analysis shows that we have independently cloned the endoglucanse gene engB originally cloned and sequenced by Doi. Our clone differs, in that we have cloned an additional 450 base pairs of DNA upstream of the putative translational start site which contains the *C. cellulovorans* promoter for engB as well as potential regulatory sites. Our characterization of the clone is consistent with Doi's in that the enzyme, EngB, encoded by the gene is secreted into the periplasmic space of *E. coli.* The enzyme is active against carboxymethylcellulose, xylan and lichenan, but not Avicel. The gene codes for a protein with a molecular mass of 48.6 kDa. Constructs are being developed to clone this gene into the pCAK1 shuttle vector for transfer to *C. acetobutylicum.*

University of Illinois
Urbana, IL 61801-4798

79. Mechanism and Structure of the Plant Plasma Membrane Ca^{2+}-ATPase
D.P. Briskin, Department of Agronomy *$156,000 (FY 91 Funds/2 years)*

The plasma membrane Ca^{2+}-ATPase couples ATP hydrolysis to the extrusion of Ca^{2+} at the plasma membrane of the plant cells. This process is important for the maintenance of the low cytoplasmic Ca^{2+} concentration required for the function of this divalent cation as a second messenger in bioregulatory signal transduction. At present, relatively little is known regarding the structure, enzyme reaction mechanism and transport mechanism of this important transport protein. In this project, research is being conducted to characterize the mechanism and structure of the plant plasma membrane Ca^{2+}-ATPase using red beet (*Beta vulgaris* L.) storage tissue as an experimental system. Studies on the activity of the plasma membrane Ca^{2+}-ATPase using alternative purine nucleoside triphosphate substrates have shown the usefulness of CA^{2+} dependent ITP hydrolytic activity as an assay for this enzyme which is independent of Ca^{2+} transport measurements. Such an assay will be utilized during purification and reconstitution of the enzyme. Protein modification studies have also shown the presence of an essential arginine in the mechanism of the plasma membrane Ca^{2+}-ATPase. When alternative substrates (ITP or GTP) were utilized to drive Ca^{2+} transport in isolated plasma membrane vesicles, no evidence for production of either an interior positive or negative membrane potential could be found using fluorescent probes. Such results would suggest that Ca^{2+} transport mediated by the plasma membrane Ca^{2+}-ATPase occurs in an electroneutral manner. Our preliminary work would support the concept that the plasma membrane Ca^{2+}-ATPase mediates Ca^{2+} transport coupled to electroneutral H^+ exchange.

University of Illinois
Urbana, IL 61801

80. Photosynthesis in Intact Plants
A.R. Crofts, Department of Physiology and Biophysics
$220,000 (FY 91 Funds/2 years)

The main goal of the project is to understand the mechanism and control of photosynthesis in intact plants under field conditions. We have developed portable and laboratory based instrumentation, and methodology for studying photosynthesis in intact plants, and a substantial program of field based research using these instruments is underway. The project also includes a laboratory based program

of research to establish in simpler systems the parameters through which observation of photosynthetic electron transport and energy coupling in intact plants can be measured. We are studying the partial reactions of the oxygen evolving complex, the two-electron gate of the acceptor side of photosystem II, and the intermediate electron transfer chain, including the b6/f complex and its interactions with the quinone pool, the cycle around photosystem I, activation and turn-over of the ATP-ase, the role of the thioredoxin system in control of C-metabolism, and the down-regulation of photosynthesis under high light. We are using a protocol involving molecular modelling, site-directed (and site-selected) mutagenesis, and biophysical assay of mutant strains, to explore the structure-function relationship in the two-electron gate of photosystem II, using Chlamydomonas as a model for the higher plant system, and anticipate extending this approach to studies of the donor side. We are studying the temperature dependence of the secondary donor and acceptor reactions of photosystem II, both from a thermodynamic perspective (to identify and measure activation barriers), and to identify sites of inhibition on damaging the oxygen evolving complex at high and low temperatures. We will continue the development of novel instrumentation, and we will continue and extend the research programs under way, and the collaborations established around the instrumentation developed.

University of Illinois
Urbana, IL 61801

81. Mechanism of Proton Pumping in Bacteriorhodopsin
T.G. Ebrey, Department of Physiology and Biophysics *$83,000*

The purple membrane of *Halobacterium halobium* probably represents the simplest biological solar energy conversion system. Light absorbed by bacteriorhodopsin, a small protein whose chromophore is retinal, directly leads to the transport of protons across the cell membrane. The resulting chemiosmotic potential can be used to make ATP. An additional feature of the purple membrane is its ability to pump protons over a wide variety of salt concentrations including extreme saline environments. This project investigates the relationship between the transport of protons across the membrane and structure of bacteriorhodopsin. Our focus in this research is on a) proton movement as detected with pH sensitive dyes or photocurrent measurements, b) the protonable groups of bacteriohodopsin, especially tyrosines, lysines, and aspartic acids, and c) the ability of protons to have access to these protonable groups.

University of Illinois
Urbana, IL 61801

82. Studies on the bo-type Ubiquinol Oxidase From Escherichia coli
R.B. Gennis, Department of Biochemistry *$232,000 (2 years)*

The *bo*-type ubiquinol oxidase is the predominant respiratory oxidase present in the bacterial membrane of *Escherichia coli* when the cells are grown with high aeration. This enzyme, also known as the cytochrome *o* complex, is a coupling site. This means that the redox reaction catalyzed by the enzyme, i.e. the 4-electron reduction of oxygen to water, is coupled to the generation of a proton and voltage gradient across the cytoplasmic membrane. Our primary interest is to learn how this is done.

The *cyo* operon encoding each of the five subunits of the oxidase has been cloned and sequenced. Remarkably, three of the oxidase subunits demonstrate a strong similarity to the mitochondrially encoded subunits of the eukaryotic cytochrome *c* oxidase. Recent biophysical studies have confirmed and strengthened the relationship between these two enzymes. Each enzyme contains one low spin heme, as well as a binuclear center consisting of a high-spin heme and a copper. Our research program is currently emphasizing the use of site-directed mutagenesis in combination with spectroscopic techniques to obtain structural and functional information. For example, the low spin heme b_{562} component of the *bo*-type oxidase has been shown to be ligated by His106 and His 421 in subunit I. Of particular value has been the use of Fourier transform infrared spectroscopy (FTIR), carried out in the laboratory of Dr. J. Alben (Ohio State University). We have confidence based on FTIR data in assigning other residues as ligands to the heme *o* and Cu_B components of the binuclear center. Our current model places six of the twelve transmembrane spans of subunit I in a manner consistent with the perturbations caused by mutations in numerous sites.

Other mutations are designed to search for residues involved in proton transfers that accompany catalysis. Protons are used chemically to form water from oxygen and are, in addition, pumped across the membrane. A proton-conducting channel is almost certainly present, and our mutagenesis experiments are targeting residues that might be part of this structure.

University of Illinois
Urbana, IL 61801

83. Regulation of Cell Division in Higher Plants
T. Jacobs, Department of Plant Biology *$82,000*

Cell division is arguably the most fundamental of all developmental processes. In higher plants, mitotic activity is largely confined to foci of patterned cell divisions called meristems. From these perpetually embryonic tissues arise the plant's essential organs of light capture, support, protection and reproduction. Once an adequate understanding of plant cell mitotic regulation is attained, unprecedented opportunities will ensue for analyzing and genetically controlling diverse aspects of development, including plant architecture, leaf shape, plant height, and root depth. The mitotic cycle in a variety of model eukaryotic systems is under the control of a regulatory network of striking evolutionary conservation. Homologues of the yeast *cdc2*gene, its catalytic product, p34, and the cyclin regulatory subunits of the MPF complex have emerged as ubiquitous mitotic regulators. We have cloned *cdc2, cdc2*-like and cyclin genes from pea. As in other eukaryotic model systems, p34 of *Pisum sativum* is a subunit of a high molecular weight complex which binds the fission yeast p13 protein and displays histone H1 kinase activity *in vitro*. Our primary objective in this study is to gain baseline information about the regulation of this higher plant cell division control complex in non-dividing, differentiated cells as well as in synchronous and asynchronous mitotic cells. We are investigating *cdc2* and cyclin expression at the levels of protein abundance, protein phosphorylation and quaternary associations.

University of Illinois
Urbana, IL 61801

84. Genetics of the Methanogenic Bacterium, Methanococcus voltae With Attention to Genetic Expression Mechanisms

J. Konisky, Department of Microbiology *$182,000 (2 years)*

The objective of this research program is to study the genetics, physiology and molecular biology of *Methanococcus voltae*, a marine archaebacterium. From our recent studies, we have concluded that the energetics of *M. voltae* is based on a chemiosmotic mechanism in which a sodium motive force is coupled to ATP synthesis. The sodium motive force is generated by a primary sodium pump whose activity is coupled to a late step in methanogenesis. Most interestingly, we have found that while cells grown at pH6.5 do not generate a transmembrane sodium concentration gradient, cells grown at pH8.2 generate a gradient in which the intracellular level of sodium is maintained at a concentration which is twelve fold below the sodium concentration present in the growth medium.

In an effort to characterize the biochemistry and molecular biology underlying the difference in the energetic schemes operative in cells grown at pH6.0 and 8.2, we have begun to examine the differential expression of the *M. voltae* atpP gene which encodes a membrane associated ATPase which we believe translocates sodium ions. For this purpose we are characterizing the levels of atpP mRNA present in cells grown under a variety of physiological conditions including the pH of growth and the level of sodium present in the medium. We are also characterizing the promoter structure of the atpP gene and identifying those DNA sequences which interact with RNA polymerase as well as with potential transcription factors.

It is our hope that our studies will provide new information on the mechanism of gene expression in methane producing microorganisms. In particular, our studies should be helpful in defining how methanogens respond to changes in their environment.

University of Illinois
Urbana, IL 61801

85. Genetic and Molecular Studies on Cytoplasmic Male Sterility in Maize

J.R. Laughnan and S. Gabay-Laughnan, Department of Plant Biology *$85,000*

The objective of this project is to determine the basic mechanisms of cytoplasmic male sterility (CMS) in maize and to understand the genetic and molecular bases for both nuclear and cytoplasmic reversions to male fertility. Genetic studies involve attempts to identify cases of insertion of transposable controlling elements into nuclear *cms-T* and *cms-S* restorer gene sites and, in the case of restorers of *cms-S*, also a search for cases in which a transposable restorer-of-fertility (*Rf*) gene is inserted into a wild-type maize gene. A number of spontaneous *Rf* genes have arisen in inbred nuclear backgrounds and these fall into two classes--functional and nonfunctional. When plants carrying nonfunctional *Rf* genes (*Rf-nf*) are crossed as male parents, there is no seed set on the ears. To date, eight independently-occurring spontaneous *Rf-nf* genes have been identified. The allelic relationships between all newly-arisen *Rf* genes is under study. Molecular analysis will be conducted in which the protein profiles of germinating pollen grains from *Rf-nf* strains will be compared with normal pollen grains from isogenic controls. *Rf-nf* genes have been found to be functional in hybrid

backgrounds and the genetic nature of this hybrid vigor effect is under study. In *cms-S* plants, a number of newly-arisen *Rf* genes have been shown to be transposable, as has the standard *Rf* gene. Efforts to characterize these transposition events continue. Genetic studies will indicate whether *Rf* elements transpose to a large number of different sites or whether there are preferred sites for insertion. The molecular determination of CMS is in the mitochondrial DNA (mtDNA). We have shown that in *cms-S* the organization of the mtDNA is controlled by the nucleus of the cell, that significant changes in mtDNA organization occur when one nuclear genotype is substituted for another, and that similar reorganizations of mtDNA accompany cytoplasmic reversion of *cms-S* to male fertility. We have characterized cytoplasmic revertants from different inbred backgrounds and have found mtDNA alterations common to all reversion events. All revertants analyzed contain rearrangements at the termini of the mitochondrial genome linearized by recombination between S-episome inverted repeats (IR) and IR-homologous sequences present in the main mitochondrial genome. The region adjacent to the integrated IR is designed R. The transcriptional profile of this region changes upon cytoplasmic reversion to fertility. Revertants in all nuclear backgrounds lack a 1.39kb transcript present in their sterile progenitors. The nuclear restorer gene *Rf3* restores fertility to *cms-S* plants and also affects expression of the R region, while the two spontaneously-arisen restorer genes studied thus far do not. This is the first molecular evidence that these two nuclear restorer genes are functionally different from *Rf3*. Additional molecular studies are aimed at characterizing the control the nucleus has over the organization of the mitochondrial genome.

University of Illinois
Urbana, IL 61801

86. Exploratory Studies on the Bacterial Formation of Methane
R.S. Wolfe, Department of Microbiology *$95,108 (2 years)*

The microbial formation of methane is carried out by a unique group of bacteria known as methanogens. These strict anaerobes are widespread in nature, and are found in diverse habitats wherever active anaerobic degradation of organic matter occurs. Methanogens are sensitive to certain wavelengths of light in the visible blue area of the spectrum. We are studying this light sensitivity to define the specific light labile compounds. Under certain conditions methanogens may exhibit a bright red fluorescence instead of the typical blue-green fluorescence. We are studying this phenomenon to isolate and characterize the red-fluorescent compound and determine whether or not it is an intermediate in the synthesis of the deazaflavin coenzyme, F420. We are exploring ways of simplifying the culture of methanogens on liquid and solid media, so that these organisms may be more readily employed as research tools by the scientific community. We are conducting exploratory studies designed to encourage the role of protozoa in methanogenic biomass digesters. One of the key enzymes in the reduction of CO_2 to methane is formylmethanofuran:tetrahydromethanopterin formyltransferase. This enzyme has been cloned, sequenced, and expressed in an active form; so the stage is now set for a more detailed study of the reaction mechanism of this enzyme and for attempts to obtain crystals suitable for X-ray crystallography. The long linear coenzyme, methanofuran, with its highly polar structures at one end and the formyl-furan group at the other should occupy a unique active site in the enzyme.

University of Illinois
Urbana, IL 61801

87. Anthropogenic Impacts on Photosynthetic Activity: A Multidisciplinary Context for Research Training

C.A. Wraight, D.R. Bush, J.McP. Cheeseman, A.R. Crofts, P.G. Debrunner, E.H. DeLucia, Govindjee, W.L. Ogren, D.R. Ort, A.R. Portis, J. Whitmarsh, R.E. Zielinski

$495,760 (2 years)

This research training program directs students towards the pressing issues of plant biology, broadly defined as anthropogenic impacts on photosynthesis and plant productivity. The curriculum and laboratory training inform them of current problem areas, and arm them with the broad-based expertise necessary to tackle them. The interdisciplinary nature of much biological research is especially strong in photosynthesis, which spans the range from physics to agronomy, and is exemplified by the research programs of the participating faculty. For success in the future, a new research training paradigm must be developed, to provide students with an interdisciplinary outlook and multidisciplinary technical abilities. In the training program, students and young associates experience research as a multidisciplinary enterprise, and are specifically apprised of areas of most societal concern and in most need of innovative research contributions through an interactive seminar program. Mechanistic knowledge, derived from laboratory advances in basic photosynthesis research at the molecular level, is integrated with the physiology of cells and whole plants and, ultimately, placed in an ecological and agronomic context. Graduate and postdoctoral trainees work on projects coordinated between two or more faculty laboratories, with distinct conceptual approaches. In addition to graduate training, the involvement of undergraduates in hands-on research is encouraged through summer fellowships. A summer program is run for high school and community college science teachers to gain expertise in easily transferable techniques to take back to their own classrooms. Special efforts are made to target predominately minority schools and to coordinate with similar programs in place at the University.

Indiana University
Bloomington, IN 47405

88. Phylogenetic Analysis of Hyperthermophilic Natural Populations Using Ribosomal RNA Sequences

N.R. Pace, Department of Biology *$100,000*

Hyperthermophilic organisms (growth optima >80°C) are a largely unexplored pool of biota with substantial potential for biotechnology. We know little about the diversity of life at high temperatures because of general reliance upon establishing laboratory cultures for assessment of organisms. It is well-known, however, that only a minor fraction of naturally occurring microorganisms is routinely culturable. The main goal of the program is, therefore, to survey phylogenetically and quantitatively the microbial biodiversity in selected high-temperature ecosystems using methods that do not rely upon cultivation. Phylogenetic information allows inference of some properties of organisms.

16S rRNA genes in DNA isolated from high-temperature environmental samples are being isolated by cloning, directly or following amplification by polymerase chain reaction, for sequencing and phylogenetic analysis. Hybridization probes prepared from recombinant 16S rRNA genes are used to evaluate the quantitative representation of particular sequence representatives and to identify the

corresponding organism in environmental samples. Environments include diverse Yellowstone settings and deep-sea hydrothermal vents. Several novel hyperthermophiles have already been discovered using this approach.

The program uses existing methods and continues to develop new ones for rapid analysis of natural communities. One goal for development is a new hybridization method for quantitative analysis of population constituents. The method, based on nuclease-protection, promises to be considerably more reliable than oligonucleotide hybridization. Additionally, 16S rRNA sequence and phylogenetic analyses are carried out for selected hyperthermophilic organisms already in culture. These sequences make possible phylogenetic analysis of the organisms from which the sequences derive and contribute to the data base to which natural populations are compared. The sequences also are used for comparative analyses of hyperthermophilic and related mesophilic 16S rRNAs, to identify primary and secondary structural elements that may contribute to extreme thermostability.

University of Iowa
Iowa City, IA 52242

89. Molecular Biology of Anaerobic Aromatic Biodegradation
C.S. Harwood, Department of Microbiology *$55,000*

We are identifying and characterizing genes required for the anaerobic degradation of benzoate and 4-hydroxybenzoate by the bacterium *Rhodopseudomonas palustris.* These aromatic acids are intermediates in the degradation of structurally diverse aromatic compounds, including lignin monomers and toxic compounds, by many metabolic types of anaerobic bacteria. They are also the starting compounds for central pathways of anaerobic benzene ring reduction and ring fission. We have recently identified a gene termed *aadR* (for anaerobic aromatic degradation regulator) that encodes a transcriptional activator of benzoate/4-hydroxybenzoate degradation, and we are currently subcloning, sequencing, and characterizing two genes that appear to be regulated by AadR. These encode benzoate-CoA ligase and 4-hydroxybenzoate-CoA ligase, the enzymes that catalyze the initial reactions of benzoate and 4-hydroxybenzoate degradation. We then plan to identify genetic elements and environmental signals that are important for the regulated expression of the ligase genes. We are also seeking to identify additional benzoate/4-hydroxybenzoate degradation genes that may be linked to the ligase genes. Our ultimate aim is to use the genes to help elucidate the precise sequence of enzyme reactions in the pathways, to determine how the genes are physically organized on the *R. palustris* chromosome, and how they are regulated. This information will be valuable should it at any point become desirable to transfer these genes to other bacteria with characteristics that may be particularly well suited for specific applications.

Johns Hopkins University
Baltimore, MD 21218

90. Analysis of Thermally Stable Electron Transport components in the Hyperthermophilic Bacterium Pyrodictium brockii
R.J. Maier, Department of Biology *$72,563*

The mechanisms by which hyperthermophilic archaebacteria grow and carry out metabolic functions at elevated temperatures have yet to be determined. The objectives of the work include developing an understanding of the enzymes involved in hydrogen/sulfur transformation by hyperthermophilic archaebacteria. Efforts are focused primarily on the autotrophic bacterium, *Pyrodictium brockii*, which has a reported optimum growth temperature (105°C) in pure culture. Biochemical and genetic

characterization of enzymes involved in the hydrogen oxidizing electron transport pathway to S° in *P. brockii* are being pursued. The H_2 oxidizing hydrogenase has been purified. It is composed of two subunits that are similar to those of mesophilic H_2 oxidizing bacteria. Interestingly, the membrane bound form of the enzyme exhibits considerably more tolerance to thermal destruction than the purified de-lipidated enzyme. Other electron transport components between H_2 and S° are being characterized. These include a quinone, a c-type cytochrome, and the S-reducing complex. The membrane bound c-type cytochrome has a molecular mass of 13-14 kDa and is located on the oxidizing side of the electron transport chain with respect to hydrogenase and the quinone. Comparisons of the biochemical and genetic properties of these electron transport components will be made with mesophilic counterparts. The gene encoding a c-type cytochrome is also being cloned and sequenced, for comparison with the predicted amino acid sequences of c-type cytochromes of mesophiles. These first characterizations of the strictly anaerobic, presumably primitive *P. brockii* electron transport chain suggest that the hydrogenase operates at a relatively high redox potential and that the H_2-oxidizing chain more closely resembles those of aerobic eubacterial H_2-oxidizing bacteria than those of H_2-metabolizing systems of anaerobes or the hyperthermophile *Pyrococcus furiosus*. The long-term goal is to understand the biochemical basis of extreme thermophily.

Johns Hopkins University
Baltimore, MD 21218

91. Transport of Ions Across the Inner Envelope Membrane of Chloroplasts
R.E. McCarty, Department of Biology *$210,000 (2 years)*

The inner envelope membrane of chloroplasts is the main permeability barrier between the cytoplasm and chloroplast stroma. This membrane is known to contain several specific proteinaceous transport systems. SO_2^-, NO_2^- and NH_4^+ are metabolized within chloroplasts, but are generated in the cytoplasm. How these ions cross the envelope is unknown. The mechanisms of Ca^{2+} and Cl^- transport are also unknown, as is that involved in the regulation of stromal pH. We have shown that the mediated transport of glycolate or glycerate may be conveniently studied by a stopped-flow fluorescence method. An impermeant pH indicator was trapped inside envelope vesicles and transport initiated by the addition of glycerate or glycolate by stopped-flow mixing. The rapid ($T_{½}$ about 120 msec) changes in the internal pH of the vesicle were monitored by the quenching of indicator fluorescence. In principle, the transport of any substance that is linked to either proton or hydroxyl movements may be studied by this technique. Using this assay, we hope to establish whether NO_2^-, SO_2^-, and NH_4^+ transport is mediated by specific transport systems. Using different fluorescent probes, we will examine Cl^- and Ca^{2+} transport.

University of Kentucky
Lexington, KY 40546-0091

92. Photoinhibition of PSII Reaction Centers; Photoactivation, Reconstitution and Resynthesis of PSII Reaction Center/Water Oxidizing Complex Components
G.M. Cheniae, Department of Agronomy *$206,000 (2 years)*

We study Photosystem II reactions causing the assembly of Mn^{2+} into tetra-$Mn^{\geq 3+}$ complexes with formation of active O_2-evolving "enzymes". We also study the kinetics/mechanisms of photodamages affecting donor side PSII Reaction Center components (Y_Z/Y_D/Chl) and causing increased turnover of

the D_1>D_2 polypeptides of the Reaction Center. Inhibition/inactivation of O_2- evolution causes the donor side components to become highly susceptible to photodamage, presumably as a consequence of the accumulation of strongly oxidizing ($E_o \geq 1.OV$) Reaction Center radicals ($P_{680}^+/Y_Z^+Chl^+$) formed during illumination. However, we believe this view may be overly simplistic based on analyses made with Mn-free PSII membranes in which measurements of P_{680}^+ relaxation (Y_Z--> P_{680}^+), formation of a quencher of flash-induced Chl*a* variable fluorescence, and the quantum yield of photooxidation of Mn^{2+} at a high affinity site (K_m~400 nM) were measured following illumination of the membranes at various conditions/durations. Protection from photodamage(s) is obtained by addition of exogenous e^- donors to Y_Z^+ [e.g. 100 μM NH_2NH_2 plus 1 Mn/RC (K_m~400 nM)], a result lending support to the above hypothesis. However, in the absence of exogenous e^- donors and under aerobic conditions, the photodamage(s) also are prevented/inhibited by addition of acceptors of electrons from Q_B^-, oxy-radical scavengers and superoxide dismutase. Similarly, photodamages are inhibited by the exclusion of O_2 during illumination of membranes with an e^- acceptor. Such results suggest that the photoinhibition mechanism possibly may involve a reaction between cation radicals on the donor side of PSII with superoxide radicals formed at the acceptor side of PSII.

University of Kentucky
Lexington, KY 40546-0091

93. The Role of Purine Degradation in Methane Biosynthesis and Energy Production in Methanococcus vannielii

E. DeMoll, Department of Microbiology and Immunology *$68,000*

Purine degrading metabolic pathways have been discovered in *Methanococcus vannielii.* We are currently studying the relationships between purine degradation, methane biosynthesis, and tetrahydromethanopterin (MPT) biochemistry in *M. vannielii.* The purine degrading pathway of *M. vannielii* resembles that described for the clostridia, however in the final steps MPT replaces tetrahydrofolic acid. *M. vannielii* can use certain purines as partial carbon and energy and sole nitrogen source. The 2-, 6-, and 8-carbons atoms of xanthine may all be eventually converted by *M. vannielii* to methane. The 2- and 6-carbons enter the methane biosynthetic pathway at the beginning as CO_2, however the 8-carbon apparently enters the methane pathway in the form of 5, 10-methenyl-MPT. We are specifically trying to determine exactly how this carbon is converted to methane. Glycine is a product of clostridial purine degradation. Its further metabolism by clostridia generates one mole of ATP. Another goal of our studies is to examine possible further metabolism of glycine by *M. vannielii.* In *M. vannielii* glycine is cleaved to CO_2 and ammonia. The fate of the 2-carbon is not known, however we have evidence that it is transferred to MPT forming 5, 10-methylene-MPT. We are also examining whether various forms of MPT freely exchange between enzymes of the methane biosynthetic pathway and other enzymes that likely would use one of these forms of MPT, such as thymidylate synthetase, or whether an MPT molecule remains more or less dedicated to the methane biosynthetic pathway. A final objective of our work is to measure the extent to which carbon atoms released during purine degradation appear in biosynthetic and energy producing pathways in *M. vannielii.*

University of Kentucky
Lexington, KY 40546

94. Mechanism and Significance of Post-Translational Modifications in the Large Subunit of Ribulose Bisphosphate Carboxylase/Oxygenase
R.L. Houtz, Department of Horticulture *$179,000 (2 years)*

The large subunit (LS) of higher plant ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) is post-translationally processed by removal of Met-1 and Ser-2, acetylation of Pro-3, and in many instances methylation of the ε-amine of lysyl residue 14. The significance of N-terminal proteolysis, acetylation and methylation is unknown. X-ray crystallography has shown that an amino acid residue adjacent to the Lys-14 methylation site (Phe-13) is located at the interface between small subunits and LS dimers, has an indirect interaction with an active-site lysyl residue, and along with residues Ala-15, Gly-16, Val-17 is at the interface between carboxy- and amino-terminal domains of LS dimers. Proteolysis studies have shown that the N-terminal region of the LS undergoes dynamic changes in solvent accessibility during catalysis, is required for maximum levels of catalytic activity, and can influence the K_m for ribulose-1,5-bisphosphate. Thus, structural modifications in this region may affect the catalytic activity, assembly, or stability of rubisco *in vivo*. The methylation of Lys-14 is catalyzed by a chloroplast localized S-adenosylmethionine (AdoMet):rubisco LS (lysine) $^{\epsilon}$N-methyltransferase that is highly specific for Lys-14 in the LS of rubisco. Partially purified preparations of this methyltransferase are capable of stoichiometrically methylating rubisco preparations *in vitro* that have unmodified lysyl residues at position 14 in the LS. The objective of our studies is to define the function of Lys-14 methylation, *in vitro* and *in vivo*, and provide a detailed enzymological and structural characterization of AdoMet:rubisco LS $^{\epsilon}$N-methyltransferase at the protein and DNA level.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

95. Enzymatic Synthesis of Materials
M.D. Alper, M. Bednarski, H.W. Blanch, M. Callstrom, D. Clark, J.F. Kirsch, B. Novak, P.G. Schultz and C.-H. Wong, Center for Advanced Materials
$155,000

The goal of this research (jointly funded by the Division of Materials Sciences) is the use of natural, engineered and "created" enzymes to synthesize new materials. The unique stereochemical control exerted by enzymes and their ability to catalyze reactions at low temperature will allow the synthesis of materials with structures and therefore properties that cannot be achieved using conventional synthetic routes.

Efforts are focused on the design of reaction conditions for the enzymatic synthesis of polymeric materials; engineering of enzyme structure and activity to allow the binding and polymerization of novel monomers; characterization and processing of the polymer products of these reactions and understanding the structure/function relationships of this new class of materials. Work is also progressing on the synthesis of organic thin films on metal and semiconductor surfaces to alter adhesive and other interfacial properties and to fabricate sensor devices. Genes have been cloned and expressed to allow production of natural and engineered enzymes. Active sites have been altered through site-directed mutagenesis and other techniques to help understand enzyme/substrate binding

and provide a rational basis for modifications required for binding and reaction with unusual substrates. A novel polymer has been synthesized that can be bound to enzymes thus allowing them to retain activity in unbuffered water, high temperature and organic solvents. It has allowed peptide synthesis with high yield. Catalytic antibodies have been developed for non-biological reactions involving C-C bond synthesis.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

96. Characterization of Carotenoid and Bacteriochlorophyll Biosynthesis Genes from a Photosynthetic and a Non-Photosynthetic Bacterium

J. Hearst, Chemical Biodynamics Division *$290,000*

Our laboratory has determined the nucleic acid sequence for all of the genes involved in the biosynthesis of bacteriochlorophyll (Bchl) and carotenoid (Crt) photosynthetic pigments in *Rhodobacter capsulatus*. As many as twenty genes may be required for the synthesis of Bchl, at least eight of which act in the early portion of the Bchl biosynthesis pathway in reactions common to both Bchl and chlorophyll (Chl) biosynthesis. Several of the gene sequences have provided inroads into a molecular understanding of pigment biosynthesis in other organisms, as well as offering unexpected challenges in deciphering their modes of expression. This is especially true for the genes encoding the reductive steps of Bchl synthesis: *bchB* and *bchL* for the reduction of protochlorophyllide and *bchX*, *bchY*, and *bchZ* for the reduction of chlorophyllide *a*. The sequences of both *bchL* and *bchX* show strong sequence similarities (32%) with the nitrogenase Fe protein, with much stronger conservation in the neighborhood of the MgATP-binding and [4Fe4S] cluster binding domains. Thus we refer to the *BchL* and *BchX* proteins as "Chl Fe proteins." By comparison with the known mechanism of nitrogenase Fe proteins, we infer that the reductive steps in Chl and Bchl biosynthesis involve transfer of electrons from a [4Fe4S] cluster held by a homodimer of *BchL* (or *BchX*) proteins to the substrate-binding, catalytic portion of the complex in *BchB* (or *BchY/BchZ*, respectively).

The Chl Fe protein sequences from *R. capsulatus* also matched with the published sequence of an open reading frame of unknown function, previously designated *frxC*, from the chloroplast of the nonvascular plant, *Marchantia polymorpha*. The great similarity between it and *bchL* (49%) strongly imply that *frxC* is a Chl Fe protein that serves the same function in chloroplasts as does *bchL* in purple bacteria, namely in the reduction of protochlorophyllide. We are therefore proposing that *frxC* be renamed as *chlL*. We next undertook to determine whether *chlL* sequences are present in other photosynthetic organisms. Using PCR primers based on sequences conserved between *bchL* and *chlL*, we succeeded in amplifying and sequencing appropriate-sized fragments from the cyanobacterium *Synechococcus sp 7002* and from the chloroplast of the fern *Polystichum acrostichoides*. The cyanobacterial and chloroplast protein sequences are all nearly identical (85-95%). Using the amplified material as a probe, we were then able to show by hybridization that homologous sequences are present in the chloroplasts of gymnosperms. Since *chlL* is not present in the sequenced chloroplasts of rice and tobacco, we are now investigating whether it is nuclear encoded in angiosperms.

The expression of Chl Fe proteins is also under investigation. Whereas *bchL* and *bchB* are located far apart from each other, *bchX*, *bchY*, and *bchZ* are all contained within a region of the bacterial chromosome ascribed to the *bchA* genetic locus. Although complementation studies have thus far shown the *bchA* locus as a single complementation group, our data clearly demonstrate the presence

of three coding segments (*bchX*, *bchY*, and *bchZ*). Part of our effort is being directed toward resolving the actual number of gene products. Preliminary evidence suggests that there are in fact three genes within the *bchA* gene cluster and that mRNA secondary structure may be confounding the complementation studies by imposing stringent translational coupling upon the three segments, making each one difficult to complement *in trans.*

In addition to the Chl Fe proteins, there is another gene, *bchK*, that shares similarity (29%) with an unidentified chloroplast open reading frame. *bchK* is an early gene in Bchl synthesis, acting at the combined Mg-chelatasae/methyl-transferase step. Again, this sequence is present in the *Marchantia* chloroplast but not that of rice or tobacco. We are exploring the possible existence of a *chlK* gene in higher plants.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

97. Center for the Analysis of Plant Signal Transduction
S.-H. Kim, Chemical Biodynamics Division *$39,000*

This center will address the molecular and biochemical mechanisms by which plants perceive signals and the subsequent signal transduction events and gene expression that occurs in response to such diverse stimuli as light, plant hormones, and plant pathogens. This project is still at a formative phase; it is too early to report results.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

98. Mechanism of Water Splitting and Oxygen Evolution in Photosynthesis
M.P. Klein, Chemical Biodynamics Division *$230,000*

Oxygen evolution in photosystem II of green plants is thought to involve reactions through a cycle of four states by which electrons are removed from two H_2O and donated to the oxidized P680 reaction center. The most successful interpretation of the data regarding this cycle is Kok's S-state scheme, which postulates a series of five (S_0-S_4) states through which electrons are cycled during oxygen evolution. Manganese is thought to play a central role in these reactions. X-ray absorption spectroscopy using synchrotron radiation is used to determine the structural and electronic state(s) of the manganese sites. In photosystem II particles of both spinach and the cyanobacterium *Synechococcus* sp., we have determined that the manganese occur minimally as a binuclear complex with Mn-Mn separation of ~2.7Å. We observe a Mn K-edge shift of ca 1 eV to higher energy upon advancing from the S_1 to the S_2 state, implying an oxidation state increase of Mn. Creation of an S_0-like state produces a K-edge shift in the opposite direction indicating a reduction of Mn between S_1 and S_0. There is no further oxidation of Mn upon advancing from the S_2 state to the S_3 state although the EPR signal characteristic of the S_2 state disappears. The structures of the Mn complex in the S_1, S_2 and S_3 states, determined by EXAFS, are similar and well described as pairs of di-μ-oxo binuclear centers with Mn-Mn distances of 2.69A and 2.79A containing Mn(III) and Mn(IV). The distance between at least one of the Mn in each of these centers is 3.3Å. Recent data of very high

quality indicate small differences between the structures in the S_1 and S_2 states. Polarized EXAFS data using oriented PSII membranes shows that the Mn dimers lie in the membrane plane while the 3.3Å vector is perpendicular to the membrane plane. Replacement of Ca by Sr has permitted us to establish that there is also a Mn-Sr(Ca) interaction at -3.3Å. Mn EXAFS of PSII particles prepared from *Synechococcus* grown exclusively on Br suggest that there is one Br (Cl) at -2.2Å. Combining these results yields the first tentative structural model of the Mn cluster of the OEC.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

99. Chemistry of Phycobiliproteins and Phytochrome Division
H. Rapoport, Chemical Biodynamics *$20,000*

A complete understanding of the chemistry and stereochemistry of phycobiliproteins and of phytochrome is sought to facilitate full understanding of the role of light in regulation of gene expression in green plants. The chemical structures of phycobiliproteins and phytochrome are being determined, including the detailed nature of the covalent attachments of chromophore to protein, by stereospecific synthesis of model chromophores. Chromophore peptides also are being synthesized to ascertain the effect of the peptide-protein on solution conformations and energy transfer.

Knowledge of this mechanism is required for future improvement in plant growth and quality based on genetic engineering at the molecular level.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

100. Photosynthetic Membrane Structure and Photosynthetic Light Reactions
K. Sauer, Chemical Biodynamics Division *$325,000*

Excitation transfer and trapping in reaction centers of photosynthetic membranes occurs in less than one nanosecond following the absorption of visible light photons. We are investigating the kinetics and energetics of this process using wavelength-resolved transient absorption change and fluorescence decay measurements applied to well defined preparations of antenna pigment proteins or reaction center complexes. X-ray crystallographic structural information for several of these proteins has enabled us to carry out excitation transfer calculations using exciton theory and/or Förster inductive resonance transfer applied to pigment arrays of known geometry. The cyanobacterial pigment C-phycocyanin (C-PC) coordinates serve as a basis for our exploration of the role of exciton delocalization in both the normal C-PC and a mutant in which one of the three types of chromophores is deleted. Structurally, this mutant pigment protein is similar to allophycocyanin, for which we have recently completed a detailed study of excitation transfer dynamics. We are now able to resolve components to the picosecond limit of resolution using pulse-probe absorption bleaching and recovery measurements and using a fluorescence up-conversion approach that we have recently instituted. Photosynthetic reaction centers convert absorbed photon energy into charge separation between electron donors and acceptors. We are investigating the effects of applied trans-membrane electric fields on the kinetics and the efficiency of this charge separation process. By monitoring both the

steady-state and the time-resolved changes in fluorescence associated with the application of electric fields, we have been able to address the question of whether the initial charge separation in Photosystem II of higher plants occurs by a two-step mechanism or one that involves super-exchange. We will extend these studies to photosynthetic bacterial systems where detailed structural information on the reaction centers is available, and then attempt to account for the observed behavior using appropriate theoretical modelling of the influence of the applied electric fields on the reaction centers. This information should be very useful for the design of biomimetic synthetic devices for solar energy conversion.

Lehigh University
Bethlehem, PA 18015

101. Post-Transcriptional Regulation of Chloroplast Gene Expression by Nuclear Encoded Gene Products

M.R. Kuchka, Department of Molecular Biology *$84,000*

Gene products encoded by the plant cell nucleus are required for the expression of chloroplast genes. Our understanding of the interplay between these two genetic systems comes in great part from the characterization of mutant strains of the unicellular green alga *Chlamydomonas reinhardtii*. We have been studying the expression of a single chloroplast encoded protein, the Photosystem II reaction center polypeptide D2, in photosynthetic mutants of the alga. By genetic analyses we have identified at least three and possibly four nuclear genes whose products are required for the successful expression of the D2 protein. When these nuclear genes are mutated, synthesis of D2 is blocked at one of two different post-transcriptional stages. In some mutants, the mature D2 protein is either not synthesized or is rapidly degraded, whereas in others, the psbD message encoding D2 is unstable and turns over at a greatly accelerated rate. Our goal is to understand how the nuclear gene products altered in mutant cells normally work to promote D2 synthesis. We now know that the psbD message is associated with large classes of polysomes in all mutants which have normal amounts of the message but fail to synthesize the protein. This suggests that the nuclear factors mutated in these strains may normally work either in late stages of D2 translation or in D2 protein stabilization. Immunoprecipitation experiments using a D2-specific antiserum are currently underway to identify putative D2 translation intermediates and/or D2 degradation products. For mutants affected in psbD mRNA stability, we are attempting to identify important cis- and trans-acting elements which regulate psbD mRNA half-life. We have genetic evidence suggesting that sequences at the 5' untranslated (UTR) region of psbD mRNA are important cis elements. Currently, we are performing mobility shift and uv-crosslinking experiments with *in vitro* synthesized psbD 5' UTR transcripts to identify proteins which may influence psbD mRNA turnover rates by their binding. Taken altogether our results point to different aspects of post-transcriptional expression as important stages in the control of D2 gene expression by nuclear encoded factors.

Los Alamos National Laboratory
Los Alamos, NM 87545

102. Carbon Metabolism in Methylotrophic Bacteria
C.J. Unkefer, Isotope and Nuclear Chemistry Division *$135,000*

Methylotrophs are aerobic microorganisms capable of growth on one-carbon (C_1) compounds more reduced than CO_2. These organisms derive their energy and reducing power from the oxidation of the reduced C_1 compounds and must condense C_1 units to form all of the macromolecular constituents of the cell. The ability of methylotrophs to grow on simple C_1 compounds gives them considerable industrial potential for they could be employed to produce a variety of useful compounds from simple precursors such as methanol or methane. In order for this potential to be realized, we must understand their fundamental biochemistry and physiology. Toward this end, we are examining the metabolism in *Methylobacterium extorquens* AM1 that is representative of a class of methylotrophs that use the icl$^-$ serine pathway for carbon assimilation. Although the initial step of the icl$^-$ serine pathway was proposed thirty years ago, the complete cycle for methanol assimilation in AM1 is as yet unknown. In particular, the mechanism for net oxidation of acetyl groups to glyoxylate, a function required for growth on methanol, remains obscure. Using ^{13}C-labeling and ^{13}C NMR spectroscopy, we are examining two carbon metabolism in *M. extorquens* AM1 and in mutants of AM1 that require glyoxylate for growth. By analyzing labeling patterns in amino acids derived from bacteria cultured in the presence of [1-^{13}C],[2-^{13}C] and [1,2-$^{13}C_2$]ethanol, we have eliminated all known and previously proposed pathways for acetate oxidation. We have demonstrated that the pathway for oxidation of glyoxylate in *M. extorquens* AM1 involves a symmetrical intermediate and are currently attempting to identify that compound.

University of Maryland
Baltimore, MD 21202

103. Structure and Regulation of L-Glutamate Dehydrogenase in Hyperthermophilic Archaea (Archaebacteria)
F.T. Robb, Center of Marine Biotechnology *$181,993 (2 years)*

Hyperthermophilic microorganisms are defined as having the unusual property of being able to grow optimally above 100°C. They originate mainly from marine hydrothermal areas, including deepsea volcanic vents, and recently two strains have been described that grow at temperatures up to 110°C. There is little information available on the molecular basis of the adaptations required for enzyme catalysis and gene regulation at extremely high temperature. The major pathways of carbon, nitrogen and energy metabolism in hyperthermophiles are largely unexplored. The enzyme glutamate dehydrogenase (GDH) is at a metabolic junction between carbon and nitrogen metabolism and is likely to be subject to regulation of enzyme activity and synthesis. The objective of the research is to characterize and compare (GDH) from two hyperthermophiles, *Pyrococcus furiosus* and ES4, an unclassified isolate from a deep hydrothermal vent. These organisms have maximal growth temperatures of 103 and 110°C respectively. GDH is an abundant protein (>1% of total soluble protein) in *P. furiosus* and has been purified. Enzyme activation occurs at 55°C, accompanied by a reversible conformational change which has been detected by microcalorimetry using pure enzyme. Rapid, irreversible denaturation occurs at 112°C. Microsequencing of GDH has provided aminoterminal sequence information for future use in cloning the gene from *P. furiosus*. After cloning

the gene for GDH, the DNA sequence will be determined in order to identify the promoter region and the structural features that may be responsible for the extreme thermostability and the heat activation/cold denaturation of the enzyme. Continuous cultures of *P. furiosus* will be grown with varying maltose/protein ratios and the regulation of GDH activity (which we know to be subject to repression) and synthesis will be measured by using the cloned gene as a probe to determine the level of mRNA by Northern blotting.

University of Maryland
College Park, MD 20742

104. Identifying Calcium Channels and Porters in Plant Membranes
H. Sze, Department of Botany *$86,286*

Many signal-induced responses in plant cells are linked to transient changes in cytosolic calcium concentration. The role of Ca^{2+} as an intermediate messenger in cells requires regulation of the cytosolic ion level. This is accomplished by coordination of passive Ca^{2+} fluxes via channels that increase cytosolic Ca^{2+}, and energy-dependent Ca transport via Ca^{2+}-pumping ATPases or H^+/Ca^{2+}, antiporters that lower cytosolic [Ca^{2+}]. As part of our long range goal to understand the mechanism and regulation of Ca^{2+} transporters in plants, we are identifying and characterizing a Ca^{2+}-pumping ATPase, a vacuolar H^+/Ca^{2+} antiporter and the inositol-1,4,5-trisphosphate sensitive calcium channel using biochemical and molecular biology techniques. We have recently identified a 120 kda Ca^{2+}-pumping ATPase in endoplasmic reticulum-enriched membranes of suspension-cultured carrot cells. The characteristics of the Ca^{2+}-dependent phosphoenzyme would suggest that this plant Ca^{2+}-ATPase has some unique features different from the major animal Ca^{2+} pumps. These studies to understand Ca transport pathways, their regulation and their role in signal transduction will lead to a better understanding of plant growth and development.

Massachusetts Institute of Technology
Cambridge, MA 02139

105. Genetic and Biophysical Analyses of the Photosynthetic Reaction Center
D.C. Youvan, Department of Chemistry *$116,000*

Mutagenesis has been applied to the bacterial photosynthetic reaction center (RC) to identify the structural region of the protein responsible for unidirectionality of electron transfer and to determine the role of accessory bacteriochlorophyll molecule (B_A) in electron transfer. It now appears that several residues may act in concert to generate unidirectional electron flow. To test this reasoning, homologous sequences were exchanged between the symmetrical L and M subunits in order to "symmetrize" the RC. The D helices were chosen as the first target since they pass through the center of the RC perpendicular to the membrane, with many side chains interacting with or in the vicinity of the chromophores. The D_{LL} mutant lacks the pheophytin acceptor (H_A) and shows vibrationally coherent decay of the excited dimer (P^*). In other experiments, the amphiphilic cd-helices have also been exchanged. These helices form a major portion of the monomeric BchII binding pockets and include the axial His ligands for these cofactors. The two helix duplications cd_{LL} and cd_{MM} assemble reduced levels of RCs which are not (significantly) photochemically active. Neither of these mutants, nor the helix switch, cd_{LM}, grow photosynthetically. However, photosynthetically competent revertants have been obtained for these helix duplication mutants. These revertants, as well as D_{LL} revertants, are candidates for bidirectional electron transfer.

University of Massachusetts
Amherst, MA 01003

106. Cellulose Fermentation by Nitrogen-Fixing Anaerobic Bacteria
E. Canale-Parola, Department of Microbiology *$189,400 (FY 91 Funds/2 years)*

In anaerobic natural environments cellulose is degraded to methane, carbon dioxide and other products by the combined activities of many diverse microorganisms. We are simulating processes occurring in natural environments by constructing biologically-defined, stable, heterogeneous bacterial communities (consortia) that we use as *in vitro* systems for quantitative studies of cellulose degradation under conditions of combined nitrogen deprivation. These studies include the investigation of i) metabolic interactions among members of cellulose-degrading microbial populations, and ii) processes that regulate the activity or biosynthesis of cellulolytic enzymes. In addition, we are studying the sensory mechanisms that, in natural environments, may enable motile cellulolytic bacteria to migrate toward cellulose. This part of our work includes biochemical characterization of the cellobiose chemoreceptor of cellulolytic bacteria. Finally, an important aspect of our research is the investigation of the mechanisms by which multienzyme complexes of anaerobic bacteria catalyze the depolymerization of crystalline cellulose and of other plant cell wall polysaccharides. The research will provide fundamental information on the physiology and ecology of cellulose-fermenting, N_2-fixing bacteria, and on the intricate processes involved in C and N cycling in anaerobic environments. Furthermore, the information will be valuable for the development of practical applications, such as the conversion of plant biomass (e.g., agricultural, forestry and municipal wastes) to automotive fuels such as ethanol.

University of Massachusetts
Amherst, MA 01003

107. Genomic Plasticity and Catabolic Potential of Pseudomonas cepacia
T.G. Lessie, Department of Microbiology *$169,860 (FY 91 Funds/2 years)*

The aim of the project is to gain information about the overall organization of the *P. cepacia* genome and to understand the contributions of the many insertion sequences identified in this bacterium to its extraordinary catabolic potential. We have undertaken the construction of a macrorestriction map of the *P. cepacia* genome which will be used as a framework to determine the distribution of various IS elements and genes related to amino acid, carbohydrate and aromatic compound degradation. The restriction enzymes *Swa*I and *Pac*I, which recognize 8-bp sequences comprised exclusively of A and T residues, cleaved *P. cepacia* DNA (ca 70% G+C) into 4 and 6 fragments, respectively. Other enzymes such as *Dra*I, *Spe*I, and *Afl*II, which recognize similar 6-bp sequences, cleaved *P. cepacia* DNA into between 25 and 41 fragments. DNA fragments were resolved by pulsed-field gel electrophoresis using a CHEF gel apparatus, and the organization of *Swa*I and *Pac*I fragments was determined by Southern hybridization analyses using overlapping *Ase*I, *Afl*II, *Spe*I and *Xba*I fragments as probes. The results indicate that *P. cepacia* contains two chromosomes of 3.8 and 0.9 Mb. Both replicons appeared to carry r-RNA genes. Similar results were reported by Suwanto and Kaplan in 1989 for *Rhodobacter sphaeroides* (*J. Bacteriol* 171: 5850). The majority of *P. cepacia* IS elements were clustered in a particular region of the 3.8 Mb replicon. We are using Tn*5-751*, which carries a trimethoprim resistance marker as well as *Dra*I and *Spe*I sites, to map genes related to the degradation of various compounds. To facilitate such mapping we are constructing derivatives of Tn*5-751* carrying *Swa*I and *Pac*I sites.

Meharry Medical College
Nashville, TN 37208

108. Enzymes of Respiratory Iron Oxidation
R. Blake II, Biochemistry Department *$79,000*

Aerobic respiration on reduced iron is a principal metabolic activity exhibited by certain chemolithotrophic bacteria that inhabit iron-bearing geological formations exposed to the atmosphere. The aim of this research is to continue the systematic isolation and characterization of the respiratory enzymes expressed by these bacteria when grown at pH 1.5 on soluble ferrous ions. Recent studies have revealed that phylogenetically distinct groups of iron-oxidizing bacteria express biochemically distinct mechanisms for autotrophic growth on iron. Each different mechanism is characterized by the principal redox-active biomolecules expressed during aerobic respiration on iron. Thus, the respiratory chain of *Thiobacillus ferrooxidans* is dominated by a blue copper protein called rusticyanin. The amino acid sequence of rusticyanin has been determined and efforts to determine the three dimensional structure of the protein are in progress. The respiratory chain of *Leptospirillum ferrooxidans* is dominated by a novel red cytochrome characterized by reduced peaks at 441 and 579 nm. The respiratory chain of *Sulfobacillus thermosulfidooxidans* is dominated by a novel yellow chromophore that resembles a flavin. The respiratory chain of *Metallosphaera sedula* is dominated by a novel yellow cytochrome characterized by reduced peaks at 434 and 572 nm. These latter chromophores represent new enzyme cofactors unique to aerobic respiration on Fe(II). A pattern of remarkable diversity in the biochemical mechanisms for bacterial respiratory iron oxidation has begun to emerge from these studies. It is anticipated that this project will provide useful information toward manipulating *T. ferrooxidans* and related organisms for commercial use.

Meharry Medical College
Nashville, TN 37208

109. Biochemistry of Dissimilatory Sulfur Oxidation
R. Blake II, Biochemistry Department *$83,701*

Dissimilatory sulfur-oxidizing bacteria obtain all of their energy for metabolism from the aerobic oxidation of reduced inorganic sulfur compounds. Despite the environmental and economic importance of these organisms, there is still much uncertainty regarding the actual metabolic pathways and the stoichiometries of these bacterial oxidation reactions. The aims of this research are to initiate the systematic identification and quantification of the relevant sulfur-transformation enzymes encoded and expressed by different species of the thiobacilli. The organisms currently under investigation include, but are not limited to, *Thiobacillus neapolitanous* and *Thiobacillus denitrificans*. Two major experimental goals are proposed: (1) to purify 4 known sulfur-transformation enzymes (bisulfite reductase, adenosine-5'-phosphosulfate reductase, sulfite oxidase, and sulfur oxygenase) to electrophoretic homogeneity; and (2) to perform immunochemical analyses of protein expression using cell-free extracts and polyclonal antibodies directed against each protein purified in goal number one. The project is expected to help define the actual enzymes and thereby the metabolic pathways of sulfur oxidation in individual species of the thiobacilli. It is anticipated that this information will be of value in the eventual manipulation of the thiobacilli and related organisms to benefit both the environment and the mining industry.

Michigan Biotechnology Institute
Lansing, Michigan 48909

110. One Carbon Metabolism in Anaerobic Bacteria: Regulation of Carbon and Electron Flow During Organic Acid Production
J.G. Zeikus and M.K. Jain *$103,000*

The overall objective of this project is to understand the fundamental biochemical mechanisms that control and regulate carbon and electron flow in anaerobic chemosynthetic bacteria that couple the metabolism of single carbon compounds and/or hydrogen to the production of organic acids and alcohols. Fermentation, enzymes, electron carriers and genetic studies in *Butyrilbacterium methylotrophicum, Anaerobiospirillum succiniciproducens,* and *Sarcina ventriculi* are used as model systems. In *B. methylotrophicum* the metabolic pathway for conversion of H_2 plus CO_2, glucose or CO into butyrate or butanol is under study with emphasis on understanding how pH regulates C & E flow and how CO_2 is reduced to formate. In *A. succiniciproducens* CO_2 serves as a catabolic electron acceptor and its addition converts glucose metabolism from a lactate fermentative process into a succinate respiratory process. Focus is placed on characterization of the key CO_2 fixing enzymes and genes. In *S. ventriculi* low pH alters one carbon metabolism and C & E flow during glucose fermentation from formate and acetate to ethanol and CO_2 production. Focus is placed on the influence of low pH on membrane structure function properties and on characterization of CO_2 reductase and pyruvate decarboxylase genes and enzymes.

Michigan State University DOE Plant Research Laboratory
East Lansing, MI 48824

111. Molecular Basis of Symbiotic Plant-Microbe Interactions
F.J. de Bruijn *$229,800*

The induction of nitrogen-fixing root nodules and stem nodules on leguminous plants by soil bacteria belonging to the *Rhizobiaceae* is a highly evolved, complex process, requiring a fine-tuned interaction between the bacteria and their host. We wish to understand the regulatory signals, between rhizobia and plants, that play a role in the symbiotic control of gene expression in both partners, during nodule establishment and in the mature nodule. Specifically we are characterizing the physiological signals and regulatory circuits which play a role in controlling free-living versus symbiotic expression of rhizobial nitrogen-fixation and assimilation genes. In addition, we are examining the bacterial- and plant-derived signals which are involved in the nodule-specific expression of plant genes encoding nodulins. We are also studying environmental (stress-) control of gene expression in rhizobia, and are investigating the role of nodule-specific opine-like compounds in competition and the potential of using the corresponding synthesis and catabolism genes to create "biased rhizospheres".

Michigan State University DOE Plant Research Laboratory
East Lansing, MI 48824

112. Enzymatic Mechanisms and Regulation of Plant Cell Wall Biosynthesis
D. Delmer *$128,200*

The goal of this task is to elucidate the mechanisms and regulation of synthesis of the plant cell wall with emphasis on the biosynthesis of cellulose and callose. Although no activity has ever been discovered for a higher plant cellulose synthase, we have recently detected several polypeptides in cotton fibers which bear marked similarity to subunits of the bacterial cellulose synthase. Using PCR, we have amplified specific regions of two genes from the bacterium *Acetobacter xylinum* which code for subunits of the cellulose synthase. These amplified regions are being used as probes to screen a cotton fiber cDNA library in an effort to clone the corresponding genes from plants; several clones have been identified and are being characterized. We also continue to seek reasons why no *in vitro* activity can be detected for the plant enzyme. Regarding callose synthesis, we are isolating and characterizing a Ca^{2+}-binding annexin-like protein which reversibly associates with plasma membranes and modulates callose synthase activity; we also are characterizing factors which induce callose synthase *in vivo*, and have now selected two *Arabidopsis* mutants incapable of callose synthesis. Interestingly, both mutants are sterile, and we are beginning searches for temperature-sensitive mutants blocked in synthesis of callose, cellulose, or lignin. We also continue our studies on the unusual cellulose-deficient cell walls of plant cells adapted to growth on the cellulose synthesis inhibitor DCB; notable differences in wall structure between adapted monocot and dicot lines have been found, and the results shed new light on how primary cell wall polymers are cross-linked, limit porosity, and contribute to wall strength.

Michigan State University DOE Plant Research Laboratory
East Lansing, MI 48824

113. Molecular Mechanisms That Regulate the Expression of Genes in Plants
P. Green *$234,800*

The steady state level of an mRNA depends both on its rate of synthesis (transcription) and its rate of degradation. Rapidly accumulating data indicate that degradation rates of mRNAs in eukaryotes vary over a wide range and can be regulated by a variety of stimuli. However, in contrast to transcription, the mechanisms that control mRNA stability are largely unknown. A major objective of our work is to identify and characterize the molecular components that control the rates of mRNA degradation in plants (e.g., RNases and the mRNA sequences that they recognize) and determine how they interact. Another goal is to understand how certain components respond to plant growth regulators and to environmental stimuli so as to alter selectively the rates of mRNA degradation. We have found that degradation rates of specific transcripts can be measured directly in stably transformed tobacco cells grown in suspension cultures. This system is presently being used to identify sequences that act as stability or instability determinants within natural plant transcripts or those encoded by reporter genes. To determine if results obtained with cultured cells hold true for regenerated plants, we are also exploring ways to measure rates of mRNA degradation in transgenic tobacco. In addition, we have begun to identify the RNases of *Arabidopsis* as a first step towards differentiating between the RNases that play a role in mRNA degradation and those that have other roles in RNA metabolism.

Michigan State University DOE Plant Research Laboratory
East Lansing, MI 48824

114. Action and Synthesis of Plant Hormones
H. Kende *$253,800*

The objective of this project is to gain knowledge on the synthesis and mode of action of the plant hormones ethylene and cytokinin. We have been studying the enzymes that catalyze the synthesis of ethylene from S-adenosyl-L-methionine. 1-Aminocyclopropane-1-carboxylate (ACC) synthase is usually the limiting enzyme in this pathway. It can be induced by a variety of chemicals and environmental conditions, including stress. We have cloned a cDNA encoding an isoform of ACC synthase from tomato fruits (ACCSYN-2) and are studying the expression of genes encoding this and other isoforms of ACC synthase using gene-specific cDNA probes. We showed that expression of ACCSYN-1 is induced by stress, e.g., by wounding of fruits and by infection of leaves with a pathogen. The level of mRNA encoding both, ACCSYN-1 and 2, is increased during fruit ripening. We are also investigating expression of the ethylene-forming enzyme (EFE) in pea stems and rice internodes; we have isolated cDNA clones encoding EFE from cDNA libraries of peas and rice and are localizing the expression of the corresponding genes by tissue printing and *in situ* hybridization. This will enable us to determine which tissues and cells have ethylene-forming capacity. The role of stress ethylene is being investigated in deepwater rice where low-oxygen stress during flooding induces ethylene biosynthesis. Ethylene, in turn, enhances rapid growth of the plants by increasing their sensitivity to gibberellin (GA). Recent results indicate that this is due to ethylene-mediated reduction in the level of a growth inhibitor, abscisic acid. GA induces first cell elongation in the intercalary meristem, which is followed by enhanced cell-division activity. We have returned to earlier work on the induction of nitrate reductase (NR) by nitrate and cytokinins in *Agrostemma githago*. We are trying to understand whether cytokinins and nitrate regulate the same or different NR genes. First experiments using cDNA probes for NR indicate that the regulation of NR activity is at the transcriptional level and that the two effectors act independently of each other.

Michigan State University DOE Plant Research Laboratory
East Lansing, MI 48824

115. Cell Wall Proteins
D.T.A. Lamport *$37,000 (Project Discontinued January 1992)*

This program is an approach to primary cell wall function from the perspective of the wall as a pleiomorphic organelle possessing structural integrity and enzymic autonomy. The structure and function of the hydroxyproline-rich structural wall protein extensin is the focal point of the research. Thus, our recent isolation of several types of extensin monomer suggests an extensin network involving covalent intermolecular crosslinks. Additional evidence based on isolation of monomeric extensin precursors to the putative covalently linked extensin network, suggests that extensin is a transmural protein. Hence the "warp-weft" hypothesis: a crosslinked extensin network ("weft") of defined porosity is penetrated by a "warp" of cellulose microfibrils creating a true molecular fabric. This simple model based on interpenetrating networks is analogous to an "angle-interlock" composite and has a profound implication - it could mechanically couple load-bearing wall polymers thereby distributing stress among the coupled wall components. Is it possible that cell extension, so often described as "biochemically controlled creep", can be explained as a slippage of cellulose microfibrils

through the pores of an extensin network?

Current work seeks to test the major premises of the warp-weft hypothesis. Do extensin networks of defined porosity exist? If so, do cellulose microfibrils penetrate the pores? We are pursuing three lines of enquiry: (1) structural elucidation of extensin crosslink sites and network porosity; (2) enzymic generation of the network *in vitro*; and (3) comparative biochemistry of newly isolated extensins from graminaceous monocots (Maize), nongraminaceous monocots (Asparagus), primitive dicots (Sugarbeet), and the gymnosperms (primitive and advanced) *Ginkgo, Pseudotsuga, Pinus, Gnetum* and *Ephedra.*

Michigan State University DOE Plant Research Laboratory
East Lansing, MI 48824

116. Interaction of Nuclear and Organelle Genomes
L. McIntosh *$253,800*

Energy is the currency of growth and development, and in plants this currency is obtained by a balance between chloroplast energy capture and mitochondrial oxidative reactions. The flow of energy through these organelles is maintained by expression of genes encoding membrane complexes embedded in organelle membranes. Our goal is to unravel the molecular events controlling energy balance - and thus growth and yield - in plants.

We employ the cyanobacterium *Synechocystis sp. PCC 6803* to study the reaction centers of Photosystem (PS) I and PSII. Site-specific mutagenesis is being used to identify specific amino acid residues responsible for oxygen production by PSII. Recently, we have demonstrated that it is possible to undertake site-directed modifications in the "core" of PSI, the PSA-A and PSA-B polypeptides. Therefore, we have begun to use site-specific modifications to study the structure of the PSI core iron-sulfur center F_X and the biogenesis of the PSI.

Plants contain a "normal" cytochrome *c* oxidase along with an "alternative" oxidase where electron flow is not linked to the production of a transmembrane potential. In order to explore the function of alternative pathway respiration in higher plants we have cloned the gene, *aox1*, encoding the alternative oxidase. This gene is being used to make "transgenic" plants where the levels of alternative oxidase will be both repressed and elevated.

Michigan State University DOE Plant Research Laboratory
East Lansing, MI 48824

117. Sensory Transduction in Plants
K.L. Poff *$234,800*

The primary objective of this project is to understand the mechanisms for the acquisition of environmental information via light and gravity reception. We are studying the blue light photoreceptor pigment system(s) which control(s) numerous light responses such as phototropism in flowering plants; in addition, we are studying the mechanism for the perception of gravity in gravitropism. For these studies, we have developed a genetic system with which we can dissect the initial steps in the transduction sequences. Screening procedures have been devised and used to identify mutants of

Arabidopsis with altered phototropism and gravitropism. One strain exhibits a threshold fluence for phototropism increased by a factor of 50. The fluence response relationship for this strain shows that the threshold fluence for one of the two photoreceptor pigments for phototropism has been altered. Thus, this strain represents a probable candidate as a photoreceptor pigment mutant. This strain exhibits approximately 5% of the wild-type amounts of blue light-phosphorylatable protein in the plasma membrane. Thus, phosphorylation may be an early step in phototropism. Under conditions of long term irradiation, plants have the capacity to adapt to the irradiation, such that their responses at the beginning and end of a long irradiation are not equal. Thus, an understanding of adaptation is necessary to understand the plant's response to long term irradiations. Our approach to the study of the pathways for phototropism and gravitropism includes mutant isolation, and genetic, physiological and biophysical characterization. This genetic/biophysical approach should permit positive identification of the receptors, access into the transduction sequence, and eventual understanding at the molecular level of the events from reception to the bending responses.

Michigan State University DOE Plant Research Laboratory
East Lansing, MI 48824

118. Molecular Mechanisms of Trafficking in the Plant Cell
N.V. Raikhel *$234,800*

Maintenance of separate subcellular compartments in eukaryotic cells depends on the correct sorting and targeting of newly synthesized proteins. We are interested in understanding the molecular determinants of differential protein compartmentalization and in identifying the components of the molecular machinery which carry out the sorting process.

Recently, our group has defined the vacuolar targeting signal from barley lectin (BL). Using transient assay systems and stable transformation of tobacco plants, we have shown that the carboxyl-terminal propeptide of proBL is involved in delivering BL to the plant vacuole. The addition of these 15 amino acids as linker to the C-terminal end of the secreted protein cucumber chitinase causes the retention of this protein in vacuoles of transgenic tobacco plants. We are continuing to analyze the molecule(s) involved in the CTPP recognition with the goal of isolating putative receptor(s) which recognize these sorting sequences and subsequently mediate protein transport to vacuoles.

Using a transgenic approach we examined the transport of two maize trans-acting factors: the O2 and R proteins. The O2 and R proteins have now been functionally characterized for the presence of nuclear localization signals (NLSs). Both of these proteins contain multiple NLSs which, when fused to the GUS reporter gene, can redirect products from the cytoplasm to the nuclei of transgenic tobacco and onion epidermal cells. By taking advantage of these plant NLSs, we will continue to study nuclear transport in plants in order to provide an in-depth understanding of the molecular components involved in nuclear targeting.

Michigan State University DOE Plant Research Laboratory
East Lansing, MI 48824

119. Physiological and Molecular Genetics of Arabidopsis
C.R. Somerville *$257,800*

Our research is focused on a molecular genetic dissection of two areas of plant biochemistry which have proven refractory to analysis by conventional methods of biochemical analysis. One class of problems concerns the elucidation of the mechanisms by which plants adjust the fatty acid composition of membranes and storage oils. In order to characterize the mechanisms regulating these pathways we have isolated a large family of mutants of *Arabidopsis* with altered lipid composition. We are currently exploiting map-based cloning methods to isolate the genes which complement the mutations which regulate fatty acid composition. In conjunction with these studies, we are examining the structural basis of catalysis of a cloned fatty acyl desaturase which has been expressed at high levels in *E. coli*. A second initiative concerns a genetic dissection of the structural complexity of the polysaccharide components of the cell wall. As a first step, we have isolated a large family of mutants of *Arabidopsis* with altered cell wall composition. We are currently placing these mutants in genetic complementation groups as a prelude to a detailed analysis of the biochemical basis, and structural consequences, of each of the mutants.

Michigan State University DOE Plant Research Laboratory
East Lansing, MI 48824

120. Molecular Basis of Disease Resistance in Barley
S.C. Somerville *$232,800*

We have chosen to work with two complementary host-pathogen systems. One is the powdery mildew disease of barley caused by the fungal pathogen *Erysiphe graminis* f. sp. *hordei* and the other is the interaction between *Arabidopsis thaliana* and bacterial pathogens. With the barley powdery mildew disease, our objective is to recover the gene(s) at the *Ml-a* resistance locus. The characterization of a race-specific disease resistance gene product, like the *Ml-a* gene product, will be an important step in determining a key biochemical component of disease resistance. In addition, understanding the genetic mechanism that is responsible for the highly polymorphic nature of the *Ml-a* locus is of interest, since the genetic structure of this locus will dictate the range of novel resistance alleles that may be created *in vitro* for the purpose of genetically engineering disease resistance. *Arabidopsis* is a model plant species that offers many technical advantages for isolating and analyzing genes for which biochemical information is lacking, as is true for most disease resistance genes. We have identified a toxic, secondary metabolite in *Arabidopsis* that has the properties of a phytoalexin. We plan to test the hypothesis that this phytoalexin plays an important role in disease resistance to a range of pathogens in *Arabidopsis.*

Michigan State University DOE Plant Research Laboratory
East Lansing, MI 48824

121. Biochemical and Molecular Aspects of Plant Pathogenesis
J.D. Walton *$234,800*

The objective of this project is to understand the biochemical events that are important to the interaction between fungal plant pathogens and their host plants. We are studying examples of factors that control specificity as well as factors that are necessary for basic compatibility. As our model organism we are using *Cochliobolus* (*Helminthosporium*) *carbonum*, which causes leaf spot disease of maize. *C. carbonum* can be transformed with exogenous DNA and particular genes disrupted. It also has a sexual stage with two mating types. Race 1 of *C. carbonum* is highly virulent on maize that is homozygous recessive at the nuclear *Hm* locus due to its ability to produce HC-toxin, a host-selective toxin. We have cloned the *Tox2* gene cluster that controls HC-toxin production and are studying its structure and relation to the enzymology of HC-toxin biosynthesis. In order to study the specificity of HC-toxin against maize we have prepared radiolabelled HC-toxin. Maize leaves can metabolize HC-toxin to the inactive 8-alcohol. Susceptible plants appear to be lacking the enzyme, and NADPH-dependent reductase, that catalyzes this reaction. We are studying, as examples of basic compatibility factors, cell wall-degrading enzymes made by *C. carbonum*. These enzymes have been proposed, but never shown, to have an important role in penetration, nutrient assimilation, and tissue invasion, as well as in triggering host defenses. We have cloned the single endopolygalacturonase gene and used it to make a mutant of *C. carbonum* with a disrupted polygalacturonase gene. The mutant was as pathogenic as the wild-type fungus. We have also isolated xylanase and laminarinase and are cloning the corresponding genes.

Michigan State University DOE Plant Research Laboratory
East Lansing, MI 48824

122. Developmental Biology of Nitrogen-Fixing Cyanobacteria
C.P. Wolk *$253,800*

Anabaena and related cyanobacteria utilize solar energy to fix nitrogen gas within cells called heterocysts. We used derivatives of transposon Tn*5* to mutagenize the *Anabaena* genome. Mutants unable to fix nitrogen in the presence of oxygen (Fox$^-$ phenotype) were identified and further characterized. These included mutants blocked at intermediate stages of heterocyst differentiation, mutants affected in O_2-protection mechanisms, and mutants in which nitrogenase itself is affected. Additionally, promoterless bacterial luciferase genes, *luxAB*, were put under the control of the promoter of a specific gene, the resulting strain was transposon-mutagenized, and many resulting colonies were screened to identify regulatory mutants in which luminescence was prevented or rendered constitutive. Fusions of the transposons to contiguous genomic DNA were cloned. In several instances, the mutations were regenerated from the cloned fusions. Further analysis and pairwise recombination of the Fox$^-$ and presumptive regulatory mutants and mutations is being pursued to elucidate the processes of differentiation and aerobic fixation of nitrogen gas in *Anabaena*. We are developing techniques to analyze the physiology of nitrogen-fixing cyanobacteria. This work will facilitate understanding of cellular differentiation and of biological conversion of solar energy.

Michigan State University DOE Plant Research Laboratory
East Lansing, MI 48824

123. Environmental Control of Plant Development and Its Relation to Plant Hormones
J.A.D. Zeevaart *$237,800*

Plant growth and development are affected by environmental factors such as daylength, temperature, and moisture. Hormones often function as chemical messengers between the perception of an environmental factor and the morphological expression. The objectives of this project are to characterize the hormones involved, how their synthesis and catabolism are regulated by the environment, and how these hormones are distributed in the plant, and how they act. Studies with growth retardant BX-112, an inhibitor of 3ß-hydroxylation, indicate that in the long-day rosette plant spinach, GA_1 (3ß,13-hydroxylated) is the bioactive GA. However, in *Arabidopsis* GA_1 is consistently less active in causing stem elongation than GA_4 (3ß-hydroxylated), suggesting that in this species 3ß-hydroxylation alone is optimal for bioactivity. Stem elongation induced by long days in rosette plants is preceded by increased cell division in the subapical meristem. In *Silene*, a 30-fold increase in the GA_1 content of the subapical region was observed after plants were transferred from short to long days.

Several lines of evidence have established that in higher plants the hormone abscisic acid (ABA) is a breakdown product of xanthophylls. In stressed leaves incubated in $^{18}O_2$, one ^{18}O atom is rapidly incorporated in the carboxyl group, whereas isotopic enrichment is much less in the oxygen atoms of the ring. The ^{18}O-labeling of the xanthophylls closely parallels that of ABA in the same tissues. Furthermore, the ABA-deficient *aba* mutant of *Arabidopsis* is deficient in the epoxycarotenoids, violaxanthin and neoxanthin, and accumulates the biosynthetic precursor zeaxanthin. These observations indicate that ABA is synthesized by oxidative cleavage of epoxycarotenoids (the "indirect" pathway). Further work is focused on determining the chemical nature of the cleavage substrate(s), the cellular location of the cleavage process, and the limiting step in the pathway that is stimulated by loss of turgor.

Michigan State University
East Lansing, MI 48824

124. A Structural Analysis of the Role of the Rhizobium Cell Surface Carbohydrates in the Rhizobium/Legume Symbiosis
R. Hollingsworth, Department of Biochemistry *$155,000 (2 years)*

In our laboratory, we are studying the role(s) of the bacterial surface carbohydrates in the establishment of a viable symbiotic relationship between legume plants and bacteria of the genus *Rhizobium*. The cell surface carbohydrates include the capsular polysaccharide (CPS), the lipopolysaccharide (LPS) and other surface carbohydrates which occur in association with lipids. One important aspect of this study is the effects of external influences such as oxygen tension, pH, carbon source and plant flavones on the bacterial surface chemistry. We use a combination of chemical, immunochemical and cytochemical methods. The immunochemical and cytochemical methods employ polyclonal antibodies raised to define surface components of the bacterium. These antibodies are then used to probe for the presence of the original antigen in *in vitro* conditions under which environmental parameters are changed or *in planta* by excision and sectioning of nodules using a secondary antibody detection system. Both light and electron microscopy (immunogold) methods are used. This work also utilizes mutants which are incapable of synthesizing one or more of the bacterial cell surface carbohydrates and correlating this defect with the actual defective symbiotic step.

Michigan State University
East Lansing, MI 48824

125. Control of Triacylglycerol Biosynthesis in Plants
J. Ohlrogge, Department of Botany and Plant Pathology *$84,000*

Triacylglycerol (TAG) is the major form of carbon storage in seeds of many important crops and the oils extracted from these plant seeds represent a $20 billion dollar commodity with a wide variety of uses in the food and chemical industries. The overall objective of this research project is to understand how triacylglycerol biosynthesis is controlled in plants. Whereas leaves, roots and other tissues usually contain less than a few percent of their total lipid in the form of TAG, seed lipids frequently contain over 95% TAG. A metabolic or biochemical explanation for this dramatic difference in lipid composition has not yet been established. In order to begin to provide such an explanation we will examine the relative importance of possible alternative control mechanisms by examining the *in vivo* pool sizes of key intermediates in the pathway for TAG biosynthesis. In particular, methods will be developed to measure the plastid pools of acetyl-CoA and malonyl-CoA. In addition, the size and fatty acid composition of the acyl-CoA and diacylglycerol pools in plants which produce different fatty acids will be determined. Leaf and seed tissue will be compared to establish correlations between the different lipid metabolism of these tissues and the pools of the key pathway intermediates. The role of diacylglycerol acyltransferase will be evaluated by examining its expression and kinetic properties in seeds and leaves and the specificity of the CDP: choline phosphotransferase will be examined to determine its ability to provide specific partitioning of unusual fatty acids into triacylglycerol and their exclusion from phospholipids.

Michigan State University
East Lansing, MI 48824

126. A National Cooperative for Genetic Engineering of Plant Lipids
J. Ohlrogge, Department of Botany and Plant Pathology *$54,000*

In order to explore the wide range of potential applications of genetic engineering techniques to the production of useful new plant lipids, it will first be necessary to develop a detailed mechanistic understanding of most aspects of plant lipid metabolism and to acquire genes for the key enzymes and structural proteins. Progress toward these general goals will be greatly facilitated by the creation of a National Plant Lipid Cooperative (NPLC). The NPLC will provide an efficient mechanism for facilitating exchange of ideas, information and research materials among all members of the North American community. Exchange of ideas will occur through the development of an electronic newsgroup and sponsorship of participation of young scientists in regular meetings and workshops devoted to plant lipid metabolism. A major objective, in this regard, will be to encourage young scientists to initiate research programs on ignored topics of strategic importance to the whole field by allocating resources to the development of these areas. Exchange of information will be stimulated by the development and distribution of several databases containing all published information about plant lipid metabolism and the chemical composition of lipids from different plant species. Finally, the NPLC will commission the production and distribution of essential research materials which are not commercially available but which are required in order to pursue new avenues of research.

Michigan State University
East Lansing, MI 48824-1101

127. Physiology and Molecular Biology of Extracellular Peroxidases and H_2O_2-Generating System of Phanerochaete chrysosporium

C.A. Reddy, Department of Microbiology — *$74,000*

Phanerochaete chrysosporium is lignin-degrading white-rot basidiomycete, that is the object of intense world-wide research not only because of its ability to degrade lignin but also because of its ability to degrade a broad spectrum of environmental pollutants. It produces two key families of extracellular peroxidases designated lignin peroxidases (LIPs) and manganese-dependent peroxidases which play a key role in various degradative activities catalyzed by this organism. We will continue to focus our studies on obtaining a better understanding of the ecology, physiology, and molecular biology of these enzymes. Attempts to isolate specific mutants that lack MNPs will continue as such biochemical mutants have proven to be invaluable in the past. The basic studies on *LIP* genes have recently been expanded to include those of *Trametes versicolor,* the second best studied fungus after *P. chrysosporium.* Recent Northern blot studies indicate that *VLG2* is perhaps the most highly expressed of the *LIP* genes of *T. versicolor.* This gene is being sequenced and will be compared to the published genes of *P. chrysosporium.* We will also be studying the pattern of expression of different *LIP* genes in defined low nitrogen media as well as during colonization on different species of wood. Furthermore, studies on the extent of distribution of *LIP* gene homology in selected genera of wood-rot fungi will be continued. The initial studies on degradation of chlorolignols have been expanded to included several other classes of compounds to better understand the biodegradative ability of this organism. Experiments have also been initiated to investigate the molecular regulation of expression of *LIP* genes.

University of Michigan
Ann Arbor, MI 48109-1048

128. Molecular Genetics of Myosin Motors in Plants

J. Schiefelbein, Department of Biology — *$163,000 (2 years)*

The normal growth and development of plant cells is dependent on a variety of intracellular transport systems which serve to organize the contents of the cytoplasm, influence cell shape, and control cell polarity. The molecular "motor" that drives intracellular transport along actin filaments is myosin, an actin-activated ATPase. At present, almost nothing is known about the types of myosin in plant cells or the precise role of myosins in plants. The objective of this project is to use a molecular genetic approach to begin to understand the structure, distribution and function of myosin in plants. DNA clones representing five different myosin-like genes have been isolated from *Arabidopsis thaliana.* These myosin DNA clones will be used to: (1) determine the amino acid sequence of several different myosins, (2) analyze the expression patterns of the myosin genes by RNA blotting and reporter gene fusions, (3) study the intracellular distribution of myosin by immunolocalization methods, and (4) inhibit the expression of specific myosin genes by antisense RNA methods. These studies will focus on the role of myosins during the morphogenesis of the *Arabidopsis* root, because of the numerous advantages that the root possesses as a model for post-embryonic development. The results from this project will lead to a better understanding of the structure and distribution of myosin motors in plants. In addition, these studies will serve to examine the role of myosin in actin-dependent transport processes like cytoplasmic streaming, chloroplast and nuclear migration, and directed secretory vesicle transport,

University of Minnesota
Minneapolis, MN 55455-0312

129. The Mechanism of Switching from an Acidogenic to a Butanol Acetone Fermentation by Clostridium acetobutylicum

P. Rogers, Department of Microbiology *$178,000 (FY 91 Funds/2 years)*

The objective of this project is to elucidate the detailed mechanism by which the solvent-forming bacterium, *Clostridium acetobutylicum*, regulated the shift in fermentation metabolism between butyric and acetic acid formation during exponential growth and butanol-acetone production triggered by accumulation of butyric acid. Experiments are designed to identify and describe the regulatory genes and protein elements that govern the rate of synthesis of solvent-pathway enzymes. How this regulatory system interacts with induction of sporulation and related formation of intra-cellular granulose or other cell changes will also be investigated.

The research is centered upon the technique of employing transposable elements that create gene fusions and mutations due to insertion in the chromosome of gram positive bacteria. Our approach is based on recent demonstration in our laboratory and by others of transconjugation of transposon Tn916 into *C. acetobutylicum* and its insertion into the chromosome. A panel of strains with Tn916 inserts that are also solvent-negative and/or asporogenic will be used to identify specific regulatory genes. Recently, we have obtained electroporative transformation of the *Bacillus subtilis* plasmid pIM13 (2.2 Kb) into *C. acetobutylicum.* Cloned DNA fragments carrying suspected regulatory genes will be ligated into pIM13 and transformed into Tn916 insert-mutants. Complementation of sporulation and solventogenesis will be investigated in order to identify regulatory genes.

University of Minnesota
Navarre, MN 55392

130. Genetics of Bacteria that Utilize One-Carbon Compounds

R.S. Hanson, Gray Freshwater Biological Institute *$162,000 (FY 91 Funds/2 years)*

Methylotrophic bacteria are those bacteria that grow on one-carbon compounds including methane, methanol, methylamines, some halomethanes, and a few other compounds that do not contain C-C bonds. Those bacteria that grow on methane (methanotrophs) contain particulate methane monooxygenases (MMO's). Some also synthesize a soluble MMO (sMMO) when grown with limiting amounts of copper. Soluble MMO's oxidize a wide variety of substrates including halogenated low molecular weight hydrocarbons which are not oxidized by pMMO's. All synthesize methanol dehydrogenases (MDH's). sMMO's and MDH's each may comprise 15-20% of the cellular protein. MDH synthesis is inducible in some facultative methylotrophs including *Methylobacterium organophilium* strain xx.

We have cloned genes encoding sMMO components and we have mapped several genes required for the synthesis of active MDH. We have identified regulatory genes and a protein that binds specifically to sequences located upstream of the MDH structural gene. The role of this protein in the regulation of MDH synthesis will be examined using a broad host range mobilizable vector in which a 250bp sequence upstream of the MDH was fused to a *xylE* reporter gene.

Regulatory sequences that regulate expression of genes that encode the sMMO components have been identified and sequenced. The mechanism of copper regulated expression of soluble MMO will be examined using methods similar to those used to define the regulation of MDH synthesis.

University of Missouri
Columbia, MO 65211

131. Dosage Analysis of Gene Expression in Maize
J. Birchler, Division of Biology *$186,000 (2 years)*

The goal of this project is to understand further the basis of dosage sensitive regulatory effects on gene expression in maize. These effects act directly or inversely on the quantitative level of gene products when the dosage of specific chromosomal segments is varied. There are six specific questions addressed in this project. First, the structural and regulatory genes for the anthocyanin pigment pathway, which have been previously cloned, will be analyzed via Northern blots in a comprehensive set of segmental dosage series to examine the relationship of the direct and inverse effects on the pathway with the effects of other regulatory genes already defined. These studies should help clarify how dosage regulators interact in regulatory hierarchies. Secondly, the dosage regulators will be tested as to whether they exhibit evidence of parental imprinting in the endosperm, as has been suggested for other regulatory genes. This will be tested by comparing dosage series produced maternally versus paternally and then examining the degree of effects on the collection of proteins expressed in the endosperm. Thirdly, combinations of multiple inverse and direct acting chromosomal segments, that are effective on the mRNA level expression of the *Globulin-1* gene, will be combined to learn the interaction properties as to whether they are cumulative, nonadditive or synergistic. Fourthly, larger aneuploids will be examined for threshold effects on gene expression as opposed to modulations found in smaller segmental aneuploids. Fifth, a collection of RFLP loci selected for expressed genes in leaves will be tested in the respective monosomics and segmental dosage series to determine the generality of structural gene dosage effects, dosage compensation, direct effects and inverse effects. Lastly, the patterns of gene expression will be examined in an extensive ploidy series from 1-7x as a comparison to the aneuploidy studies. The dosage sensitive nature of the studied effects bears not only on the problem of gene regulation but also on the molecular bases of aneuploid syndromes and dosage compensation.

University of Missouri
Columbia, MO 65211

132. Molecular Analyses of Nuclear-Cytoplasmic Interactions Affecting Plant Growth and Yield
K.J. Newton, Division of Biological Sciences *$178,957 (FY 91 Funds/2 years)*

The biogenesis and functioning of mitochondria depends on the expression of both mitochondrial and nuclear genes. One approach to investigating the role of nuclear-mitochondrial cooperation in plant growth and development is to identify combinations of nuclear and mitochondrial genomes that result in altered but sublethal phenotypes. Plants carrying certain maize nuclear genotypes in combination with cytoplasmic genomes from certain teosintes (wild relatives of maize) can exhibit "incompatible" phenotypes, such as reduced plant growth and yield and cytoplasmic male sterility, as well as altered mitochondrial gene expression. The characterization of these nuclear-cytoplasmic interactions is the

focus of this project. We are investigating the effects of two maize nuclear genes, *Rcm1* and *Mct*, on mitochondrial function and gene expression. We will attempt to clone and characterize each of these genes and to analyze how the products of recessive and dominant alleles at each locus coordinate with products of the teosinte mitochondrial genes to give aberrant plant phenotypes.

University of Missouri
Columbia, MO 65211

133. Genetics of the Sulfate-Reducing Bacteria
J.D. Wall and B.J. Rapp-Giles, Biochemistry Department *$77,000*

The genetics of the strictly anaerobic sulfate-reducing bacteria, in particular *Desulfovibrio desulfuricans*, are being developed for use in the analysis of the metabolic functions that are critical to the roles of the SRB in the sulfur cycle, biocorrosion of metals and mineralization of biomass. During the past year, we have identified a number of limitations in extrapolating from enteric bacteria to the SRB as well as from one strain of *D. desulfuricans* to another. Conjugation is readily demonstrated with IncQ broad-host-range plasmids into strain G100A. IncP and IncN plasmids appear to transfer but are not stable. No evidence for transfer of other incompatibility group plasmids to this strain has been obtained. In contrast, with strain ATCC 27774, no stable transfer of any plasmids can be demonstrated. The possibility that some plasmids are being transferred and not maintained allows the development of delivery systems for transposons for random insertional mutagenesis. To date, transposons Tn*5* and Tn*9* do not appear to undergo transposition in *D. desulfuricans* G100A. Tn*10* and Tn*916* are now being tested.

Potential shuttle vectors have been constructed from Km^R-tagged cointegrates of pBG1, isolated from *D. desulfuricans* G100A, and pTZ18U. Constructs with pBG1 interrupted at three different positions have all apparently been unable to be stably maintained in G100A. Attempts to cure G100A of its endogenous pBG1 for use as a recipient are underway.

Mount Sinai School of Medicine
New York, NY 10029

134. The Respiratory Chain of Alkaliphilic Bacteria
T.A. Krulwich, Department of Biochemistry *$202,999 (2 years)*

Extremely alkaliphilic *Bacillus* species possess high membrane concentrations of respiratory chain complexes that extrude protons, and do not act as primary Na^+ pumps. There are multiple terminal oxidases, one of which is a caa_3-type oxidase that is up-regulated during growth at highly alkaline pH. Having now cloned and sequenced the operon encoding this complex, we will investigate the apparent pH-dependent increase in mRNA and the possible heterogeneity among the transcripts. In addition, mutations in this oxidase will be characterized at a molecular biological level, and studied with respect to their impact on alkaliphile physiology. Current data suggest that the caa_3-oxidase has a special role in growth at especially high pH values, not entirely related to its role in producing a bulk protonmotive force; mutational loss of this oxidase, however, can be compensated by very high levels of a *o*-type terminal oxidase. During the coming project year, we will begin to characterize this phenomenon in detail and investigate its basis.

National Renewable Energy Laboratory
Golden, CO 80401

135. The Water-Splitting Apparatus of Photosynthesis
M. Seibert, Photoconversion Branch *$164,000*

Photosynthetic water-splitting function in photosystem II (PS II) is catalyzed by a bound tetrameric manganese cluster. Bridging ligands hold the cluster together, but terminal ligands bind it to at least some of the proteins comprising the isolated PS II reaction center (RC) complex. Both diphenylcarbazide (DPC) and Mn^{2+} are independently capable of donating electrons to PS II devoid of the functional manganese cluster; however, binding of Mn^{2+} to a PS II RC already occupied by DPC can result in the inhibition of electron donation to that center. At high DPC concentration, added Mn^{2+} (low micromolar levels) acts primarily as a non-competitive inhibitor of DPC electron donation rather than as an alternative electron donor to PS II. This inhibition phenomenon has been used as an assay for detection of four high affinity, Mn^{2+}-binding sites in PS II. Direct metal analysis confirmed the binding of >3.2 Mn per PS II RC under the assay conditions. Two of the four high affinity sites are associated with ligands contributed by carboxyl amino acid residues and two by histidyl residues. Only the carboxyl sites are observable when two of the four functional Mn that are more loosely bound to the membrane are removed. One of the histidine sites has been identified as His-337 on the D1 protein by both biochemical and immunological approaches. Preliminary studies with site-directed mutants of *Synechocystis* 6803 suggest that His-336 on the D2 protein may be the other histidine site detectable by the DPC/Mn^{2+} assay. The four high affinity, Mn-binding sites identified by this approach could be sites associated with four Mn-binding sites that normally form sequentially in the photoactivation process and may also be related to amino acids that provide some of the terminal ligands for binding functional Mn.

University of Nebraska
Lincoln, Nebraska 68588-0118

136. Physiology and Genetics of Metabolic Flux Regulation in Zymomonas mobilis
T. Conway, School of Biological Sciences *$77,200*

The enzymology of glycolytic pathways is now well established, but the molecular mechanisms that control carbon flux are only beginning to be understood. The metabolically simple bacterium, *Zymomonas mobilis,* has been chosen for this work because it is amenable to genetic studies and the variables involved in regulation of flux can be easily manipulated. This organism uses the Entner-Doudoroff pathway exclusively for conversion of carbohydrates to the sole fermentation products ethanol and carbon dioxide. The role of gene expression in regulating high level synthesis of the glycolytic enzymes in a balance that allows proper glycolytic flux control is being elucidated. A growing body of evidence indicates that several layers of genetic control, perhaps in a hierarchal arrangement, act in concert to determine the relative abundance of the enzymes. The genes all possess canonical ribosome binding sites and display high codon bias. Enzyme abundance generally correlates well with the relative quality of the ribosome binding sites. Co-translation of the overlapping *zwf* and *edd* genes might be important for controlling the balance of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydratase. Transcript half-lives as long as 18 minutes appear to be important for achieving high level expression of the genes. The relative stabilities of the

mRNAs are proportional to the respective enzyme concentrations and therefore play a primary role in coordinating expression of the glycolytic genes. Likewise, post-transcriptional mRNA processing appears to be important for dictating an appropriate balance of the glycolytic enzymes. Promoter strength and the relative rates of transcription of the glycolytic genes, which apparently form a separate, high expression class, are likely to be important. Ongoing research involves the relationship of these and other features of glycolytic gene regulation to physiological aspects of glycolytic flux control.

University of Nebraska
Lincoln, NE 68588-0118

137. Characterization of a Defective Interfering RNA That Contains a Mosaic of a Plant Viral Genome

T.J. Morris, School of Biological Sciences, University of Nebraska and A.O. Jackson, University of California, Berkeley

$182,745 (FY 91 Funds/2 years)

Our research emphasizes identification of viral sequences effecting viral pathogenicity by characterization of a unique class of RNAs called defective interfering RNAs. DIs are linear deletion mutants of viral genomes that interfere with helper virus and reduce disease severity. Many more plant viral DIs have now been reported since our first discovery of DIs in tomato bushy stunt tombusvirus (TSBV). We have now constructed cDNA clones from which infectious RNA transcripts for both the viral and DI genomes can be produced. Mutagenesis of DI clones has identified essential regions of viral sequence in natural DIs necessary for replication, and their role in symptom attenuation is continuing. Studies on the biogenesis of DI RNAs using DI free transcripts of cloned TBSV, cucumber necrosis (CNV) and chimeric constructs of both genomes followed by PCR analysis of inoculated plants has conclusively demonstrated that DIs arise *de novo* shortly after inoculation of plants and that DIs evolve rapidly. Deletion mutagenesis of TBSV has produced artificial defective RNAs that were replicated by the virus. Unlike native DIs lacking coding sequence, insertion of foreign coding sequences (CAT gene) into regions of artificial DIs resulted in a high level of replication and CAT gene expression upon co-transfection with wild-type transcripts demonstrating that gene expression can be activated using artificial DIs. The study of the expression of DI sequences from transgenic plants is continuing.

University of Nebraska
Lincoln, NE 68583

138. Mechanistic Enzymology of CO Dehydrogenase from Clostridium thermoaceticum

S.W. Ragsdale, Department of Biochemistry *$158,668 (FY 91 Funds/19 months)*

Acetogenic bacteria perform a novel mechanism of CO_2 and CO fixation called the acetyl-CoA pathway. Carbon monoxide dehydrogenase (CODH) assembles acetyl-CoA via methyl-CODH, CODH-CO, and acetyl-CODH intermediates. These intermediates all appear to be organometallic species involving a Ni-Fe containing site on CODH. CoA binds to and then is condensed with acetyl-CODH in the final steps of the synthesis. The rates of formation of these intermediates are markedly accelerated by prior reduction of the Ni-Fe active site at low redox potentials (<-450mV). Our focus

is on elucidation of the reaction mechanisms of the individual steps in the pathway by steady-state and presteady-state kinetic analyses combined with electrochemical and spectroscopic methods. Measurement of the rates of the individual intermediates should allow us to establish their kinetic competence and determine the rate-limiting step(s) in the pathway. The structures and roles of the metal centers in CODH are being investigated with a major focus on the Ni-Fe_{3-4}-S_4 center which appears to play a key role in the assembly of the acetyl moiety of acetyl-CoA.

New York University
New York, NY 10003

139. Asparagine Synthetase Gene Expression and Plant Nitrogen Metabolism
G.M. Coruzzi, Department of Biology *$198,000 (2 years)*

We are using molecular-genetic approach to study the role of asparagine synthetase (AS) in plant nitrogen metabolism. We have cloned two AS genes (AS1 and AS2) from *Pisum sativum* and are performing experiments designed to address three broad aims. The first aim is to understand the function of each encoded AS isoenzyme. The second aim is to understand the molecular mechanisms which regulate the expression of individual members of the AS gene family. We have recently discovered that AS gene expression is negatively regulated by light and will dissect the molecular basis for this regulation. The third aim is to integrate our findings on AS gene regulation and isoenzyme function by manipulating AS gene expression in transgenic plants and monitor effects on nitrogen metabolism and/or plant growth. Our specific goals are to: 1) Express plant AS genes in microorganisms and utilize dominant negative mutants to dissect the subunit structure and substrate specificity of each encoded AS enzyme. 2) Examine the mechanism of light-repressed expression of AS1 in non-photosynthetic organs. 3) Characterize the cis-acting elements and trans-acting factors involved in the light-repressed and cell-specific expression of AS1 and AS2 during plant development. 4) Modulate the AS gene expression in plants and monitor changes in plant nitrogen metabolism and/or development. The studies proposed are multifaceted and attempt to integrate gene expression studies with physiology. The results of our studies on the plant AS genes should therefore not only uncover novel mechanisms of gene regulation but will also have significance to plant nitrogen metabolism.

North Carolina State University
Raleigh, NC 27695-8008

140. Transcription Factors Regulating Lignin Biosynthesis in Xylem
R. Sederoff, Department of Forestry *$194,910 (2 years)*

About one fourth of woody plant biomass is lignin, therefore, the biosynthesis of lignin requires a major fraction of the biosynthetic resources of living woody plants. Lignin forms part of the embedding matrix of the plant cell wall, reinforcing cellulose microfibrils and imparting rigidity to the wall. Lignification of the cell wall is essential for the production of a functional water conducting system. The pathway for the production of lignin from primary metabolic precursors (phenylalanine) is understood, but relatively little is known about the regulation of genes that code for the enzymes in the lignin biosynthetic pathway. Our efforts will be directed to understand the mechanisms regulating two specific genes coding for PAL (phenylalanine ammonia-lyase), the first enzyme in the pathway, and CAD (cinnamyl alcohol dehydrogenase), the enzyme forming coniferyl alcohol, the final precursor for

lignin. In this project, we will verify the identity and map the genes coding for PAL and CAD enzymes involved in lignin formation in xylem, isolate the promoters for these genes, and identify transacting factors involved in genetic regulation. Our experiments should lead to information on the nature of the signal transduction pathways regulating these enzymes in development and in response to environmental induction of lignification. Understanding of these mechanisms are important for strategies of genetic engineering of wood properties in forest trees.

Ohio State University
Columbus, OH 43210

141. Structure and Regulation of an Archaebacterial Promoter: An In Vivo Study
C.J. Daniels, Department of Microbiology *$157,575 (FY 91 Funds/2 years)*

The archaebacteria (Archaea) have become a subject of great interest to the biologist and the biochemist. As inhabitants of some of the most extreme environments known to support life, and members of a third evolutionary line of descent, these organisms have the potential to provide novel biochemical systems and insight into the evolution of cellular processes. The goal of these studies is to identify sequence elements controlling the initiation and termination of transcription in the halophilic archaebacteria. For these studies we have developed a *Haloferax volcanii-E. coli* shuttle expression vector. This plasmid carries a *H. volcanii* tRNALys gene promoter-containing fragment that faithfully directs transcription *in vivo*. The essential transcription initiation element of this fragment has now been reduced to a 40 base pair fragment and we are investigating the role of individual nucleotides in the initiation process. Regulation of this promoter, in response to growth rate and nutrient composition, is being evaluated. We have also observed that polypyrimidine tracts, located 3' to a reporter gene, lead to the production of transcripts with discrete 3' termini, suggesting that these sequences play a role in transcription termination. The ability of these sequences to direct termination will be tested by constructing a transcription unit where the potential termination signal is located between the promoter and the reporter gene. Finally, we are investigating whether the halophilic transcriptional machinery will recognize the promoter elements of non-halophilic genes. These later studies will indicate the compatibility of archaebacterial transcriptional signals and show whether the halophiles can act as surrogate hosts for the genes of other archaebacteria, particularly the hyperthermophiles, which are not readily amenable to genetic analysis.

Ohio State University
Columbus, OH 43210

142. Transmethylation Reactions During Methanogenesis from Acetate in Methanosarcina barkeri
J.A. Krzycki, Department of Microbiology *$160,000 (FY 91 Funds/2 years)*

Acetate is the primary precursor of methane in many environments of economic and environmental importance. We are investigating the reactions in this pathway following the cleavage of acetyl-CoA by carbon monoxide dehydrogenase and prior to the methylation of coenzyme M. When the terminal enzyme of the pathway is inhibited in methanogenic cell free extracts of *Methanosarcina barkeri*, methyl groups accumulate on three different proteins. Therefore, these proteins may participate in the transmethylation reactions of acetate dependent methanogenesis. Two proteins have been isolated with molecular sizes of 480 kDa and 29 kDa and both carry the methyl groups on bound corrinoids.

The smaller monomeric protein comprises about 1% of the soluble protein in cells grown on acetate, and is also present in cells grown on methanol. The larger protein has two subunits of 44 and 31 kDa and possesses Ca, Co, and Zn; but not Fe. The 480 kDa protein comprises 3% of the soluble protein in cells grown on acetate, but is less abundant in cells grown on methanol or H_2/CO_2. It thus appears to be a novel, regulated, corrinoid protein with a unique role in aceticlastic cells. We are continuing to characterize both proteins, and define their roles in this methanogen using monoclonal antibodies, inhibitor studies, and interactions with potential substrates such as coenzyme M or tetrahydrosarcinapterin. In addition, we are involved in the determination of the stereochemistry of methyl transfer from acetate to these two coenzymes.

Ohio State University
Columbus, OH 43210

143. The Molecular Characterization of the Lignin-Forming Peroxidase: Growth, Development, and Response to Stress
L.M. Lagrimini, Department of Horticulture *$126,530*

The tobacco anionic peroxidase, which is expressed in epidermal and xylem-forming tissues, is thought to have a critical role in lignin synthesis, however, other functions this enzyme may possess are unknown, To better understand how this enzyme functions, a cDNA clone for this peroxidase was joined in one of two orientations to the CaMV 35S promoter to direct the specific over- or under-expression of this enzyme in transgenic plants. Lignin levels in leaf, root (thick), and stem (woody) tissue from plants which overproduce the anionic isoenzyme were found to be 200-300% higher than in control plants. This indicated that in control plants either peroxidase is limiting in certain tissues, or the over-expression of peroxidase results in elevated levels of cinnamyl alcohols. Using antisense RNA produced by the 35S promoter, endogenous peroxidase activity was reduced by 20-fold. However, these plants were found to have no less lignin than control plants. it is suspected that the antisense RNA is not being expressed in lignifying tissue. Currently, transgenic plants are being constructed with antisense RNA being produced from a xylem-specific promoter. Plants over-producing peroxidase were found to chronically wilt upon reaching sexual maturity. This was a result of insufficient root surface area for an equivalent leaf surface area. Roots but not shoots stop growing as the plants mature. The root tissue was found to have low levels of auxin, and root explants were unresponsive to exogenous auxin.

Ohio State University
Columbus, OH 43210

144. Structure and Regulation of Methanogen Genes
J.N. Reeve, Department of Microbiology *$319,629 (2 years)*

The long-term goals of this project are to characterize the genetic organization and mechanisms of regulation of gene expression in methanogens, to use gene cloning and the techniques of molecular biology to dissect and understand the biosynthesis of methane and to develop genetic exchange systems for methanogens. Regulation of synthesis of methyl coenzyme M reductase (MR) in *Methanococcus vannielii* and methyl-viologen reducing hydrogenase (MVH) in *Methanobacterium thermoautotrophicum* are being studied. Although five, closely-linked genes (*mcr*BDCGA) form the MR operon only the products of the *mcr*B, *mcr*G and *mcr*A genes are components of the purified Mr

holoenzyme. We have shown that there are ~150 intact, non-processed transcripts of this operon with half-lives of ~15min in exponentially growing *M. vannielii* cells and that transcription is inhibited by H_2-limitation or by addition of the inhibitors BES, levulinic acid, monesin, puromycin, pseudomonic acid and virginiamycin. The product of the *mcr*D gene has been purified and antibodies raised against this polypeptide shown to decrease the rate of methanogenesis *in vitro* in cell extracts. The polyferredox encoded by the *mvh*B gene in *M. thermoautotrophicum* has been purified and its role in electron transport during methanogenesis is being investigated. Several plasmids based on the methanogen-derived pME2001 replicon have been constructed carrying puromycin or pseudomonic acid resistance genes. These plasmids are being used as donor DNAs to develop and evaluate transformation protocols for *M. thermoautotrophicum*.

Ohio State University
Columbus, OH 43210

145. Photosynthetic Electron Transport in Genetically Altered Chloroplasts
R.T. Sayre, Departments of Biochemistry and Plant Biology *$208,000 (2 years)*

It is generally accepted that the structural organization of the photosystem II (PS II) reaction center complex is analogous to the bacterial photosynthetic reaction center. The primary and secondary structure of the reaction center core polypeptides as well as the primary reactions catalyzed by PS II and bacterial reaction center core complexes have many features in common. In this study, we use the bacterial reaction center crystal structure as a model to guide us in the characterization of protein-chromophore interactions which regulate and/or participate in charge transfer processes in the PS II complex. Our strategy is to target select residues of the PS II reaction center D1 and D2 core polypeptides for site directed mutagenesis followed by characterization of the mutant phenotypes. We will identify and characterize amino acid residues which: 1) are involved in the binding and orientation of chromophores, 2) regulate charge transfer by electrostatic effects, and 3) directly participate in charge transfer. The unique feature of our approach will be the generation and characterization of mutants in the eukaryotic alga *Chlamydomonas. Chlamydomonas* has many advantages as a genetic and biochemical system for the characterization of the PS II complex in chloroplasts. It will serve as a model system for the genetic manipulation of higher plant chloroplasts.

Ohio State University
Columbus, Ohio 43210-1292

146. Regulation of Alternative CO_2 Fixation Pathways in Procaryotic and Eucaryotic Photosynthetic Organisms
F.R. Tabita, Department of Microbiology *$180,000 (FY 91 Funds/2 years)*

We are investigating the control of alternative pathways of CO_2 metabolism in anaerobic photosynthetic bacteria and aerobic eucaryotic marine diatoms. Such procaryotic and eucaryotic organisms are representative of important and environmentally significant CO_2 utilizing organisms and each has been proposed to assimilate CO_2 by routes that differ from the usual Calvin reductive pentose phosphate pathway. Our studies employ molecular biological and biochemically-oriented experiments to elucidate the molecular basis for switches in CO_2 metabolic paths. Our initial studies have been with mutants of purple nonsulfur photosynthetic bacteria and have provided clear-cut evidence of significant non-Calvin-type CO_2 fixation. Constructs were initially prepared in *Rhodobacter sphaeroides* such that the

separate chromosomal RubisCO genes were deleted; the resultant strain was incapable of photoheterotrophic or photolithoautotrophic growth using CO_2 as electron acceptor. However, from this RubisCO deletion strain, a second mutant was isolated, strain 16-PHC; this strain was capable of photoheterotrophic growth with CO_2 as electron acceptor. Subsequent studies showed that strain 16-PHC catalyzed substantial rates of whole-cell CO_2 fixation, but retained the inability to synthesize RubisCO. Strain 16-PHC thus possessed the same phenotype as a RubisCO deletion mutant of *Rhodospirillum rubrum*, strain I-19. Strains I-19 and 16-PHC are the focus for further genetic and enzymological studies designed to determine the mechanism and regulation of the alternative CO_2 assimilatory path. Control of CO_2 fixation in the two RubisCO deletion strains is also being compared to the CO_2 fixation process of green photosynthetic bacteria (two species of *Chlorobium*), since these organisms exhibit active and well described alternative CO_2 fixation pathways.

Oklahoma State University
Stillwater, OK 74078

147. The Structure of Pectins from Cotton Suspension Culture Cell Walls
A. Mort, Department of Biochemistry *$87,741*

In this project we are trying to understand the complexities of the structure of pectins. At present we are using cotton suspension culture as our source of cell walls for pectin isolation but will extend our studies to "real" plant tissues in the near future. The main emphasis, at present, is to characterize the rhamnose rich part of pectin (RGI in the classification of pectins used by Albersheim and coworkers), including the homogalacturonan segments of RGI. We isolate RGI by sequential digestion of intact cell walls with a purified endopolygalacturonase followed by "cellulase" digestion. After fractionation of the cellulase-solubilized material we obtain ~50% of the RGI of the cell wall. By partially degrading the RGI with liquid HF we find that it contains a high proportion of the repeating disaccharide galA→rha. H^1 NMR shows that these repeats are naturally acetylated with about 1 acetate residue per repeat on average. Mass spectrometry of the oligosaccharides representing from 1-5 repeats indicates that there can be up to two acetates per repeat. We are trying to pinpoint the location of the acetates by 2D NMR and collisionally induced fragmentation coupled with linked B/E scanning mass spectrometry. In addition to the galA-rha repeat sequences, there appear to be homogalacturonan segments in cotton RGI-like material. By treating the material with HF we obtain a range of oligomers of galacturonic acid with rhamnose at their reducing end. These oligomers contain a little xylose and are about 50% methyl esterified. Interestingly, if we treat intact cell walls in liquid HF to cleave all rhamnosyl linkages (but not galacturonic linkages) we can solubilize two distinct classes of homogalacturonan. 1) ~10% methyl esterified with little ramnose (rha:galA::<1:50). 2) ~50% methyl esterified with rha:galA::~1:25. The second class appears to be the homogalacturonan solubilized in the RGI fraction with the enzymic digestion.

We have devised a scheme for determining how the methyl esters are distributed within homogalacturonans and are applying it to the cotton pectins as well as to commercial apple and citrus pectins. The fruit pectins appear to be randomly methyl esterified but in preliminary experiments the pectins from cotton walls are far from randomly esterified.

University of Oklahoma
Norman, OK 73019

148. Effect of Community Structure on Anaerobic Aromatic Degradation
M.J. McInerney, Department of Botany and Microbiology *$80,000*

The effect of acetate on the rate and extent of benzoate degradation by an anaerobic syntrophic bacterium, *Syntrophus buswellii*, in coculture with a hydrogen-using sulfate reducer was studied. The addition of acetate decreased the initial rate and the extent of benzoate degradation by the coculture. In the presence of 20 mM acetate or more, benzoate consumption reached a threshold of 2 to 29 μM benzoate with no further degradation observed even after extended incubation periods. Thermodynamic calculations indicated that the change in free energy approached a value close to that required for the synthesis of ATP suggesting that a minimum change in free energy may be required for the metabolism of substrates by syntrophic bacteria. Threshold values were not observed when a thermodynamically more favorable electron acceptor, nitrate, was used. This suggests that the threshold was the result of a thermodynamic constraint rather than end product toxicity.

The biochemistry of fatty acid degradation and poly-ß-hydroxyalkanoate (PHA) synthesis in *Syntrophomonas wolfei* was studied. *S. wolfei* forms D-(-)3-hydroxybutyryl-coenzyme A (CoA) required for PHA synthesis by an acetoacetyl-CoA reductase activity that uses either NADH or NADPH as the electron donor. Thus, the NADH formed during ß-oxidation can be used directly for PHA synthesis without the need for a transhydrogenase activity or some other mechanism to interconvert NADH and NADPH. The acetoacetyl-CoA thiolase was purified to homogeneity as indicated by native gel electrophoresis. However, the pure enzyme still had detectable 3-hydroxyacyl-CoA dehydrogenase and crotonase activities suggesting that the ß-oxidation enzymes form a multienzyme complex.

Oregon Graduate Institute of Science and Technology
Beaverton, OR 97006-1999

149. Biochemical Genetics of Lignin Degradation by Phanerochaete chrysosporium
M.H. Gold, Department of Chemical and Biological Sciences *$251,998 (2 years)*

White-rot fungi are primarily responsible for initiation of lignin degradation in wood. The best-studied lignin-degrading organism, *Phanerochaete chrysosporium*, secretes two extracellular heme enzymes--lignin peroxidase (LiP) and manganese peroxidase (MnP)--which catalyze the oxidative degradation of lignin. The long-term objective of our research is to further our understanding of the lignin degradative system of *P. chrysosporium* and selected other white-rot fungi. We are studying the roles of LiP, MnP, laccase, and other oxidases in the depolymerization of lignin; and the pathway and enzymes involved in the further degradation of benzoquinones which are key lignin degradation intermediates.

A. Role of extracellular enzymes in lignin depolymerization

We are examining the ability of MnP and LiP from *P. chrysosporium* and MnP and laccase from *Dichomitus squalens* to depolymerize specifically labeled syringyl-guaiacyl synthetic lignin (DHP) copolymers and a variety of other DHPs. We are also comparing the ability of LiP, MnP, and combinations of these enzymes to degrade various ethylated and nonethylated DHPs. Short-term

depolymerization experiments will be carried out with purified enzymes in the presence and absence of competent cells to assess the role of cells in preventing repolymerization. We have recently shown the mechanism of MnP-catalyzed cleavage of free phenolic lignin model dimers containing an α-carbonyl function. This strongly suggests that α-carbonyl formation might be a major pathway for lignin biodegradation and that MnP plays an important role. Finally, gene disruption methods will be developed and used to determine the role of individual MnP and LiP isozymes in lignin degradation.

B. Further degradation of benzoquinones

Benzoquinones such as 2-methoxy-p-benzoquinone are key intermediates in lignin degradation; a unique reduction/oxidation cycle is involved. We are isolating the quinone reductase(s) involved in this cycle and characterizing its catalytic mechanism and mode of regulation. We are also investigating whether MnP or possibly another hydroquinone oxidase is involved in this cycle. We have shown that this reduction/oxidation cycle converts lignin or a series of aromatic pollutants into trihydroxy- or tetrahydroxybenzenes. Finally, we are isolating and characterizing the enzyme responsible for aromatic ring cleavage of 1,2,4-trihydroxybenzene, a key intermediate in 2-methoxy-p-benzoquinone degradation.

Oregon Graduate Institute of Science & Technology
Beaverton, OR 97006-1999

150. Oxidative Enzymes Involved in Fungal Cellulose Degradation

V. Renganathan, Department of Chemical and Biological Sciences

$166,000 (2 years)

Many cellulolytic fungi produce extracellular cellobiose-oxidizing enzymes (COE) in addition to cellulases; however, the role of these enzymes in cellulose degradation is not understood. Cellobiose dehydrogenase (CDH) and cellobiose: quinone oxidoreductase (CBQase) are two extracellular cellobiose-oxidizing enzymes produced by the cellulose-degrading cultures of *Phanerochaete chrysosporium*. We plan to develop an understanding of the role of COE in cellulose degradation with CDH and CBQase as models. We will also elucidate the structure, function and mechanism of CDH.

1. CDH enhances the *Trichoderma* cellulases-catalyzed crystalline cellulose hydrolysis. Biochemical mechanisms responsible for this enhancement will be probed. This will be useful in enhancing the rate of crystalline cellulose hydrolysis by cellulases, a rate-limiting step in the bioconversion of cellulose to glucose.

2. COE and ß-glucosidase compete for cellobiose formed from cellulose degradation. To understand the relative contributions of COE and ß-glucosidase reactions to cellobiose degradation, ß-glucosidase will be purified and its kinetics for reaction with cellobiose will be compared with that of COE kinetics for cellobiose oxidation.

3. COE inhibit lignin peroxidase reactions; mechanisms of this inhibition and the relevance to lignin-degradation will be examined.

4. CDH, a hemoflavoenzyme, contains a heme b and a FAD per molecule. FAD appears to be responsible for dehydrogenating cellobiose to cellobionolactone, whereas the heme appears to be responsible for receiving electrons from the reduced FAD and then transferring it to other electron

acceptors. To further understand this electron transfer mechanism, the redox potentials of heme and flavin will be determined spectroelectrochemically.

5. The heme structure of CBO will be elucidated by resonance Raman and EPR spectroscopic techniques.

Oregon State University
Corvallis, OR 97331

151. Catalytic Mechanism of Hydrogenase from Aerobic N_2-Fixing Microorganisms
D.J. Arp, Laboratory for Nitrogen Fixation Research
$142,000 (FY 91 Funds/2 years)

We are investigating the catalytic mechanism of the hydrogenase from the aerobic, N_2-fixing microorganism, *Azotobacter vinelandii.* This enzyme efficiently recycles the H_2 evolved by nitrogenase. Several properties of this hydrogenase (e.g., a very low rate of the back reaction, H_2 evolution, and a low Km for H_2) make it ideal to function in an environment in which all of the available substrate is generated *in situ.* This enzyme is a Ni- and Fe-containing dimer with subunits of molecular weight 65,000 and 35,000. This metal content and subunit composition are typical of a large group of H_2 oxidizing hydrogenases. We have focused on the catalytic functions of this enzyme and are combining three approaches to understanding how the enzyme functions. First, we are characterizing the mechanisms of a number of inhibitors and inactivators of this hydrogenase including C_2H_2, O_2, CO, NO, Cu^{++} and HCN. Characterizations include considerations of the competitive nature of the inhibitor, the time-dependence of the inhibition, and the dependence on the redox state of the enzyme. Second, we are using EPR and UV-vis spectroscopy to characterize the various inhibited and redox states of the enzyme with a view towards identifying the redox centers in the enzyme and their roles in catalysis. Third, we are using site-directed mutagenesis to study the roles of a number of amino acid residues (cys and his) that are conserved throughout this class of hydrogenases. This system is particularly well-suited to these investigations because the enzyme is well-characterized at the biochemical level and because the bacterium is amenable to genetic transformation.

Oregon State University
Corvallis, OR 97331-3804

152. Analysis of Potyviral Processing: A Basis for Pathogen Derived Resistance?
W.G. Dougherty, Department of Microbiology *$192,000 (2 years)*

Members of the potato virus Y group are flexuous rod-shaped viruses that are important pathogens on most crop species. The single strand RNA genome is expressed as a polyprotein that undergoes co-and post-translational proteolytic processing. Three viral-encoded proteinases are involved in processing the genomic polyprotein. One of these proteinases is a part of the NIa polyprotein and is responsible for six cleavage events. We demonstrated this proteinase is a 240 amino acid protein and comprises the carboxyl-terminal half of the small nuclear inclusion [NIa] polyprotein. The proteinase has a *cis*, or auto catalytic, and *trans* or bi-molecular activities. The auto catalytic activity of the proteinase has been examined. Cleavage is rapid and the proteinase can cleave itself from all proteins tested. We also examined the *trans* activity of the 27kDa proteinase. We were interested in determining if flanking sequences would affect proteolytic activity. In general, regardless of the

polyprotein the NIa proteinase was a part of, it processed two different substrates with the same specificity and activity as the 27kDa proteinase alone. Collectively, the data suggest it will be difficult to interfere with the auto catalytic activity of the proteinase but bi-molecular processing events can be targets in a viral inhibition strategy.

University of Oregon
Eugene, OR 97403-1229

153. Transposon-Induced Nuclear Mutations that Alter Chloroplast Gene Expression
A. Barkan, Institute of Molecular Biology *$172,000 (FY 91 Funds/2 years)*

The biogenesis of chloroplasts is genetically complex, involving hundreds of genes distributed between the nucleus and organelle. In multicellular plants, developmental parameters confer an added layer complexity upon the genetic control of organelle biogenesis: the properties of plastids differ dramatically in different cell types. The long term goal of this project is to use mutant phenotypes as a guide to nuclear genes that determine the timing and localization of chloroplast development.

Studies are being conducted with nuclear mutants in maize that are defective in chloroplast translation or RNA metabolism. These mutants are green, but non-photosynthetic due to the absence of several of the enzymatic complexes involved in photosynthesis. To determine the molecular basis for the protein deficiencies, we analyzed the structure and abundance of chloroplast mRNAs, and the association of these mRNAs with ribosomes. In this way we have identified seven mutants whose protein deficiencies result from aberrant chloroplast translation or RNA metabolism. Three mutants exhibit unique alterations in chloroplast mRNA metabolism: *hcf*136 appears to be defective in a site-specific ribonuclease whose activity is required to generate translatable mRNAs from an inactive primary transcript. *hcf*142 seedlings lack the predominant *pet*A transcript, and therefore fail to synthesize or stabilize this mRNA. *hcf*143 over-accumulates several RNA species that normally accumulate to only low levels. This mutant may be defective in a factor that destabilizes specific RNAs. Four mutants have a general defect in chloroplast
translation initiation; these mutants are likely to lack components of the chloroplast translation machinery.

The mutations causing defects in chloroplast mRNA metabolism define genes with previously undescribed roles in modulating chloroplast gene expression. The precise roles of these genes will be investigated through further biochemical and genetic studies, molecular cloning of the normal alleles (by exploiting the transposon tag), and analyses of the normal and mutant gene products.

Pennsylvania State University
University Park, PA 16802

154. Rapid Regulatory Control of Plant Cell Expansion and Wall Relaxation
D.J. Cosgrove, Department of Biology *$88,000*

Plant cell expansive growth is controlled by biochemical processes that loosen the cell wall and induce stress relaxation, and consequently water uptake and wall expansion. This project is aimed at elucidating the physical, cellular and molecular mechanisms which control wall loosening and relaxation. High-resolution studies of cell expansion and wall relaxation indicate that both expansion

and relaxation exhibit complex dynamics indicative of a rapid feedback-controlled process. By perturbing growth rates with pressure sinusoids, we are able to find clear evidence for feedback, resonance, harmonics and period doublings in the growth responses to these perturbations. We are also analyzing the spontaneous variations (spatial and temporal) in growth rate and relaxation to characterize the dynamics of this feedback. Alterations by chemical agents, water stress and genetic loci that influence cell growth are being studied to give us clues about the cellular and molecular elements of this feedback. Our results require two surprising additions to the conventional model of plant cell growth, namely there must be a growth-rate sensor which integrates growth over 1 min or less, and also a means to alter wall relaxation rapidly - within 2-3 min in some plants - in response to the signal from the growth-rate sensor. Further work is being directed at identifying the nature of the growth rate sensor and its signal. Our working model invokes stretch-activated ion channels in the plasma membrane as the growth rate sensor and cytoplasmic calcium as one component of the signal from the sensor.

Pennsylvania State University
University Park, PA 16802

155. Role of Ca^{++}/Calmodulin in the Regulation of Microtubules in Higher Plants
R. Cyr, Department of Biology *$79,000*

The cytoskeleton, and in particular its microtubule (Mt) component, participates in several processes that directly affect growth and development in higher plants. Normal cytoskeletal function requires the precise and orderly arrangement of Mts into several cell cycle and developmentally specific arrays. One of these, the cortical array, is notable for its role in somehow directing the deposition of cellulose, the most prominent polymer in the biosphere. Unfortunately, little molecular information is available regarding the formation of these arrays, or the cellular signals to which they respond. It is therefore important to acquire information regarding the molecules which regulate Mts within the different arrays. Experimental data has been obtained to suggest that plant cells use calcium, in the form of a Ca^{++}/calmodulin complex, to affect the dynamics of Mts within the cortical array. Owing to the importance of Ca^{++} as a regulatory ion in higher plants we are probing for a putative Ca^{++}/Mt transduction pathway which may serve to integrate Mt activities within the growing and developing plant cell. To aid in our investigations we are using a lysed cell model, (made from carrot protoplasts) that permits functional assays to be performed upon cortical Mts. We are also using an *in vitro* Mt assembly assay to investigate the effect of calcium/calmodulin and calmodulin binding proteins on Mt-dynamics. We are also in the process of identifying and characterizing membrane proteins that bind Mts to investigate if Ca^{++}/calmodulin affects their ability to interact with Mts. The information gained in these studies will be useful in understanding how developmentally important signals are transduced into morphogenic events during plant growth and development.

Pennsylvania State University
University Park, PA 16802

156. Characterization of Lignin and Mn Peroxidases from Phanerochaete chrysosporium
M. Tien, Department of Molecular and Cell Biology *$100,000*

Lignin and Mn peroxidases are extracellular lignin-degrading enzymes secreted by the wood-degrading fungus *Phanerochaete chrysosporium.* These two enzyme catalyze the oxidation of lignin and lignin

model compounds via a free radical mechanism. The role of these two enzymes and their isozymes in lignin biodegradation is the focus of our research. We are addressing this question by characterizing the enzymology and regulation of these isozymes. Although highly homologous in structure and catalytic activities, these isozymes are differentially regulated. This suggests that they serve different roles in lignin biodegradation. We have isolated the cDNAs encoding these isozymes and have been able to express these isozymes in active form in baculovirus. Heterologous expression has allowed us to continue our mechanistic and structural studies. These studies are aimed at determining the structural aspects which confer these peroxidases with their unique reactivities.

University of Pennsylvania
Philadelphia, PA 19104

157. Circadian Rhythms in CAB Gene Expression
A.R. Cashmore, Plant Science Institute, Department of Biology *$122,500*

Many biological systems exhibit daily rhythms. Some of these phenomena respond not simply to diurnal cues, but are driven by an endogenous clock. An example of such a phenomena is the rhythmic expression of CAB genes exhibited in many plant species. Here, it has been observed that there are pronounced diurnal changes in levels of expression, with maximum levels observed around midday and minimum expression occurring at approximately midnight. This rhythmic expression does not simply reflect the light dependence of CAB gene expression, as continued cyclical expression is observed if plants are kept for several "days" in darkness.

We have previously demonstrated that this rhythmic expression in CAB gene expression--first observed for steady-state RNA--reflects pronounced changes in the level of transcription. In order to further our understanding of the molecular basis of this transcriptional response, we are continuing our studies of the promoter elements of the *cab-E* gene from *Nicotiana plumbaginifolia.* Of primary interest in these studies are experiments aimed at determining the minimal element required to mediate cyclical expression and whether or not this element is sufficient to mediate light-induced transcription. Related studies are concerned with characterizing DNA binding proteins that bind to these regulatory elements.

In our studies of the cis-acting regulatory elements we have primarily depended on the use of transgenic plants. We will explore the prospect that transient assays using particle bombardment may be a convenient and complementary approach to the study of this system.

University of Pennsylvania
Philadelphia, Pa 19104-6018

158. Membrane-Attached Electron Carriers in Photosynthesis and Respiration
F. Daldal, Department of Biology *$205,635 (FY 91 Funds/2 years)*

The overall aim of this project is to contribute to a clear and complete understanding of the various energy transduction pathways that operate during respiration and photosynthesis. Our model system is that of the facultative photosynthetic bacterium *Rhodobacter capsulatus* which provides an excellent experimental system for multi-disciplinary approaches. In this organism, electron transfer during

photosynthetic growth had been thought to proceed only via a cyclic pathway through the membrane-bound cytochrome (cyt) bc_1 complex to the photochemical reaction center, mediated by the soluble cyt c_2. Our previous genetic and biochemical experiments involving the deletion of the gene encoding this protein have firmly established that both respiratory and photosynthetic growth continues in the absence of the soluble cyt c_2. Electrons are still transferred from the cyt bc_1 complex to the reaction center by an electron carrier of unknown nature. Using a genetic approach based on the complementation of Ps^- mutants lacking cyt c_2 we have now revealed that a new membrane-associated cytochrome, called cyt c_y, structurally distinct from, an yet functionally similar to, cyt c_2 operates as such an electron carrier. Studies are in progress to characterize the structure of this gene, its product and its biochemical properties as electron donor. Mutants lacking either cyt c_2 or cyt c_y are being constructed to dissect and analyze the physiological role and the properties of the "soluble carrier-dependent" (i.e., via cyt c_2) and soluble carrier-independent (i.e., via cyt c_y) electron transfer pathways both in respiration and in photosynthesis.

University of Pennsylvania
Philadelphia, PA 19104-6018

159. Transport Function and Reaction Mechanism of Vacuolar H^+-Translocating Inorganic Pyrophosphatase

P.A. Rea, Department of Biology *$171,998 (FY 91 Funds/2 years)*

The vacuolar membrane of plant cells contains two primary H^+ pumps: a H^+-ATPase and a H^+-translocating inorganic pyrophosphatase (H^+-PPase). Both enzymes catalyze inward, electrogenic H^+-translocation (from cytosol to vacuole lumen) to establish a H^+-electrochemical gradient across the vacuolar membrane capable of acting as energy source for the secondary, H^+-coupled transport of a wide range of solutes.

Our research program is directed at defining the transport capabilities and reaction mechanism of the vacuolar H^+-PPase through biochemical analyses of native membrane vesicles isolated from *Vigna radiata* - one of the richest known sources of the enzyme - and by reconstitution of the isolated enzyme into artificial membranes of low background ionic conductance. Towards this end, we have optimized procedures for purification of the enzyme from *Vigna*, inserted it into artificial liposomes and reconstituted its capacity for MgPPi-energized H^+-translocation. In the light of the results of recent patch clamp studies, performed in collaboration with Dr. Dale Sanders (University of York, England), which show that the H^+-PPase is vectorially activated by extravacuolar K^+ and the reversal potential of the pump is critically dependent on the transtonoplast K^+ concentration ratio, experiments are being conducted on the reconstituted preparation to determine its capacity for direct MgPPi-energized K^+-translocation. The basic question of why there should be two transport systems (the ATPase and PPase) pumping the same ion (H^+) into the same intracellular compartment may be reconcilable if rather than simply acting as a supplementary H^+ pump, the PPase serves also to translocate K^+ into the vacuole.

Current reaction mechanistic studies center on the identification of sequence motifs implicated in substrate-binding - on the basis of sequence and topographic data derived from our recent molecular cloning of the genes encoding the catalytic subunit of the H^+-PPase from *Arabidopsis thaliana* and *Beta vulgaris* - through the development of strategies for selective cleavage of the M_r 66,000 polypeptide and the mapping of affinity ligand-modified peptide fragments.

H^+-translocating PPases have been identified in the energy-coupling membranes of mitochondria, chloroplasts and bacteria but the vacuolar H^+-PPase is the first example of a PPi-dependent H^+-translocase in a "non-energy" coupling membrane. In view of the apparent ubiquity of the H^+ the H^+-PPase in the vacuolar membrane of plant cells, the unique status of PPi as the limiting case of a high energy phosphate and the increasing recognition of PPi as a key metabolite in plant cells, it is expected that many of the conclusions stemming from this work will be of broad bioenergetic applicability.

Purdue University
West Lafayette, IN 47907

160. Crystallographic Studies of Nitrogenase and Hydrogenase
J.T. Bolin, Department of Biological Sciences *$202,761 (2 years)*

The ultimate objective of this project is to determine and analyze the crystal structures of enzymes that play key roles in microbiological metabolic processes significantly related to the production and consumption of energy resources. The project will focus on the bacterial enzymes nitrogenase and hydrogenase. Nitrogenase is the multi-protein bio-catalyst responsible for the conversion of dinitrogen to ammonia, the central reaction in biological nitrogen fixation and a key process in the global nitrogen cycle. Knowledge of the structure of nitrogenase is a necessary component of any attempt to understand the biochemical mechanism by which ammonia is produced, and thus is a crucial step in attempts to enhance biological nitrogen or transfer the chemistry of the enzyme to non-biological processes. An immediate goal of this project is to determine the crystal structure of MoFe-protein, the catalytic component of nitrogenase, at atomic resolution. Initially, the structure of the wild-type enzyme will be determined and analyzed with respect to a large body of biochemical and biophysical data. Subsequently, the structures of mutant proteins that demonstrate altered functional properties will be examined. Hydrogenases are a diverse group of metalloenzymes that catalyze an oxidation-reduction reaction which evolves or consumes molecular hydrogen. Enzymes from this group are involved in a number of significant metabolic processes including nitrogen fixation, carbon fixation, and methanogenesis. In this case, determination of the crystal structure of a Clostridial, all-Fe hydrogenase is the first major goal. This study will provide the first opportunity to examine the three-dimensional structure of any hydrogenase.

Purdue University
West Lafayette, IN 47907-1155

161. Structure and Biosynthesis of the Mixed-linkage ß-D-Glucan of Grasses
N.C. Carpita, Department of Botany and Plant Pathology
$166,000 (FY 91 Funds/2 years)

Membranes of the Golgi apparatus from maize (Zea mays L.) are used to synthesize *in vitro* the mixed linkage (1→3,1→4)-ß-D-glucan that is unique to the cell wall of the Poaceae. Activated charcoal, added to the membrane preparation before sucrose density gradient centrifugation, eliminated interference by endogenous phenolic compounds and stimulated synthesis of (1→3,1→4)-ß-D-glucan by several fold in a fraction enriched with endoplasmic reticulum and Golgi apparatus. Synthesis of

this macromolecule was proven by several analytical techniques. The (1→3,1→4)-ß-D-glucan was separated from (1→3)-ß-D-glucan (callose) by gel-permeation chromatography on Sepharose 4B. The peak molecular mass was about 250 kDa, similar to (1→3,1→4)-ß-D-glucan purified from barley bran. Separation by gas-liquid chromatography of partly methylated alditol acetate derivatives of the reaction product and detection of radioactivity in each derivative using gas proportional counting showed (1→3)- and (1→4)-linked glucosyl units. Oligosaccharides, released by an endo-(1→4)-ß-D-glucanase (E.C.3.2.1.73) from *Bacillus subtilis*, were separated by high pH anion exchange-high performance liquid chromatography, and the principal radioactive products co-eluted with the trisaccharide, ß-D-Glc*p*-(1→4)-ß-D-Glc*p*-(1→3)-D-Glc, and the tetrasaccharide [ß-D-Glc*p*-(1→4)]$_2$-ß-D-Glc*p*-(1→3)-D-Glc. The pentamer, [ß-D-Glc*p*-(1→4)]$_3$-ß-D-Glc*p*-(1→3)-D-Glc, and hexamer [ß-D-Glc*p*-(1→4)]$_4$-ß-D-Glc*p*-(1→3)-D-Glc were also synthesized in proportions similar to those found in purified (1→3,1→4)-ß-D-glucan. These and other data verified the *in vitro* synthesis of macromolecular, mixed-linkage (1→3,1→4)-ß-D-glucan. Work now is devoted to characterization of the synthesis of the ß-D-glucan on membranes of the isolated Golgi apparatus, and to establish facile means to partially solubilize the synthase in zwitterionic detergents and preserve the faithful synthesis of the mixed-linked ß-D-glucan. Preliminary results indicate that the synthase, like cellulose synthase, may be converted to a synthase capable only of the synthesis of (1→3)-ß-D-linked glucosyl units.

Purdue University
West Lafayette, IN 47907

162. Ca^{++} Gated Proton Fluxes in Energy Transducing Membranes
R.A. Dilley, Department of Biological Sciences *$80,000*

Our research on chloroplast bioenergetics focuses on the proton electrochemical potential gradient that forms as an intermediary energy storage in, or across, the thylakoid membrane, during the conversion of absorbed sunlight energy into the chemical energy forms needed for plant life. The proton gradient-akin to a battery but powered by protons (H^+ rather than electrons-drives the energy-requiring synthesis of adenosine triphosphate (ATP) as the protons flow down the energy gradient, through a special membrane protein complex that "couples" the energy-releasing H^+ flow to the energy-requiring ATP formation reaction. The molecular mechanisms for H^+ ion movement in and through membranes are not understood, and represent an important, unsolved question in biology.

We have evidence that the proton flow through the energy coupling complex is a "gated" flux, with calcium ions providing part of the mechanism for switching the H^+ flux gate between the open or closed condition. It appears that when calcium ions are tightly bound to the 8 kDa subunit of the H^+ channel part of the coupling complex, the H^+ stays within localized domains, but when the calcium ions are displaced, the H^+ ions flow out of the postulated gate into the inner volume of the thylakoid and form a proton gradient over a larger volume, thus the term delocalized proton gradient is used. We are studying the biochemical parameters that control the calcium gating action on the H^+ gradients.

Another question we are investigating concerns learning how the proteins associated with the putative Ca^{++}-binding/gating site are arranged to produce the H^+ flux control gate. Two membrane-associated proteins are implicated in the gating function of 6 kDa and 8 kDa molecular mass.

Purdue University
West Lafayette, IN 47907

163. Analysis of the PSII Proteins MSP and CP43
L.A. Sherman, Department of Biological Sciences *$178,000 (2 years)*

The major objectives of this project are to analyze gene regulation under different environmental conditions and to determine the role of the *psbO* protein (MSP, the manganese stabilizing protein, the 33 kDa protein) in O_2- evolution. These objectives are studied in the transformable cyanobacteria *Synechococcus* sp. PCC7942 and *Synechocystis* sp. PCC6803, respectively. We have produced a deletion strain (Δ*psbO*) in *Synechocystis* that completely lacks the gene or the gene product, and which can grow photosynthetically at about 2/3 the normal rate. We have used this deletion strain to construct site-directed mutations at specific externally-located, charged residues. In particular, we are analyzing mutations made at residues 9, 20, 91-96 and 159 using thermoluminescence and O_2-flash yield procedures.

We have identified and cloned the gene which codes for the novel chlorophyll-protein complex which is synthesized during iron deficiency. The apoprotein, *isiA*, has an amino acid sequence very similar to that of the gene product of *psbC* (CP43) except for the lack of most of the large lumenal loop E. We have found that an insertion mutant in *isiA* can grow normally in regular iron-sufficient medium and in partially iron-deficient cultures. We will use this mutant and others to investigate the chlorophyll-protein complexes in regular and iron-deficient membranes and how the cell regulated its response to stresses such as iron-deficiency.

Rice University
Houston, TX 77251

164. Genetic and Biochemical Analysis of Solvent Formation in Clostridium acetobutylicum
G.N. Bennett, Department of Biochemistry and Cell Biology *$180,000 (2 years)*

The anaerobic organism *Clostridium acetobutylicum* has been used for commercial production of important organic solvents due to its ability to convert a wide variety of crude substrates to acids and alcohols in high yield. Current knowledge concerning the molecular genetics, cell regulation and metabolic engineering of this organism is still rather limited. The objectives of the research are to improve our knowledge of the molecular genetics and enzymology of Clostridia that will be beneficial toward generating genetic alterations of cell metabolism for a variety of applications. Two factors that limit butanol production in continuous culture are: 1) the degeneration of the culture, with an increase in the proportion of cells which are incapable of solvent production. The molecular mechanism of degeneration will be analyzed to determine if it is due to a genetic loss of solvent related genes. 2) The production of hydrogen which uses up reducing equivalents in the cell. If the reducing power could be more fully directed to the reduction reactions involved in butanol production the process would be more efficient. We propose to analyze the enzymatic properties of the hydrogenase and to clone a gene encoding a hydrogenase subunit.

Rutgers University
New Brunswick, NJ 08903-0231

165. Cellulase - A Key Enzyme for Fermentation Feedstocks
D.E. Eveleigh, Department of Biochemistry and Microbiology *$69,993*

Feedstock chemicals can be obtained from biomasss, cellulose forming the prime substrate with regard to its abundance. Cellulose would be best utilized through fermentation, but must first be converted to sugars. For this purpose we have been studying the cellulase of a thermophilic bacterium, *Microbispora bispora*. This actinomycete produces a cellulase that is effective towards crystalline cellulose, is thermally stable and is comprised of endo- and exo-splitting glucanases and also beta-glucosidase. The latter is resistant to end-product inhibition which makes it potentially attractive for industrial application. We find two beta-glucosidases - two distinct genes and gene products. The latter differ in their substrate specificities. These genes are being sequenced in order to clarify understanding of mechanism of action of the enzyme. *M. bispora* additionally produces an exo-splitting glucanase, to date a relatively rare bacterial enzyme. It is important as it is synergistic with endo-glucanases in its action towards crystalline cellulose. Through cloning one cellobiohydrolase has been gained, selected through the use of a sensitive monoclonal antibody assay. The basic characterization of the cellobiohydrolase produced in *E. coli* has been accomplished. Yields were low, but through cloning into *Streptomyces lividans* as a host, an increase in yield of 10-15 fold was gained. The Streptomycete cloned enzyme is slightly larger than that from *E. coli* probably due to glycosylation, and it is also more thermally stable. Its gene sequence has been determined. Such sequences allow evolutionary analysis of cellulase genes - 60 sequences are now known. Our analysis of them indicates that the domains (binding and catalytic) could have evolved independently, and later merged. The application of these unique cellobiohydrolases and beta-glucosidases is being improved and should considerably aid in total cellulolysis.

Rutgers University
New Brunswick, NJ 08903

166. The Role of Alternative Respiration in Plants
I. Raskin, AgBiotech Center *$154,000 (2 years)*

Plants are generally considered poikilotherms (organisms whose temperature is determined by the environment). Thermogenic plants, which can generate large amounts of heat in their inflorescence via the cyanide-insensitive or alternative respiratory pathway (AP), are the only accepted deviation from this rule. In partial challenge to this view, we propose that, while most plants do not effectively thermoregulate at the organismic level, they still do so at the mitochondrial level. This implies that the difference in heat production and thermoregulation between so-called "thermogenic" and "non-thermogenic" plants is quantitative rather than qualitative. We suggest that the increased "heating" of the inner mitochondrial membrane at low temperatures is accomplished by a larger electron flux through the AP, which dissipates most of the energy stored in the respiratory substrates as heat. This localized thermoregulation counteracts the deleterious effects of chilling on the fluidity of the mitochondrial membrane, which determines the activity of the respiratory enzymes vital for plants. We will use a state-of-the-art heat-conductance microcalorimeter, adapted for use with biological samples, to measure AP activity and heat evolution. We will also study the regulation of the AP activity by endogenous and exogenous salicylic acid.

Rutgers University
Piscataway, NJ 08855-0759

167. Corn Storage Protein: A Molecular Genetic Model
J. Messing, Waksman Institute *$212,000 (2 years)*

Our studies of regulation of gene expression are focused on raising the methionine storage in the maize kernel. Most of its methionine accumulated in the endosperm during development of the kernel due to the expression of the 10kD zein gene (chromosome 10) with 23% of its codons methionine. An interesting *trans*-acting factor in BSSS53 (chromosome 4), called Zpr10/(22), that is either absent or suppressed in most inbreds is able to cause overexpression of the 10 kDa zein. In its unsuppressed state, it does not change the transcriptional rate of the 10 kDa zein gene, but raises the accumulation of 10 kDa mRNA which in turn leads to the accumulation of the 10 kDa protein. This increased accumulation simply serves as a sink for free methionine that otherwise would be lost when seeds are dried and stored. For the first time, it was possible to demonstrate in a feeding trial that the high methionine corn can serve as a protein source and can substitute free methionine supplements. Since in most crosses overexpression of 10 kDa zein is suppressed, it now becomes very attractive to dissect the various *cis*- acting elements of its and other zein genes to combine them in a novel ways leading to a stably transmitted overexpression phenotype. A critical step towards the biochemical analysis of these genes was achieved by developing a homologous expression system based on immature endosperm tissue that remains differentiated in culture and expresses tissue-specific genes.

Salk Institute for Biological Studies
San Diego, CA 92186-5800

168. Signal Transduction Pathways that Regulate CAB Gene Expression.
J. Chory, Plant Biology Laboratory *$211,000 (2 years)*

The process of greening, or chloroplasts differentiation, involves the coordinate regulation of many nuclear and chloroplast genes. The cues for the initiation of this developmental program are both extrinsic (e.g., light) and intrinsic (cell-type and plastid signals). Several regulatory photoreceptors are involved in the perception of light signals; however, the exact mechanisms by which light and other signals are perceived by plant cells and converted into physiological responses are not understood.

The proposed research program focuses on the genetic, biochemical, and molecular characterization of new *Arabidopsis thaliana* mutants that have been previously isolated in our laboratory. These mutants were identified using a novel molecular genetic approach in which we identified mutations by aberrant gene expression patterns, rather that by predicted phenotype. We chose the nuclear-encoded chlorophyll a/b binding protein promoter (*cab3)* of *Arabidopsis* as an indicator of light-regulated developmental gene expression. We have utilized *cab3* promoter-marker gene chimeras to select for new mutants in which the *cab3* promoter is aberrantly expressed with respect to light, tissue-specificity of expression, and in response to signals from the chloroplast. Most information is available for mutants that fall into two classes: (1) *doc (**d**ark **o**verexpression of **c**ab)* mutants in which there are elevated levels of *cab* gene expression when the seedlings are germinated in the dark; and (2) *gun* (*genomes **u**ncoupled)* mutants in which the expression of *cab* genes has become uncoupled from the expression of photosynthetic chloroplast genes. Detailed biochemical and genetical characterization of these regulatory mutants is in progress. Based on the results from the genetic and physiological studies, we will choose one *DOC* and one *GUN* gene for further molecular analysis.

Scripps Research Institute
La Jolla, CA 92037

169. Genetic Engineering with a Gene Encoding a Soybean Storage Protein
R.N. Beachy, Department of Cell Biology *$180,000 (FY 91 Funds/2 years)*

The ß-conglycinins are soybean storage proteins encoded by genes that are tightly regulated both temporally and spatially. During studies to characterize the *cis* DNA sequence elements we identified several sequences that are responsible for maintaining the expected degree of promoter regulation in transgenic petunia and tobacco (*Nicotiana tabacum* and *N. benthamiana*) plants. These studies were carried out in the context of several different core promoters and upstream regulating sequences. During these studies we identified DNA sequences that were essential to maintaining spatially and temporally regulated expression of the ß-conglycinin promoters. The sequences CATGCAC and CATGCAT were determined to be important to both quantitative aspects of gene expression and for maintaining tissue specificity of gene expression. It was apparent from the studies that these and other sequence elements are influenced by other contextual sequence components and the core promoter used for the analyses.

We are currently attempting to isolate the proteins and the corresponding cDNAs that represent the Soybean Embryo Factors (i.e., SEF proteins) that bind upstream sequences in the α and ß-subunit genes of ß-conglycinin. To date no *trans* factors have been found that bind the CATGCAC/T sequences, and we are continuing studies to identify the role of these sequences to limit the expression of the ß-conglycinin genes to seeds in transgenic *Nicotiana* spp. and in *Arabidopsis*.

University of South Carolina
Columbia, SC 29208

170. Exploration of New Perspectives and Limitations in Agrobacterium-Mediated Gene Transfer Technology
L. Márton, Department of Biological Sciences *$200,000 (2 years)*

Motivated by the demand for controlled genetic manipulation of plants, we have focused on further application of *Agrobacterium* mediated gene transfer (AMGT) technology, specially considering the application for gene targeting, homologous gene replacement. The objective of this project is the development of a special Agrobacterium binary vector and corresponding plant recipient system for the elucidation of interrelations of homologous recombination, insertion, mutagenesis by the T-DNA and AMGT-related mutagenesis events which all could be scored in the same experiment.

The vector will carry the *Arabidopsis nia2* major nitrate reductase apoenzyme (NR) gene as a source of homology interrupted by a kanamycin resistance gene. An *Arabidopsis* recipient heterozygous for the *chl3-5* deletion allele (G5 X RLD) will be used in the AMGT experiments. Due to its hemizygous nature for the *nia2* gene there will be a single target for gene targeting and mutagenesis.

Our facile *Arabidopsis* transformation protocol of sustained root culture and the possibility of positive selection for the NR-deficiency allow the practically unlimited production of the desired genotypes. The appropriateness of different negative selection genes for enrichment of homologous recombination as well as the molecular mechanisms involved in the AMGT-related mutagenesis process are addressed as well.

We provide a blueprint for analogous approaches to altering other genes of interest.

Southern Illinois University
Carbondale, IL 62901

171. Regulation of Alcohol Fermentation by Escherichia coli
D.P. Clark, Department of Microbiology *$172,000 (FY 91 Funds/2 years)*

The purpose of this project is to elucidate the way in which the synthesis of ethanol and related fermentation products are regulated in the facultative anaerobe *Escherichia coli.* We are also investigating the roles of certain genes which are induced during fermentative growth. Structural and regulatory mutations affecting the expression of the fermentation alcohol dehydrogenase have been isolated. The *adh* structural gene has been cloned and sequenced; at present the upstream sequences responsible for anaerobic induction are being characterized by means of gene fusion. The build up of reduced NADH during anaerobic fermentative conditions appears to play a major role in regulating the *adh* gene. The upstream regulatory region is presently being dissected by the PCR approach. The protein encoded by the *adh* gene expresses both alcohol and acetaldehyde dehydrogenase activities. In addition it acts as the deactivase for pyruvate formate lyase and is thus a trifunctional enzyme. The N-terminal region is homologous to other aldehyde dehydrogenases and the C-terminus to the family of Fe activated alcohol dehydrogenases. Further delineation of the structure-function relationships of the ADH protein is being performed by the polymerase chain reactions (PCR) approach. The PCR method is also being used to generate hybrids between the *adh* genes of *E. coli* and related gram negative bacteria. The kinetics of hybrid enzymes will be assessed and correlated with sequence alterations. The *ldh* gene, encoding the fermentative lactate dehydrogenase has also been cloned and sequencing is in progress. Regulatory mutations which affect the expression of LDH are also available. Many anaerobically induced gene fusions can be switched on in air in the presence of cyanide or chelating agents. the possibility of an iron containing regulatory protein is being investigated.

Stanford University
Stanford, CA 94305-5025

172. The Effect of Oligosaccharides on Glycoprotein Stability
C.F. Goochee, Department of Chemical Engineering *$187,000 (2 years)*

The oligosaccharides of glycoproteins frequently promote resistance to irreversible thermal denaturation. The oligosaccharides could affect resistance to thermal denaturation by three independent mechanisms:

1. by affecting the equilibrium toward the folded state and reversibly unfolded states
2. by affecting the kinetics of protein unfolding and/or refolding
3. by reducing the rate of formation of irreversibly unfolded protein

The long-term goal of this project is to clarify the importance of each of these mechanisms. As a first step, we are developing a new model glycoprotein system amenable to site-directed mutagenesis to introduce glycosylation sites at selected locations on the protein surface. Our immediate experimental focus is on the expression and secretion of staphylococcal nuclease (SNase) in *Saccharomyces cerevisiae.* The SNase protein is a small 16.5 kDa protein that has a distinguished history of use as a model system for studying protein folding and unfolding events. It is likely that introduction of a

single Asn-X-Ser/Thr sequence will result (in at least some cases) in the synthesis by *Saccharomyces* of a large mannan-type oligosaccharide structure, raising the molecular weight of the resulting SNase glycoprotein to 25-30 kDa and permitting definitive conclusions concerning the effect of oligosaccharide on protein stability. The SNase glycoprotein with smaller oligosaccharides could subsequently be generated by enzymatic reduction of carbohydrate size or by glycoprotein expression in another fungal system. With SNase glycoproteins(s) in hand, we will examine the effect of oligosaccharides on the equilibrium between the folded and unfolded protein states (mechanism 1 above).

Stanford University
Stanford CA 94305-5020

173. Nodulation Genes and Factors in the Rhizobium-Legume Symbiosis
S.R. Long, Department of Biological Sciences *$246,726*

We study the symbiosis between *Rhizobium* bacteria and legume plants a major nitrogen fixation system important for conservation of petrochemicals in agriculture. The interaction is highly specific, in that particular species of bacteria form nodules only on certain plants: we study *Rhizobium meliloti* and its host, alfalfa. We are analyzing bacterial signals that cause the plant to form the root nodules, and in the mechanism of the plant response. We have isolated Nod factors and characterized them by mass spectrometry and other methods; their structure conforms to the general finding in many labs that factors are modified oligomers of N-acetyl glucosamine. The *R. meliloti* factors contain a sulfate, and we have studied the role of three *nod* genes, *nodP, nodQ,* and *nodH,* in sulfation. We have obtained *in vitro* activity for the major steps: activation of sulfate, and transfer to model oligosaccharides. Among the surprising results are that NodP and NodQ carry out not only ATP sulfurylation, but also APS kinase activity and that the process requires GTP. Our *in vitro* demonstration of NodH activity may have significance for other studies of carbohydrate sulfation. We are studying the plant reaction by developing assays that work in single root hairs. We have established that root hairs depolarize their cell membrane potential in response to Nod factors. As a means to further analysis, we have recently developed techniques that allow injection of single root hairs with marker dyes and fluorescently tagged proteins.

Stanford University
Stanford, CA 94305

174. Enzymology and Molecular Biology of Cell Wall Biosynthesis
P.M. Ray, Department of Biological Sciences *$195,000 (FY 91 Funds/2 years)*

Our objective is to identify plant cell wall polysaccharide synthases and clone the corresponding genes, so that the hormonal regulation of cell wall synthesis in relation to plant growth can be studied. Golgi-localized, auxin-regulated ß-1,4-glucan synthase (glucan synthase-I or GS-I), which produces the glucan backbone of xyloglucan, and plasma membrane-associated, Ca^{2+}-regulated ß-1,3-glucan synthase (GS-II), which synthesizes callose (as a wound response), are currently targeted. Upon glycerol gradient fractionation either of detergent-solubilized crude membranes, or of purified Golgi (for GS-I) or plasma membranes (for GS-II), certain polypeptides correlate with the respective GS activities, in pea. Our immediate goals are to identify by immunological, covalent labeling, and other methods, which of these polypeptides may be involved in GS activity, and to obtain from them amino acid sequences that can be used for gene cloning.

One such, a 55 kDa polypeptide that correlates with GS-II, copurifies with GS-II activity during isoelectric focussing and by product entrapment, wherein it becomes trapped within centrifugally pelletable glucan micelles produced by the GS-II reaction. Product entrapment eliminates a 68 kDa polypeptide that correlates with GS-II in glycerol gradients, as well as numerous other polypeptides that occur in GS-II-containing gradient fractions. The 55 kDa polypeptide can be labeled by UV irradiation of plasma membranes in the presence of α-[^{32}P]UDP-glucose, and an antiserum against it adsorbs GS-II activity. Attempts to sequence the 55 obtained from plasma membrane protein gels were initially frustrated because its N terminus is apparently blocked. From a tryptic peptide of the 55 we obtained a limited sequence that corresponds to no known sequence in the GenBank data base. However, various indications have caused us to suspect that the 55 kDa band in plasma membrane gels contains some polypeptide(s) irrelevant to GS-II activity. We are preparing to obtain more reliable sequence information for GS-II by using the 55 obtained from product entrapment, a more difficult undertaking because of the limited yield of protein from this procedure.

Antiserum raised against one of the polypetides (52 kDa) that correlate with GS-I activity during glycerol gradient fractionation did not yield evidence for its participation in GS activity. Before attempting to raise antibodies against, or sequence, other of the GS-I-correlated polypeptides, we shall try to find a method for further fractionating Golgi proteins (product entrapment does not work for GS-I) to narrow down the number of candidate polypeptides that must be examined. Attempts will also be made to obtain sequence information for the members of the Golgi-localized 40 kDa doublet that can be labeled with UDP-[^{14}C]glucose under GS-I assay conditions and may be part of the GS-I system; these proteins can be isolated by ion exchange chromatography and are therefore accessible, although with difficulty because of their low abundance.

University of Tennessee
Knoxville, TN 37996

175. Plant Recognition of Bradyrhizobium japonicum Nod Factors
G. Stacey, Microbiology Department *$172,000 (2 years)*

We are studying the nitrogen-fixing symbiosis between *Bradyrhizobium japonicum* and soybean. This system is a useful model for the study of plant-microbe interactions, especially with regard to the exchange of signal molecules. The development of nitrogen-fixing nodules on soybean is a complex process requiring the coordinate regulation of both plant and bacterial functions. This regulation is mediated, in part, by the exchange of signal molecules between the symbiotic partners. One such molecule produced by the bacterium is a substituted lipo-oligosaccharide that induces many of the early nodulation responses of the plant. We have identified the major lipo-oligosaccharide signal produced by *B. japonicum* and are continuing to characterize the full repertoire of such compounds produced by this bacterium. Recently, we have obtained evidence that the spectrum of signals produced by a particular *B. japonicum* strain is important for its interaction with specific soybean cultivars. We wish to further explore this finding and elucidate the mechanisms involved. The elucidation of the chemical structures of the active signal molecules produced by *B. japonicum* will be important for further studies to define the essential elements required for plant recognition. Our eventual goal is to elucidate the complete signal transduction pathway involved in the soybean nodulation response. Detailed knowledge of legume symbioses is important for possible extension of biological nitrogen fixation for energy conservation.

Texas A&M University
College Station, TX 77843-2128

176. Regulation of Chloroplast Number and DNA Synthesis in Higher Plants
J.E. Mullet, Department of Biochemistry and Biophysics *$155,332 (2 years)*

The long term goal of this research is to understand how chloroplast biogenesis is regulated in higher plants. This grant investigates how chloroplast number and DNA synthesis is regulated during chloroplast biogenesis in higher plant leaves. The ability of plant cells to accumulate large numbers of plastids is an important cell specialization. Chloroplast numbers increase from 10 to over 60 during biogenesis of mesophyll leaf cells. In addition, plastid numbers increase dramatically in starch storing cells of tubers and the endosperm in the form of amyloplasts. As a first step in elucidating the regulation of plastid number per mesophyll cell, mutagenized populations of Arabidopsis will be screened for variation in plastid number and DNA content. The early activation of plastid DNA synthesis will also be investigated. To elucidate how activation occurs, proteins associated with plastid DNA including the plastid DNA polymerase will be isolated and the corresponding genes characterized. *Cis* and *trans*-factors which regulate expression of these genes early in chloroplast biogenesis will be identified.

Texas Tech University
Lubbock, TX 79409

177. Characterization of a 1,4,-ß-D-glucan Synthase from Dictyostelium discoideum
R.L. Blanton, Department of Biological Sciences *$69,354*

The recent advances in understanding prokaryotic cellulose biosynthesis have not yet been matched in any eukaryote, although some aspects of the prokaryotic system have been shown to exist in higher plants. The cellular slime mold *Dictyostelium discoideum* holds great promise as a model organism for eukaryotic cellulose synthesis. It remains the only eukaryote with an *in vitro* assay for the cellulose synthase in which the product of the reaction has been extensively characterized. Additional advantages of *D. discoideum* include the inducibility of cellulose synthesis and the ability to apply methods such as gene disruption to the cellulose synthesis problem.

In the past year we studied the induction of cellulose synthesis and found that in cell monolayer cultures both cAMP and differentiating-inducing factor (DIF, the stalk cell morphogen) were required for maximal cellulose synthase activity. We developed a whole cell lysate assay that allowed the detection of cellulose synthase activity in small quantities of cells, helped us to determine that the cellulose synthase is most likely not activated by proteolysis, and will facilitate the search for soluble effectors. We continued to search for conditions that would result in higher activity in solubilized enzyme preparations. Additional screening procedures for isolating mutants deficient in cellulose synthesis were tested. In conjunction with other funded projects, we continued the characterization of potential antibody and nucleic acid probes for the cellulose synthase and studied the interactions between cellulose and *D. descoideum* extracellular matrix proteins.

Texas Tech University
Lubbock, TX 79409

178. The Interaction of Ferredoxin:NADP$^+$ Oxidoreductase (FNR) and Ferredoxin: Thioredoxin Reductase with Substrates

D.B. Knaff, Department of Chemistry and Biochemistry *$82,000*

Monoclonal antibodies against spinach ferredoxin:NADP$^+$ oxidoreductase (FNR) inhibited electron flow from NADPH to the non-physiological acceptor, 2,6-dichlorophenol indophenol (DCPIP) without inhibiting electron flow from NADPH to ferredoxin, NADP$^+$ binding or ferredoxin binding. These results suggest the presence of highly antigenic epitopes located at the site where DCPIP interacts with FNR, a site distinct from the binding sites for the two physiological substrates for FNR, ferredoxin and NADP$^+$. Complex formation between FNR and ferredoxin protects two FNR lysines (K18 and K153) against biotinylation and complex formation protects a region of FNR containing K33 and K35 from modification by S-DABITC. These lysines appear to constitute a portion of the ferredoxin-binding domain of FNR. Similar differential chemical modification studies, using taurine plus a water-soluble carbodiimide to modify carboxyl groups on ferredoxin, are being conducted to define the binding domain on ferredoxin for FNR. Cross-linking and immunological experiments have demonstrated that the ferredoxin binding site on glutamate synthase, a ferredoxin-dependent chloroplast enzyme, is similar to that on FNR. Chemical modification studies using phenylglyoxal and N-acetyl succinimide to identify arginine and lysine residues on glutamate synthase involved in binding ferredoxin are in progress. Resonance Raman, MCD and EPR spectroscopy have been used to establish the presence of a functional [3Fe-4S] cluster in spinach glutamate synthase and the Em values of this cluster and of the FMN and FAD groups of the enzyme have been measured to be -180 mV and -170 mV respectively. Spectroscopic characterization of the [4Fe-4S] cluster of thioredoxin reductase has also been carried out and evidence accumulated to support a structural rather than electron carrier role for the cluster in this ferredoxin-dependent chloroplast enzyme.

University of Utah
Salt Lake City, UT 84112

179. The Plant Mitochondrial mat-r Gene/nad1 Gene Complex

D.R. Wolstenholme, Department of Biology *$185,000 (2 years)*

The main objective of this project is to gain an understanding of the mechanism by which mature transcripts of a structurally complex plant mitochondrial gene, that for subunit 1 of the respiratory chain NADH dehydrogenase (*nad1*) are generated. The maize *nad1* gene consists of five exons. Exons B and C are jointed by a continuous group II intron, but this exon pair and exons A,D and E (each associated with partial group II intron sequences) are widely separated on the maize 570 kilobase pair mitochondrial genome. Production of mature *nad1* transcripts is postulated to involve trans-splicing (to create functional group II introns) followed by *cis*-splicing. A gene (*mat-r*) for a maturase-related protein is located in the intron upstream from the E exon: in soybean the *mat-r* gene-containing intron is continuous between exons D and E. Intron cDNA sequences will be examined to determine whether *mat-r* gene transcripts are edited and whether RNA editing might be essential for intron excision, and to elucidate the nature of intron component association during *trans*-splicing. Run-off transcripts of cDNAs will be employed to determine whether, *in vitro*, the soybean *mat-r* gene-containing intron can self-*trans*-splice, then self-*cis*-splice. Soybean and maize *mat-r* gene cDNAs will be used to

synthesize MAT-R proteins in *Escherichia coli* cells. These proteins will be assayed for RNA splicase and reverse transcriptase activities, and used to seek evidence for a mitochondrially located MAT-R protein.

Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

180. Enzymology of Acetone-Butanol-Isopropanol Formation
J.-S. Chen, Department of Anaerobic Microbiology *$85,621*

Acetone, butanol, and isopropanol (solvents) are important industrial chemicals or fuel additives. Several *Clostridium* species produce butanol as a major product. *C. beijerinckii* (also known as *C. butylicum*), which is used in this study, can produce all three compounds. Solvent production by clostridia involves a switch in metabolism from acid production to solvent production. The onset of active solvent production follows the expression of solvent-forming enzymes, which is triggered by certain environmental signals. The ratio of solvents produced is also affected by growth conditions. The objective of this project is to elucidate the mechanisms that control the product ratio and the expression of genes for solvent production. Our approach is to determine first the catalytic and structural properties of the solvent-forming enzymes, and then to use physiological and genetic means, based on the biochemical data, to elucidate the molecular mechanisms of regulation. We have examined enzymes for all solvent-forming reactions and also several related reactions. Our focus is now on (1) alcohol dehydrogenases (ADHs) that catalyze the formation of butanol, ethanol, and isopropanol, and (2) enzymes reacting with acetoacetyl-CoA that leads to either butanol or isopropanol formation. We have purified and characterized key enzymes for solvent production, including a novel primary/secondary ADH and 3-hydroxybutyryl-CoA dehydrogenase. Based on the properties of the primary/secondary ADH, we increased isopropanol production 4-fold through culture manipulations. The structural gene for the primary/secondary ADH has been cloned and sequenced, which has led to the identification of a flanking gene whose deduced product resembles a signal-transducing regulatory protein. We are studying the relationship between this putative regulatory gene and solvent production. A mechanistic understanding of the control of biological solvent production will emerge from this and related studies.

Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

181. Enzymological Studies of One-Carbon Reactions in the Pathway of Acetate Utilization by Methanogenic Bacteria
J.G. Ferry, Department of Anaerobic Microbiology *$92,850*

Several enzymes in the pathway of acetate conversion to methane and carbon dioxide have been purified from *Methanosarcina thermophila*. The mechanisms of these enzymes are under investigation utilizing biochemical, biophysical and molecular genetic approaches. Acetate kinase and phosphotransacetylase catalyzes the activation of acetate to acetyl-CoA. The primary structure of these enzymes will be determined through cloning and sequencing of the genes. Two protein components of the CO dehydrogenase complex are under investigation. The metal centers of each component have been characterized using EPR. Cloning and sequencing of the genes for the two subunits of each component is in progress. Results indicate that the Ni/Fe-S component cleaves the

C-C and C-S bonds of acetyl-CoA followed by oxidation of the carbonyl group to carbon dioxide and transfer of the methyl group to the Co/Fe-S component. The enzymes and cofactors involved in transfer of the methyl group from the Co/Fe-S component to coenzyme M will be purified and characterized. Ferredoxin is an electron acceptor for the Ni/Fe-S component and also serves to reductively reactivate methylreductase which catalyzes the demethylation of methyl coenzyme M to methane. This ferredoxin is being characterized utilizing EPR and RR spectroscopic methods to determine the properties of the Fe-S centers. Genes encoding this and other ferredoxins have been cloned and sequenced to determine the primary structures. Carbonic anhydrase is being purified and characterized to determine the function of this enzyme in the pathway.

Washington State University
Pullman, WA 99164-6340

182. Membrane Function in Lipid Mutants of Arabidopsis
J. Browse, Institute of Biological Chemistry *$203,830 (2 years)*

A diverse variety of acyl lipids are components of the hydrophobic membrane barriers that delineate the compartments of plant cells. Acyl lipids are also the major component of seed oils which are an important renewable resource. My research uses both biochemical and genetic approaches to study the pathways and regulation of plant lipid synthesis and to investigate the ways in which lipid composition affects membrane function. This project focuses on several *Arabidopsis* mutants with specific alterations in membrane composition. We have shown that the allelic mutants *fad2-2* and *fad2-3* are deficient in one of two oleate desaturases and, as a result, the plants contain reduced levels of polyunsaturated fatty acids. Plants of both *fad2-2* and *fad2-3* are indistinguishable from wild type when grown at 22°C, but are inviable at low growth temperature (6°C). In order to identify the primary lesion of this chilling sensitivity, the temperature profiles of key membrane functions will be compared between wild type and mutant plants grown at 22°C. These functions include plasma membrane integrity, plasma membrane ion transport, and mitochondrial respiration. Seed of the *fad2-2* line will be subject to further mutagenesis to allow us to select second-site mutations that result in reversion of the cold-sensitive phenotype. Characterization of these second-site revertants will provide further information on the relationship between membrane structure and function. Once genes corresponding to the mutant loci are available, it will be possible to produce tissue specific constructs and transform the mutant lines with these. This approach will provide an alternative way to obtain data on the importance of polyunsaturated lipids at different stages of development.

Washington State University
Pullman, WA 99164-6340

183. Regulation of Terpene Metabolism
R. Croteau, Institute of Biological Chemistry *$182,500 (FY 91 Funds/2 years)*

Terpenoid oils, resins and waxes from plants are important renewable resources. The objective of this project is to understand the regulation of terpenoid production using (+)-camphor metabolism in sage and (-)-menthone metabolism in mint as model systems. The pathways of biosynthesis and catabolism have been established, and the relevant enzymes have been isolated and characterized. Developmental studies relating enzyme activity levels to terpene production within, and loss from, the oil gland sites of metabolism suggest that product accumulation is controlled by the balance between

the branch-point terpene cyclases and the catabolic enzymes responsible for the synthesis of terpenyl glycoside transport derivatives. Cyclases have been purified to homogeneity in order to obtain polyclonal antibodies and amino acid sequence information with which the corresponding cDNA clones have been isolated. Immunochemical methods have been employed to localize the cyclases within oil gland cells, and to determine the temporal levels of these enzymes and their translatable messages. The corresponding cDNA clones are being used to examine transcriptional and translational control of metabolism in greater detail. A method has been developed for the isolation of oil gland cell clusters that can sustain high rates of terpenoid biosynthesis from exogenous sucrose. These structures are being exploited to examine the regulation of the common isoprenoid pathway from acetyl CoA to the branch-point metabolites geranyl, farnesyl and geranylgeranyl pyrophosphate. Results from this project will have important consequences for the yield and composition of terpenoid natural products that can be made available for industrial exploitation.

Washington State University
Pullman, WA 99164-6340

184. Towards a Detailed Understanding of Structural Variability in Lignins
N.G. Lewis, Institute of Biological Chemistry *$158,000*

The mechanisms whereby cell wall thickening (maturation) processes are regulated or controlled are poorly understood, as are the means by which the dominant cell wall polymers (lignins, cellulose, hemicellulose) are assembled and then temporally and spatially distributed into the wall itself. This particular study is focused upon three knowledge-deficient areas in lignification, namely the identity (and the mechanism of transport) of the monomer(s) being transported from the cytoplasm into the lignifying cell wall, factors regulating lignin structural heterogeneity at both the subcellular level and in different tissue types, and the structure of lignins induced as a response to wounding/fungal attack. Immunocytochemical techniques are being employed to determine the subcellular organization of enzymes and intermediates associated with monolignol biogenesis, as well as establishing the temporal and spatial transport of different *E* and *Z* monomers into the cell wall. Factors controlling lignin structural heterogeneity are being investigated using two model systems: *Pinus taeda* cell suspension cultures (an early stage of lignification) and compression wood from *Pinus taeda* plants. Additionally, lignin bonding environments and monomer composition of wound/pathogen induced lignins are being determined using solid/solution state carbon-13 NMR spectroscopy.

Washington State University
Pullman, WA 99164-4660

185. Isocitrate Lyase and the Glyoxylate
B.A. McFadden, Department of Biochemistry and Biophysics *$74,931*

Our objectives are to shed light upon the structure, regulation and catalytic function of isocitrate lyase (*icl*), an enzyme which catalyzes the first unique step in the glyoxylate cycle. In this cycle, lipids are converted to carbohydrates.

We have described the cloning and sequencing of the *icl* gene of *Escherichia coli* and markedly improved purifications of *icl* from *E. coli* and watermelon. In the present project period, studies of the

kinetic mechanism for the *E. coli* enzyme have established that the binding of glyoxylate precedes that of succinate [*Current Microbiol. 21,313 (1990)].* We have alkylated the active-site residue *cys*-195 with the substrate analog brompyruvate resulting in marked resistance to proteolysis [*Arch. Biochem. Biophys.*278,373 (1990)]. We have also obtained diffraction-quality crystals of both *icl* and pyruvyl *icl* [*J. Mol. Biol. 220,13* (1991)] and continue to work on both crystal structures. *His*-266 and -306 [*Biochemistry* 30,7451 (1991)] and *ser*-319 and -321 [*J. Biol. Chem.* 267,91 (1992)] have been placed in the active site of *E. coli icl.* Mutagenesis of the gene is being directed towards replacing these and other potentially functional residues to test our hypothesized catalytic mechanism [*Comparative Biochem.* 95B, 431 (1990)]. Inferences will be compared with our developing knowledge of the crystal structure for *icl.*

These studies will provide basic information about *icl.* The function of this enzyme is vital to microbial growth (on acetate or fatty acids) and to the growth of varied plant seedlines. mRNA has been isolated from watermelon seedlings as a first step in the generation of *icl* cDNA. Cloning, sequencing and high-level expression will enable a comparison of *icl* from bacteria and plants.

Washington State University
Pullman, WA 99164-6340

186. Enhancement of Photoassimilate Utilization by Manipulation of the ADPglucose Pyrophosphorylase Gene
T.W. Okita, Institute of Biological Chemistry *$74,000*

The goal of this project is to assess the feasibility of increasing the conversion of photosynthate into starch via manipulation of ADPglucose pyrophosphorylase, a key enzyme in starch biosynthesis. In developing storage tissues such as cereal seeds and tubers, starch biosynthesis is primarily regulated by the gene activation and expression of ADPglucose pyrophosphorylase, starch synthase, and branching enzyme, as well as by the allosteric regulation of ADPglucose pyrophosphorylase activity. To gain insight into the structure/function relationship of the plant ADPglucose pyrophosphorylase we have previously elucidated the heterotetrameric structure of the potato tuber enzyme using 2-D gel electrophoresis, isolated cDNA clones to both the small and large subunits, and conducted homology studies which revealed conserved regions important for both catalytic and allosteric function. In collaboration with G. Barry (Monsanto Corp.), both cDNAs have been overexpressed (1-2% total protein) in *Escherichia coli.* Each subunit alone was unable to complement a bacterial glycogen mutant; however, the simultaneous expression of both subunits resulted in the restoration of bacterial glycogen synthesis and the presence of significant ADPglucose pyrophosphorylase activity. Both the temporal and spatial expression of the genes encoding each subunit as well as their correlation to starch biosynthesis has been elucidated. Genomic clones to the small subunit gene have been obtained and its gene structure determined. Transgenic potato plants have been produced containing deletions of the small subunit promoter. Currently, cis acting elements and their involvement in spatial and temporal expression are under investigation.

Washington University
St. Louis, MO 63130

187. Processing and Targeting of the Thiol Protease, Aleurain
J.C. Rogers, Department of Biology *$87,000*

We are studying an unusual thiol protease, aleurain, that is the product of a single-copy gene in barley. Aleurain is synthesized as a proenzyme that is cleaved in two steps after it reaches an acidified post-Golgi compartment; in aleurone cells its final destination is a morphologically unique type of vacuole. The sequence of aleurain is 65% identical to that of mammalian cathepsin H, a lysosomal enzyme whose natural substrates are unknown. We have purified aleurain to homogeneity and find that its Km for certain synthetic substrates is essentially the same as that of cathepsin H; both enzymes are primarily aminopeptidases. In addition, aleurain has the same unusual minichain/heavy chain structure found in cathepsin H. By expressing barley proteins in tobacco suspension culture protoplasts, we find that proaleurain is targeted to the vacuole while another barley thiol protease, proEP-B, is quantitatively secreted. Fusions between the two different proteins show that the N-terminal 12 amino acids of proaleurain are necessary and sufficient to direct it to the plant vacuole. This targeting determinant can be divided into two smaller determinants, SSSSFADS and SNPIR, each of which is sufficient to target proEP-B chimeras to the vacuole, but with lower efficiency. Thus, these determinants interact in a positive manner, and the sorting mechanism for vacuolar targeting in plants must be able to recognize individual determinants that have no apparent primary or secondary structural similarities.

Washington University
St. Louis, MO 63130

188. Plant Cell Wall Architecture
J.E. Varner, Biology Department *$46,086*

The cell walls of plants contain a number of proteins having repetitive sequences, e.g., hydroxyproline-rich glycoproteins, proline-rich proteins, and glycine-rich proteins. These are presumed to have structural roles in the determination of cell wall architecture. The localization of the cell wall proline-rich proteins (PRPs) and the gene expression of the cell wall glycine-rich proteins (GRPs) and the hydroxyproline-rich proteins (HRGPs) were examined in several dicot species. The PRPs are accumulated in the corner walls of the cortex where several cells are joined together and in the protoxylem cell walls of three-day old soybean root. In one-month old soybean plants, the PRPs are specifically deposited in xylem vessel elements of the young stem; and they are accumulated in both phloem fibers and xylem vessel elements and fibers of the older stem. Likewise, the PRPs are localized in xylem vessel elements and fibers in tomato, petunia potato and tobacco stems. They are also found in outer and inner phloem fiber cell walls of tomato stem and in outer phloem fiber cell walls of petunia stem. HRGP mRNAs are abundant in outer and inner phloem regions, while GRP mRNAs are present mostly in primary xylem region and in the cambium region. Immunocytochemical localization showed that the GRPs have a localization pattern similar to that of the PRPs in tomato, petunia and tobacco stems. Because of the role of H_2O_2 in lignin deposition in xylem and phloem fibers we have devised a simple direct histochemical test for H_2O_2.

Wayne State University
Detroit, MI 48201

189. Site-directed Mutagenesis of an Energy Transducing Membrane Protein Bacteriorhodopsin

R. Needleman, Department of Biochemistry *$166,461 (2 years)*

Our goal is to understand the mechanism of proton transport in the light-driven membrane protein bacteriorhodopsin. To achieve this we will evaluate the role of particular amino acids in the structure and function of bacteriorhodopsin and use this information to develop and critically test hypotheses about the molecular mechanism of ion transport. We will generate mutants of bacteriorhodopsin by both classical and recombinant methods, using our recently described system for *Halobacterium halobium* to introduce and express the cloned bacteriorhodopsin gene (*bop*). Although mutant bacteriorhodopsins produced by site-directed mutagenesis have been studied by others, the mutant proteins have been synthesized in *E. coli* and refolded *in vitro*. It is therefore not clear that the properties of the native protein have been preserved. Indeed, we have presented evidence that several mutant proteins that are made in *H. halobium* have different properties than those synthesized *E. coli*. After expression the mutant proteins in *H. halobium* will be purified and we will determine the correlation between the amino acid substitution and the changes in spectroscopic and photochemical properties. We will also develop additional vectors for the efficient introduction and expression of the gene coding for bacterhodopsin. Our ultimate goal is to provide a detailed molecular mechanism of hydrogen ion transport.

University of Wisconsin
Madison, WI 53706

190. Regulation of Extracellular Polygalacturonase Production in Pseudomonas solanacearum

C. Allen, Department of Plant Pathology *$142,337 (2 years)*

Pseudomonas solanacearum causes bacterial wilt disease of many economically important crop plants including potato, banana, and peanut. This bacterium produces at least three extracellular polygalacturonases (PGs) that contribute to plant pathogenesis. These enzymes degrade pectin, a major component of plant cell walls. Although *P. solanacearum* produces very little PG activity when growing in rich medium, the level increases about 100-fold when the bacteria grow in plant tissue, suggesting that PG production is induced by a plant factor. Determining how virulence genes induced *in planta* (like those encoding PG) are regulated is central to understanding the plant-pathogen interaction. Thus, the general objective of this research is to investigate the genetic and biochemical mechanisms regulating PG production. To accomplish this we have cloned a locus required for PG production that appears to encode a *trans*-acting positive regulator. Mutants in this locus (*pehR*) are severely reduced in virulence. We will determine the mechanism by which *pehR* directs expression of PG structural genes. Since *pehR* has homology to highly conserved regions of protein kinases from other prokaryotic environmentally-responsive positive regulators, we will test the hypothesis that PG production is regulated by a similar signal-transduction pathway involving phosphorylation. Further, we will characterize the inducing signal from the host and determine its relation to the regulatory system. Finally, we will mutate a *pehR* strain with a transposon-reporter gene to identify other genes that are co-regulated with PG.

University of Wisconsin
Madison, WI 53706

191. Biochemical and Molecular Analysis of a Transmembrane Protein Kinase from Arabidopsis thaliana
A.B. Bleecker, Department of Botany *$80,000*

Receptor protein kinases on the plasma membrane of cells interact with various growth factors and hormonal agents, generating intracellular signals from these external signals. As such the receptors play a pivotal role in the ability of a cell to respond to its environment and develop. Although widely studied in animals, very little is known about receptor kinases in plants.

We have isolated genomic and cDNA clones encoding a novel receptor-like protein kinase from the higher plant *Arabidopsis thaliana.* This kinase is being studied by combining biochemical, molecular, and genetic approaches. Domain-specific antibodies immunodecorate a polypeptide with a molecular mass of 120,000 daltons in extracts of *Arabidopsis*, where it has been found in all portions of the plant examined including root, stem, leaf, flower, and silique. Cytochemical analysis and studies using the kinase promoter with the GUS reporter gene system should give more explicit information on localization. The kinase is glycosylated, like the animal receptor kinases, and has been partially purified from *Arabidopsis* by using lectin columns. We have been able to demonstrate kinase activity by (1) renaturing the native protein from *Arabidopsis* after blotting to nitrocellulose, and (2) expressing the kinase in *E. coli.* Transgenic plants are also now being produced that either overexpress or carry altered forms of the protein kinase gene. These experiments will help determine the natural role the kinase plays in a pathway of signal transduction.

University of Wisconsin
Madison, WI 53706

192. Enzymology of Biological Nitrogen Fixation
R.H. Burris, Department of Biochemistry *$49,850*

Two genes involved in the regulation of nitrogenase activity, *draT* and *draG*, were cloned and found to be contiguous on the *Azospirillum brasilense* chromosome, The nifH gene, encoding dinitrogenase reductase, is near to *draT* with an intervening gap of 1.9 kb. The organization of these genes in *Azospirillum lipoferum* and *Rhodospirillum rubrum* is similar, but *nifH* and *draT* are separated by only 400 bp in the organisms. *A. brasilense draTG* is very similar to *draTG* in *R. rubrum* with 91.8% similarity and 85.3.% identity at the amino acid level. Apparently *A. brasilense* uses the normal ATG initiation codon for *draT*, and *draG*. The genes for *A. brasilense* were able to restore function to appropriate mutants of *R. rubrum*. The heterologous expression of *A. brasilense draTG* in *R. rubrum* was not fully normal, as it responded more slowly to darkness and more quickly to ammonia than wild type cells. Our mutational analysis of the *draTG* region of *A. brasilense* confirms the function of these genes in the regulation of nitrogenase activity, but it also revealed minor but demonstrable differences in the control systems of *R. rubrum* and *A. brasilense*.

University of Wisconsin
Madison, WI 53706

193. Molecular Genetics of Ligninase Expression
D. Cullen and T.K. Kirk, Department of Bacteriology *$198,000 (2 years)*

Lignin depolymerization is catalyzed by extracellular peroxidases of white rot basidiomycetes such as *Phanerochaete chrysosporium.* In submerged culture, multiple isozymes of lignin peroxidase (LiP) are secreted at relatively low levels, and production is derepressed under carbon, nitrogen, or sulfur limitation. Our objectives are to elucidate the organization/regulation of the genes encoding LiP's of *Phanerochaete chrysosporium* and to investigate their expression in *Aspergillus.* Toward these goals, we have cloned and sequenced six closely related LiP genes. Three of these genes are clustered within a 30 kilobase region of the genome. We have separated the *P. chrysosporium chromosomes* using alternating field electrophoresis and localized the LiP genes to two dimorphic chromosomes. Specialized polymerase chain reaction methods have been developed which permit quantitative detection of specific transcripts. Using these techniques, we have shown that the transcriptional regulation of certain LiP genes is dramatically altered by cultural conditions. The relationship between genomic organization and transcriptional regulation is being studied in detail. In addition, the gene encoding LiP isozyme H8 has been expressed in *Aspergillus* under the control of the glucoamylase promoter. Expression of additional LiP genes is being evaluated. The long term goal, production of highly purified recombinant peroxidases may aid in the development of processes such as biological bleaching of pulps, effluent treatments, and in biopulping.

University of Wisconsin
Madison, WI 53706

194. Role of Transit Peptides in the Proper Localization of Nuclear-Encoded Chloroplast Proteins
K. Keegstra, Department of Botany *$97,000*

Most chloroplast proteins are encoded in the nucleus and synthesized in the cytoplasm as precursors containing amino acids called a transit peptide. The precursors are post-translationally imported into chloroplasts and segregated to their proper location. The objective of our work is to understand the role of the transit peptide and other topogenic sequences in directing the import and sorting processes. These processes are being studied in an *in vitro* reconstituted system. In this system, radioactive precursor proteins are synthesized by *in vitro* expression of cloned precursor genes and the resulting precursor proteins imported into isolated intact chloroplasts. The localization of imported proteins is examined by chloroplast fractionation studies. Genes for precursor proteins destined for five different locations within chloroplasts have been isolated and their transport pathways are being characterized. These five locations are: outer envelope membrane, inner envelope membrane, stroma, thylakoid membrane and thylakoid lumen. The role of transit peptides is being examined by generating hybrid precursor proteins containing the transit peptide from a precursor destined for one location fused to the mature peptide destined for a different location. At the present time we are focusing most of our efforts on proteins destined for the envelope membranes because the topogenic sequences involved are the least well characterized.

University of Wisconsin
Madison, WI 53706

195. Organization of the R Chromosome Region in Maize
J. Kermicle, Laboratory of Genetics *$65,000*

Organization of the *R* region in maize is under study with a view to determining the number, kind and arrangement of components involved in the control of anthocyanin pigmentation. *R* is organized on a modular basis and is extensively polymorphic. An allele comprises one or more functionally independent units (genic elements), each distinguished by particular tissue-specific effects. Intragenic recombination serves to place differences between genic elements relative to sites of recessive mutation associated with insertion of the transposable element *Dissociation.* Molecular characterization provides detail concerning the physical structure of regions of particular functional significance, such as those involved in tissue-specific action and paramutation. Separate attention is being given to the pattern of recombination occurring when duplications and insertions are present.

University of Wisconsin
Madison, WI 53706

196. Carbon Monoxide Metabolism by Photosynthetic Bacteria
P.W. Ludden and G.P. Roberts, Depts of Biochemistry and Bacteriology *$79,000*

This project focuses on the biochemistry, physiology and genetics of the carbon monoxide oxidation system found in the photosynthetic bacterium *Rhodospirillum rubrum.* The carbon monoxide dehydrogenase (CODH) of *R. rubrum* carries out the oxidation of CO to CO_2 with concomitant production of H_2 via an associated hydrogenase activity. CODH is a Ni-, Fe-S- enzyme that can be produced in an apo-form (lacking Ni) by starving the cells for Ni^{2+}. The purified apo-CODH can be activated by treatment with Ni^{2+}; Co^{2+}, Fe^{2+} and Zn^{2+}
will also take the place of Ni in the cluster yielding an inactive enzyme. The CO-induced hydrogenase is being purified. The genes for CODH (*cooS*), its associated hydrogenase (*cooH*) and the electron accepting ferredoxin for CODH (*cooF*) have been isolated, sequenced and mutagenized. Medium for CO-dependent growth of *R. rubrum* has been optimized and the physiology and biochemistry of CO-dependent growth are being studied. CODH has been crystallized and attempts to crystallize the electron accepting ferredoxin are being initiated.

University of Wisconsin
Madison, WI 53706

197. Starch Synthesis in the Maize Endosperm as Affected by Starch-Synthesizing Mutants
O.E. Nelson, Department of Genetics *$106,522 (2 years)*

Attention has been focused on the mutant of the newly identified Chr. 4 locus previously known as *vitreous*-8132* and now designated as *glucosidase-transferasel, (gltl)* since the *gltl-Ref* mutation under investigation markedly reduces both an alpha-1,6-glucosidase and an oligo-1,4-transferase, which appears capable of making both alpha-1,4- and alpha-1,6-linkages. These activities co-purify from extracts of either mutant or nonmutant endosperms. The glucosidase function is evidenced by its

ability to liberate maltotriose from pullulan and to render a beta-amylase limit dextrin susceptible to further attack by beta-amylase, and the transferase function by the transfer of labeled (^{14}C) glucose units from pullulan or amylopectin to amylose. The mutant endosperms have reduced starch, which is altered in a manner that is not fully understood although the I_2 complex of the amylopectin component of the mutant starch has an absorption peak that is somewhat higher than the nonmutant amylopectin. The nonmutant enzyme is present in the developing endosperms but is not detectable in germinating seeds. It differs from the mammalian glucosidase-transferase in that it appears to function only in polysaccharide synthesis and not in degradation, at least not in germinating seeds. Further, it liberates only maltose from an amylopectin phosphorylase limit dextrin and not glucose as does the mammalian glucosidase-transferase, and it cannot add glucose units to either amylopectin or phytoglycogen.

University of Wisconsin
Madison, WI 53706

198. Feedback Limitations of Photosynthesis
T.D. Sharkey, Department of Botany *$79,000*

We are studying the interaction between photosynthetic CO_2 assimilation and starch and sucrose synthesis. We have shown that photosynthesis can be limited by feedback from starch and sucrose synthesis and how this affects the maximum rate of photosynthesis. We are using plants which have altered genetic information in these studies. One plant lacks most of the cytosolic fructose bisphosphatase and so makes sucrose only very slowly. We are currently trying to find where the residual enzyme activity is located within the photosynthesizing leaf by immunolocalization. We are also studying the effect of loss of other enzymes required for starch or sucrose synthesis on the maximum capacity for photosynthesis. To examine the effect of increased enzyme activity, we are investigating a transgenic tobacco plant with excess phytochrome. We found that the extra phytochrome increased the level of sucrose-phosphate synthase which resulted in an increase in the capacity for sucrose synthesis when sufficient CO_2 was supplied. An increase in diffusion resistance was found however, which prevents increased photosynthesis at moderate CO_2 levels. We undertook a study of mesophyll diffusion resistance in a range of plants. We developed a new method for measuring the mesophyll resistance and tested it against the more difficult stable isotope method. Further studies of the effect of phytochrome on sucrose synthesis and mesophyll diffusion resistance will involve adding the phytochrome gene to other plants and growing transgenic plants under conditions which changes the phenotype of the transgenic plants and by making transgenic soybeans and tomatoes.

University of Wisconsin
Madison, WI 53706

199. Molecular Mechanism of Energy Transduction by Plant Membrane Proteins
M.R. Sussman, Department of Horticulture *$206,250 (FY 91 Funds/2 years)*

Proton pumps are membrane-bound proteins that act as biological energy transducers. The plasma membrane of plants and fungi contains an ATP-coupled proton pump (H^+-ATPase) that converts chemical into electrical energy. This enzyme is essential for the growth of plants and fungi and

provides the driving force used to catalyze the uptake and accumulation of solutes. The plant/fungal plasma membrane H^+-ATPase contains a single polypeptide of Mr=100,000. Its simple polypeptide structure makes this enzyme an attractive candidate for studying the molecular basis of energy transduction. A major aim of this project is to identify aspects of the enzymes's primary structure that are essential for converting chemical into electrical energy. DNA cloning and sequencing techniques are being used to obtain the complete amino acid sequence for ATPase structural genes present in *Arabidopsis thaliana*, a higher plant with a small genome and a rapid generation time. Using PCR and low-stringency Southern hybridization, we have identified 12 distinct genes encoding P-type ATPases. The sequence of genomic and cDNA clones is being determined for each of the several ATPase gene isoforms present in the nuclear genome. Expression of these genes is being studied using Northern blots and GUS gene fusions with putative ATPase promoter sequences. These studies on cell-specific expression of the ATPase gene will help to delineate the developmental and environmental signals that regulate activity of the plasma membrane proton pump *in situ*. In addition, these studies provide data necessary for testing hypotheses concerning the biological role of ion pumps and the molecular mechanism of protein-mediated energy transduction in plants.

University of Wisconsin
Madison, WI 53706

200. Analysis of Structural Domains Required For Phytochrome Function By In Vitro Mutagenesis

R.D. Vierstra, Department of Horticulture *$186,000 (FY 91 Funds/2 years)*

Phytochrome is a red/far-red photoreversible photoreceptor that has a central role in light-regulated development in plants. In an effort to determine how phytochrome functions at the molecular level as an activator of photomorphogenesis, we have recently developed a biological assay for active chromoproteins that involves the expression of a chimeric oat phytochrome gene in transgenic tobacco. Such ectopic overexpression induces a striking "light exaggerated" phenotype which can be exploited as a convenient *in vivo* assay of receptor function. The goal of this project is to use this transgenic system in conjunction with *in vitro* mutagenesis to identify phytochrome domains potentially important to synthesis, dimerization, chromophore attachment, Pr/Pfr phototransformation, Pfr-enhanced degradation, and biological activity. One domain of particular interest is the extreme N-terminus. This region has been proposed to be intimately involved in phytochrome function because it undergoes a conformation change during Pr/Pfr transformations and is important for many of the physical properties of the chromoprotein. To test this possibility, we have expressed oat phytochrome lacking amino acid residues 7-69 in tobacco. Although this protein attaches chromophore, is photoreversible, and capable of dimerization, it is not biologically active demonstrating that this region is indeed required for optimal function. Additional N-terminal mutations have recently been generated to identify critical residues within this 62-amino acid domain. Completion of this work will represent an important step in the identification of domains essential to the proper assembly and function of this essential photoreceptor.

Worcester Foundation for Experimental Biology
Shrewsbury, MA 01545

201. Novel Biomaterials: Genetically Engineered Pores
H. Bayley *$165,570**

Recombinant DNA technology allows the generation of new materials in microorganisms. We are constructing a selection of microscopic pores by genetic manipulation of a bacterial channel protein. The molecule we are remodeling is α-hemolysin (αHL), which is secreted by *Staphylococcus aureus*. It is a small robust polypeptide that should be relatively easy to reengineer. The single chain of 293 amino acids forms hexameric pores in membranes ~11 Å in internal diameter. The hexamer can also be assembled from the monomer *in vitro* by the addition of an inexpensive detergent. Recombinant αHL (r-αHL) has now been obtained in multimilligram amounts by overexpression of the αHL gene in *E. coli*. r-αHL is identical to αHL purified from *S. aureus* by several criteria, including its pore-forming properties in artificial lipid bilayers. Deletion mutagenesis has demonstrated that virtually the entire polypeptide chain is required for efficient pore formation. The N and C termini are particularly crucial, while small segments of a central glycine-rich loop can be removed. Now, a combination of point mutagenesis and chemical modification is being used to create pores with different internal diameters, with selectivity for the passage of molecules and ions, and with gating properties (the ability to open and close in response to a physical stimulus, e.g. an electric field or light). Ultimately, the new pores will be used to confer novel permeability properties upon materials such as thin films. Such products have potential technological applications; for example, as components of energy conversion and storage devices, selective electrodes, electronic devices, and ultrafilters.

(*funded collaboratively with Division of Materials Sciences)

Yale University
New Haven, CT 06511

202. Molecular Cloning and Structural Characterization of the R Locus of Maize
S.L. Dellaporta, Department of Biology *$90,000*

From past studies on the R-r, we have shown that the complex can be subdivided into two regions: a (P) component consisting of a simple R gene, and a (S) subcomplex consisting of a truncated R gene, termed (Q), and two (S) components, termed (S1) and (S2). (Q), (S1) and (S2) are collectively referred to as the (S) subcomplex. During the last research period, we have continued our characterization of the R-r complex of maize to include a functional assay of R components, CHEF gel mapping of component intervals and structural analysis of non-crossover (NCO) mutations. These studies have demonstrated the overall organization of the R-r complex to be (P)(Q)(S1)(S2) -- the two (S) genes are organized as an inverted repeat, with (S2) in direct orientation with respect to (P). Displaced pairing and crossing over between regions in direct orientation lead to derivative formation by unequal exchanges and the loss of the intervening segment. Pairing between the (P) promoter region and (Q) lead to formation of (P)-loss derivatives while displaced exchanges between the (P) coding region and (S2) lead to (S)-loss derivatives. The interval between (P) and the (S) subcomplex has been mapped at ca. 190 kb by CHEF analysis. Our most recent data has implicated a mobile element in the formation of the (S) subcomplex through transposon-mediated structural rearrangements of a progenitor (P) gene.

Yale University
New Haven, CT 06511-7444

203. Spatial Regulation of C4 Genes in C3, C4, and C3/C4 Intermediate Flaveria Species
T. Nelson, Biology Department *$192,000 (FY 91 Funds/2 years)*

The dicot genus *Flaveria* (*Asteraceae*) includes species utilizing C3, C4, and C3/C4 intermediate schemes of carbon fixation. In the efficient C4 scheme, neighboring photosynthetic bundle sheath (BS) and mesophyll (M) cells cooperate for carbon fixation. CO_2 is fixed initially in C4 acids in M cells, then further metabolized in BS cells, via a pathway that relies on expression of the genes for pathway enzymes in M- or BS-specific patterns. In less efficient C3 species, M cells independently fix CO_2 in a C3 compound, and the BS is not generally photosynthetic. C3/C4 intermediate species exhibit anatomical and biochemical characteristics between the C4 and C3 extremes and may represent evolutionary steps between C3 and C4 forms. In both C3 and C3/C4 species, C4 pathway genes are present, but used in different spatial patterns. The variety of *Flaveria* species provides an opportunity to compare the structure and regulation of C4 pathway genes in closely related C3 and C3/C4 species. Our ongoing work includes (1) the isolation and comparison of genes for phosphoenolpyruvate carboxylase (PEPCase) and ribulose bisphosphate carboxylase (RuBPCase) from C3 and C4 species of *Flaveria*, (2) the characterization of spatial and temporal expression patterns of these genes in C3, C4, and C3/C4 species, and (3) the expression of PEPCase- and RuBPCase-reporter gene fusions in leaf cells of C3, C4 and C3/C4 species.

Yale University
New Haven, CT 06511

204. Organization and Control of Genes Encoding Catabolic Enzymes in Rhizobiaceae
D. Parke and L.N. Ornston, Department of Biology
$166,000 (FY 91 Funds/2 years)

The goal of this project is to understand energy metabolism, gene organization, and gene control in plant-associated bacteria of the family *Rhizobiaceae*. The group includes the plant pathogen *Atrobacterium* and the legume symbionts *Rhizobium* and *Bradyrhizobium*. Bacteria of the three genera grow at the expense of a wide range of monocyclic phenolics which originate from lignin and plant root exudates. The ß-ketoadipate pathway, serving to break down diverse phenolic compounds, is universally distributed in members of the *Rhizobiaceae*. Inducible *Agrobacterium*, *Rhizobium*, and all other microbes known to possess them, most enzymes of the ß-ketodipate pathway are expressed constitutively in *Bradyrhizobium*. One enzyme in particular, ß-ketodipate succinyl CoA transferase, a product of the *pcaE* gene, is expressed at high levels in saprophytic and symbiotic *Bradyrhizobium*. Genes encoding enzymes of phenolic catabolism from *Bradyrhizobium japonicum* I-110 will be cloned, their genetic organization studied, and the genetic basis for high and low level expression of different enzymes explored. Mutant strains of *B. japonicum* blocked in the catabolism of phenolics have been isolated and will be used to investigate the selective value of the trait. The genetic organization and control of phenolic catabolism in *Agrobacterium* have been elucidated; further studies of intriguing aspects of regulation in this genus will provide a point of reference for other members of the *Rhizobiceae*. Southern hybridization using cloned genes will localize genes for phenolic catabolism to plasmids or the chromosome in diverse representatives of this bacterial family.

Yale University
New Haven, CT 06510

205. Mechanisms and Control of K^+ Transport in Plants and Fungi
C.L. Slayman, Department of Cellular and Molecular Physiology *$109,000*

The overall objective of this research has been a detailed functional description of potassium transport in model plant and fungal systems, via the adaptation and development of molecular techniques for studying membrane transport processes. The main preparations in use have been the tonoplast and plasma membrane of the yeast, *Saccharomyces cerevisiae*, the plasma membrane of the mycelial fungus, *Neurospora crassa*, and the plasma membrane of leaf mesophyll cells from the higher plant, *Arabidopsis thaliana*. Principal new experimental work in the past year has been the detailed patch-clamp study of K^+-channel structures in the plasma membranes of *Saccharomyces* and *Arabidopsis*, as well as completion of studies on *Neurospora* with fluorescent probes and lipid-soluble ions to investigate the reaction cycle of the primary ion pump (and H^+-ATPase) and more general charge-transfer characteristics of the *Neurospora* plasma membrane.

Work will be carried out over the coming year on four groups of experiments: 1) detailed characterization of the primary K^+ channel in the plasma membrane of *Saccharomyces*, which we now know to have at least three gating states, to be a strong outward rectifier, and to be modulated by voltage, calcium, and redox agents; 2) extending the patch-clamp assay of ion channels in *Arabidopsis* by testing other tissues and conditions, in order to develop procedures for surveying functional channels in plant tissues, complementary to the chemical mapping of membrane proteins and/or precursors via immunofluorescence and in-situ hybridization; 3) beginning chemical characterization of the cation channel of the yeast tonoplast membrane, using its calmodulin affinity as a means of purifying the channel; and 4) examining the physiological consequences of expressing fungal proton pumps in cultured mammalian fibroblasts.

Yale University
New Haven, CT 06511

206. Transfer RNA Involvement in Chlorophyll Biosynthesis
D. Söll, Department of Molecular Biophysics and Biochemistry *$102,000*

Chlorophyll is the major pigment in photosynthesis. One molecule of chlorophyll is synthesized from eight molecules of δ-aminolevulinic acid, the universal precursor of porphyrins. Chlorophyll synthesis is regulated at the step of δ-aminolevulinic acid formation, which occurs in the stroma of greening plastids. The mechanism of δ-aminolevulinic acid synthesis from glutamate is not completely understood. There is now solid evidence that in the chloroplasts of plants and green algae, in cyanobacteria (*e.g., Synechocystis 6803*), in some eubacteria (*e.g., E. coli* and *B. subtilis*) and archaebacteria this biosynthetic route, the C5-pathway, involves the tRNA-dependent reduction of glutamate to glutamate-1-semialdehyde which is subsequently converted to δ-aminolevulinate. There are indications which suggest the existence of an additional pathway for δ-aminolevulinic acid formation in these organisms.

The initial metabolite for the two-step C5-pathway is Glu-$tRNA^{Glu}$ which is converted by the action of an unusual enzyme, Glu-tRNA reductase, to glutamate-1-semialdehyde with the concomitant release

of $tRNA^{Glu}$. Glutamate-1-semialdehyde is the first committed precursor of chlorophyll synthesis. Thus, Glu-$tRNA^{Glu}$ is a dual-function molecule; it provides glutamate for protein synthesis and glutamate-1-semialdehyde for porphyrin synthesis. The regulation of the flow of Glu-$tRNA^{Glu}$ into the different pathways may be determined by the relative concentrations of EF-Tu and Glu-tRNA reductase, two proteins which compete in binding Glu-$tRNA^{Glu}$. The Glu-tRNA reductase can discriminate between different tRNAs in a biochemical reaction which is as yet not understood. In the second step of the pathway and aminotransferase (GSA-aminotransferase) converts glutamate-1-semialdehyde to δ-aminolevulinic acid.

We have purified the two enzymatic activities from *Chlamydomonas* and demonstrated the *in vitro* conversion of Glu-tRNA → δ-aminolevulinic acid by the action of the two purified proteins. We have cloned the genes encoding Glu-tRNA reductase from *Synechocystis 6803* and *Arabidopsis thaliana.* We have also cloned and analyzed the gene for GSA-aminotransferase from *Arabidopsis.* Our fi0nal goal is to obtain a detailed understanding of the regulation of the activities of these enzymes in higher plants and of their importance in the control of chlorophyll synthesis.

PROJECT CATEGORIZATION

What follows is a categorization of the Energy Biosciences projects into areas of investigation relevant to the overall objectives of the program. The projects listed under each category represent the efforts directed towards the various aspects of the topic. Some projects overlap between different categories and are listed in both categories. Although there is considerable activity in the development of new approaches to problem areas, there is no technology development category. Instead, projects are classified under the overall subject objectives. Likewise, there is no listing of techniques/approaches, e.g., recombinant DNA, site directed mutagenesis, nuclear magnetic resonance spectroscopy, etc. All are integrated into the specific topic areas. Each project has been assigned a number which identifies it within the report.

1. PHOTOSYNTHESIS

Photosynthesis is the central driving mechanism in the conversion of solar energy into chemical energy in living organisms leading to renewable resources production. The areas covered include dissecting of the numerous aspects of photosynthesis such as carbon fixation, oxygen evolution, photorespiration, photophosphorylation, structures of photosynthetic elements and other topics relating to photosynthesis under natural conditions. In approaching these questions a great diversity of techniques ranging from ultrafast laser spectroscopy to site directed mutagenesis are being utilized. The intent is to understand this critical biological energy conversion process upon which most life depends.

Center for the Study of Early Events in Photosynthesis
Abs. 3 Arizona State University *R.E. Blankenship, J.P. Allen, W.D. Frasch, J.D. Gust, J.K. Hoober, A.L. Moore, T.A. Moore, G.R. Seely, W.F.J. Vermaas, A.N. Webber and N.W. Woodbury*

Antenna Organization in Green Photosynthetic Bacteria
Abs. 4 Arizona State University *R.E. Blankenship*

The Chlorophyll-Binding Protein CP47 in Photosystem II
Abs. 5 Arizona State University *W.F.J. Vermaas*

Molecular Bases of Photoadaptation in Unicellular, Eucaryotic Algae
Abs. 14 Brookhaven National Laboratory *P.G. Falkowski and J. LaRoche*

2. PLANT CELL WALLS

The major component of biomass resources are plant cell walls which consist of polysaccharides, lignins, proteins and other compounds. Studies in this category include research on the synthesis, structure, function and other aspects of cell wall components.

In approaching the multiple questions encompassed by this topic, a broad diversity of techniques are employed including fast atom bombardment mass spectroscopy, Raman spectroscopy, Nuclear Magnetic Resonance spectroscopy, numerous molecular biological procedures, and others. The objective is to fully understand the nature and function of this resource in order to positively affect biomass production and utilization.

Molecular Organization in the Native State of Woody Tissue: Studies of Tertiary Structure Using the Raman Microprobe, Solid State ^{13}C NMR and Biomimetic Tertiary Aggregates
Abs. 1 USDA - Madison, Wisconsin *R.H. Atalla*

Role of Pectolytic Enzymes in the Programmed Release of Cells from the Root Cap of Higher Plants
Abs. 7 University of Arizona *M.C. Hawes*

CarbBank - A Structural and Bibliographic Database for Complex Carbohydrates
Abs. 61 University of Georgia *P. Albersheim*

The University of Georgia Complex Carbohydrate Research Center (CCRC) - A Department of Energy Unit of the USDA/DOE/NSF Plant Science Centers Program
Abs. 62 University of Georgia *P. Albersheim and A. Darvill*

The Structures and Functions of Oligosaccharins
Abs. 63 University of Georgia *P. Albersheim*

Structural Studies of Complex Carbohydrates of Plant Cell Walls
Abs. 64 University of Georgia *A. Darvill*

Mechanisms of Lignin Biosynthesis During Xylogenesis in *Zinnia elegans*
Abs. 66 University of Georgia *K-E.L. Eriksson and J.F.D. Dean*

Enzymatic Mechanisms and Regulation of Plant Cell Wall Biosynthesis
Abs. 112 Michigan State University DOE Plant Research Laboratory *D. Delmer*

Cell Wall Proteins
Abs. 115 Michigan State University DOE Plant Research Laboratory *D.T.A. Lamport*

Transcription Factors Regulating Lignin Biosynthesis in Xylem
Abs. 140 North Carolina State University *R. Sederoff*

The Molecular Characterization of the Lignin-Forming Peroxidase: Growth, Development, and Response to Stress
Abs. 143 Ohio State University *L.M. Lagrimini*

The Structure of Pectins from Cotton Suspension Culture Cell Walls
Abs. 147 Oklahoma State University *A. Mort*

Structure and Biosynthesis of the Mixed-linkage ß-D-Glucan of Grasses
Abs. 161 Purdue University *N.C. Carpita*

Enzymology and Molecular Biology of Cell Wall Biosynthesis
Abs. 174 Stanford University *P.M. Ray*

Characterization of a 1,4,-ß-D-glucan Synthase from *Dictyostelium discoideum*
Abs. 177 Texas Tech University *R.L. Blanton*

Towards a Detailed Understanding of Structural Variability in Lignins
Abs. 184 Washington State University *N.G. Lewis*

Plant Cell Wall Architecture
Abs. 188 Washington University *J.E. Varner*

3. PLANT RESPIRATION/NUTRITION

Plants require energy and inorganic nutrients as all living organisms do in order to grow and survive. Plants have many similarities in respiratory mechanisms and nutrition requirements when compared with other life forms, but there are also significant differences. To be able to use plants more effectively as renewable resources in a biotechnological context it is essential to understand how plants use the available photosynthetically-derived energy as well as how plants absorb, transport and utilize mineral nutrients in sustaining their growth, development and other biosynthetic activities. As in other categories, there is a large diversity of research technologies that are used in these studies.

Energy Capture and Use in Plants and Bacteria
Abs. 33 University of California - Los Angeles *P.D. Boyer*

Mechanism and Structure of the Plant Plasma Membrane Ca^{2+}-ATPase
Abs. 79 University of Illinois *D.P. Briskin*

Identifying Calcium Channels and Porters in Plant Membranes
Abs. 104 University of Maryland *H. Sze*

Transport Function and Reaction Mechanism of Vacuolar H^+-Translocating Inorganic Pyrophosphatase
Abs. 159 University of Pennsylvania *P.A. Rea*

4. PLANT METABOLISM

One of the greatest resources plants have to offer is the ability to synthesize a massive variety of products that can be used as food, fibers, structural components, pharmaceuticals and numerous other agents. In order to have greater ability to use plants and plant processes as a resource in the rapidly growing biotechnology industry, it is absolutely essential to build the base of understanding of not only the metabolic capabilities of plants, but how the various pathways are regulated. Future biotechnological developments utilizing plant systems will require knowledge about how plants partition the products that are synthesized. This category encompasses research directed towards these goals. The availability of newer techniques for chemical analyses, in addition to the formidable molecular biological procedures, have made it possible to probe questions that were previously almost intractable.

5. PLANT GROWTH AND DEVELOPMENT

It is clear that the development of plants for maximal biomass production requires that the nature of what controls the way in which plants produce new cells, expand tissues, and differentiate into different organ types are fully understood. This category includes studies exploring the development of entirely new molecular approaches in the last decade.

Role of Transit Peptides in the Proper Localization of Nuclear-Encoded Chloroplast Proteins

Abs. 194 University of Wisconsin *K. Keegstra*

Analysis of Structural Domains Required For Phytochrome Function By *In Vitro* Mutagenesis

Abs. 200 University of Wisconsin *R.D. Vierstra*

6. PLANT GENETIC REGULATION AND MOLECULAR BIOLOGY

The most profound advances in biology over the last decade or so have been made in gaining a greater understanding of genetic structure and expression. This has also included the development of modes of transferring genetic information between organisms as well as extremely detailed characterization of genes. In this category, efforts are included to better comprehend how certain complex genetic components are expressed, what the regulatory elements are, how exterior signals are received that affect genetic expression, plus a variety of other questions relating to the nature of and what controls the genetic apparatuses of plants. The overall importance of this information in respect to future biotechnological developments rests with the ability to provide ways of assuring the expression of desirable genes in plants that will result in the improved quantity and quality of products.

Differential Regulation of Plastid mRNA Stability

Abs. 11 Boyce Thompson Institute for Plant Research, Inc. *D.B. Stern*

Plant Molecular Genetics

Abs. 13 Brookhaven National Laboratory *B. Burr and F.A. Burr*

Genetic Analysis of *Adh1* Regulation

Abs. 21 University of California - Berkeley *M. Freeling*

Regulation of Tomato Fruit Growth by MVA and GTP-Binding Proteins

Abs. 22 University of California - Berkeley *W. Gruissem*

The Suppression of Mutations Generated by *Mu* Transposons in Maize

Abs. 41 Cold Spring Harbor Laboratory *R. Martienssen and V. Sundaresan*

Organ-Specific Gene Expression in Maize: The *P-wr* Allele

Abs. 42 Cold Spring Harbor Laboratory *T. Peterson*

Mechanisms and Genetic Control of Interspecific Crossing Barriers in *Lycopersicon*

Abs. 45 Cornell University *M.A. Mutschler*

Genetic Engineering with a Gene Encoding a Soybean Storage Protein
Abs. 169 Scripps Research Institute *R.N. Beachy*

Exploration of New Perspectives and Limitations in *Agrobacterium*-Mediated Gene Transfer Technology
Abs. 170 University of South Carolina *L. Márton*

Regulation of Chloroplast Number and DNA Synthesis in Higher Plants
Abs. 176 Texas A&M University *J.E. Mullet*

The Plant Mitochondrial *mat-r* Gene/*nad1* Gene Complex
Abs. 179 University of Utah *D.R. Wolstenholme*

Organization of the *R* Chromosome Region in Maize
Abs. 195 University of Wisconsin *J. Kermicle*

Starch Synthesis in the Maize Endosperm as Affected by Starch-Synthesizing Mutants
Abs. 197 University of Wisconsin *O.E. Nelson*

Molecular Cloning and Structural Characterization of the *R* Locus of Maize
Abs. 202 Yale University *S.L. Dellaporta*

7. PLANT STRESS

The ability of plants to grow under sub-optimal environmental conditions is a crucial advantage for renewable resource productivity. How plants contend with drought conditions, heat, salinity and other factors that deter growth is the emphasis of this category. The projects are aimed at discerning the mechanisms, genetic, biochemical and physiological, by which plants adapt to such conditions. Once again the efforts are now bolstered by the availability of new approaches such as molecular biology and others.

Modifying K^+/Na^+ Discrimination in Salt-Stressed Wheat Containing Chromosomes of a Salt-Tolerant *Lophopyrum*
Abs. 28 University of California - Davis *E. Epstein and J. Dvorak*

Tonoplast Transport and Salt Tolerance in Plants
Abs. 37 University of California - Santa Cruz *L. Taiz*

Effects of Freezing and Cold Acclimation on the Plasma Membrane of Isolated Cereal Protoplasts
Abs. 48 Cornell University *P.L. Steponkus*

Metabolic Mechanisms of Plant Growth at Low Water Potentials
Abs. 53 University of Delaware *J.S. Boyer*

Environmental Stress-Mediated Changes in Transcriptional and Translational Regulation of Protein Synthesis in Crop Plants
Abs. 67 University of Georgia *J.L. Key and R.T. Nagao*

Violaxanthin De-Epoxidase: Biogenesis and Structure
Abs. 76 University of Hawaii *H.Y. Yamamoto*

8. PLANT-PATHOGEN/VIRAL INTERACTIONS

The ultimate productivity, and oftentimes survival, of plants is frequently dependent upon how the plant is able to respond to incursions by microbes or viruses that are pathogenic. Future protection of biomass productivity by plants will depend on the ability to devise effective strategies to protect plants against the invasion by pathogens. The projects included in this category are designed to generate fundamental understandings of the workings of how pathogens invade and damage the host plants. A substantial portion of the activities are now based on using molecular biological approaches combined with the genetic mutants.

Phytoalexin Detoxifying Enzymes in the Plant Pathogenic Fungus *Nectria haematococca*
Abs. 9 University of Arizona *H.D. VanEtten*

Determination of Genes and Phenotypes of Bacteria Necessary for Epiphytic Colonization and Survival on Plants
Abs. 23 University of California - Berkeley *S.E. Lindow*

Cloning and Characterization of Genes Determining Disease Resistance in *Arabidopsis-Pseudomonas* Interactions
Abs. 25 University of California - Berkeley *B.J. Staskawicz*

Transposon Tagging of Disease Resistance Genes
Abs. 29 University of California - Davis *R.W. Michelmore*

Mechanisms of Inhibition of Viral Replication in Plants
Abs. 47 Cornell University *P. Palukaitis*

Molecular Studies of Functional Aspects of Higher Plant Mitochondria
Abs. 55 Duke University *J.N. Siedow*

Molecular Basis of Disease Resistance in Barley
Abs. 120 Michigan State University DOE Plant Research Laboratory *S.C. Somerville*

Biochemical and Molecular Aspects of Plant Pathogenesis
Abs. 121 Michigan State University DOE Plant Research Laboratory *J.D. Walton*

Characterization of a Defective Interfering RNA That Contains a Mosaic of a Plant Viral Genome
Abs. 137 University of Nebraska *T.J. Morris and A.O. Jackson(University of California, Berkeley)*

Analysis of Potyviral Processing: A Basis for Pathogen Derived Resistance?
Abs. 152 Oregon State University *W.G. Dougherty*

Regulation of Extracellular Polygalacturonase Production in *Pseudomonas solanacearum*
Abs. 190 University of Wisconsin *C. Allen*

9. NITROGEN FIXATION AND PLANT-MICROBIAL SYMBIOSIS

One of the most crucial aspects of plant nutrition is obtaining adequate nitrogen for the growth and survival. Nature has evolved the technique of nitrogen fixation for using atmospheric nitrogen to supply the needs of microbes and certain plants via symbiotic reactions. The prospect of exploiting this mechanism to avoid the use of man-made nitrogen fertilizer has been suggested, but unless the whole process of nitrogen fixation in microbes and in symbiotic relationships is better understood, no such objective can ever be considered seriously. The projects in this category cover various aspects of these processes in both microbes and plants, including studies on nodulation, relevant biochemical reactions and other topics.

Organization and Regulation of the Genes for Nitrogen Fixation in *Rhodobacter capsulatus*
Abs. 38 University of Chicago *R. Haselkorn*

Regulation of Gene Expression in the *Bradyrhizobium japonicum*/soybean symbiosis
Abs. 52 Dartmouth College *M.L. Guerinot*

Cellulose Fermentation by Nitrogen-Fixing Anaerobic Bacteria
Abs. 106 University of Massachusetts *E. Canale-Parola*

Molecular Basis of Symbiotic Plant-Microbe Interactions
Abs. 111 Michigan State University DOE Plant Research Laboratory *F.J. de Bruijn*

Developmental Biology of Nitrogen-Fixing Cyanobacteria
Abs. 122 Michigan State University DOE Plant Research Laboratory *C.P. Wolk*

A Structural Analysis of the Role of the *Rhizobium* Cell Surface Carbohydrates in the Rhizobium/Legume Symbiosis
Abs. 124 Michigan State University *R. Hollingsworth*

Catalytic Mechanism of Hydrogenase from Aerobic N_2-Fixing Microorganisms
Abs. 151 Oregon State University *D.J. Arp*

Crystallographic Studies of Nitrogenase and Hydrogenase
Abs. 160 Purdue University *J.T. Bolin*

Nodulation Genes and Factors in the *Rhizobium*-Legume Symbiosis
Abs. 173 Stanford University *S.R. Long*

Plant Recognition of *Bradyrhizobium japonicum* Nod Factors
Abs. 175 University of Tennessee *G. Stacey*

Enzymology of Biological Nitrogen Fixation
Abs. 192 University of Wisconsin *R.H. Burris*

10. LIGNIN-POLYSACCHARIDE BREAKDOWN

With the availability of abundant biomass, consisting largely of polysaccharides such as cellulose and others, it is critical that improved technologies be generated to facilitate the conversion of the resources into usable products. Accordingly, this category dwells on basic studies dealing with the organismal and enzymatic conversion of the major constituents of biomass into usable feedstocks. Clearly, organisms have been carrying out such conversions for eons, but the exact details of the mechanisms are still lacking. Thus these studies include genetic, biochemical and physiological approaches to learn how organisms break down lignocelluloses so that it may be possible to more effectively exploit these processes in a biotechnological context.

Cellulose Binding Proteins of *Clostridium cellulovorans* Cellulase
Abs. 27 University of California - Davis *R.H. Doi*

Studies of the Genetic Regulation of the *Thermomonospora* Cellulase Complex
Abs. 50 Cornell University *D.B. Wilson*

Microbiology and Physiology of Anaerobic Fermentations of Cellulose
Abs. 70 University of Georgia *H.D. Peck, Jr., L.G. Ljungdahl. L.E. Mortenson and J.K.W. Wiegel*

Hemicellulases from Anaerobic Thermophiles
Abs. 74 University of Georgia *J. Wiegel*

Cellulose Fermentation by Nitrogen-Fixing Anaerobic Bacteria
Abs. 106 University of Massachusetts *E. Canale-Parola*

Physiology and Molecular Biology of Extracellular Peroxidases and H_2O_2-Generating System of *Phanerochaete chrysosporium*
Abs. 127 Michigan State University *C.A. Reddy*

Biochemical Genetics of Lignin Degradation by *Phanerochaete chrysosporium*
Abs. 149 Oregon Graduate Institute of Science and Technology *M.H. Gold*

Oxidative Enzymes Involved in Fungal Cellulose Degradation
Abs. 150 Oregon Graduate Institute of Science & Technology *V. Renganathan*

Characterization of Lignin and Mn Peroxidases from *Phanerochaete chrysosporium*
Abs. 156 Pennsylvania State University *M. Tien*

Cellulase - A Key Enzyme for Fermentation Feedstocks
Abs. 165 Rutgers University *D.E. Eveleigh*

Molecular Genetics of Ligninase Expression
Abs. 193 University of Wisconsin *D. Cullen and T.K. Kirk*

11. FERMENTATIVE MICROBIAL METABOLISM

Organisms that live in the absence of atmospheric oxygen oftentimes have unique exaggerated biochemical pathways to derive energy. The projects included in this category focus on attempting to understand the nature of some of these pathways including the unusual pattern of aromatic compound degradation, the production of organic solvents and others. Such basic knowledge could afford new ways of converting biomass resources into useful products and also provides insights into potential applications of these organisms for the degradation of certain environmental pollutants.

Anaerobic Metabolism of Aromatic Compounds by Phototrophic Bacteria: Biochemical Aspects
Abs. 43 Cornell University *J. Gibson*

Ethanologenic Enzymes of *Zymomonas mobilis*
Abs. 57 University of Florida *L.O. Ingram*

Genetics of Solvent-Producing Clostridia
Abs. 78 University of Illinois *H.P. Blaschek*

Molecular Biology of Anaerobic Aromatic Biodegradation
Abs. 89 University of Iowa *C.S. Harwood*

One Carbon Metabolism in Anaerobic Bacteria: Regulation of Carbon and Electron Flow During Organic Acid Production
Abs. 110 Michigan Biotechnology Institute *J.G. Zeikus and M.K. Jain*

The Mechanism of Switching from an Acidogenic to a Butanol Acetone Fermentation by *Clostridium acetobutylicum*
Abs. 129 University of Minnesota *P. Rogers*

Genetics of the Sulfate-Reducing Bacteria
Abs. 133 University of Missouri *J.D. Wall and B.J. Rapp-Giles*

Physiology and Genetics of Metabolic Flux Regulation in *Zymomonas mobilis*
Abs. 136 University of Nebraska *T. Conway*

Effect of Community Structure on Anaerobic Aromatic Degradation
Abs. 148 University of Oklahoma *M.J. McInerney*

Genetic and Biochemical Analysis of Solvent Formation in *Clostridium acetobutylicum*
Abs. 164 Rice University *G.N. Bennett*

Regulation of Alcohol Fermentation by *Escherichia coli*
Abs. 171 Southern Illinois University *D.P. Clark*

Enzymology of Acetone-Butanol-Isopropanol Formation
Abs. 180 Virginia Polytechnic Institute and State University *J.-S. Chen*

Organization and Control of Genes Encoding Catabolic Enzymes in Rhizobiaceae
Abs. 204 Yale University *D. Parke and L.N. Ornston*

12. ONE AND TWO CARBON MICROBIAL METABOLISM

Microorganisms are particularly attuned to the interconversion of simple carbon compounds such as carbon monoxide, acetic acid, methanol and others. For example, the bioproduction of methane in the very large number of varied sites e.g., swamps, rumens, rice paddies largely involves using one or two carbon compounds as precursor

molecules. The studies covered include methanogenesis, methylotrophy and other systems. With such information in hand and with the capabilities of genetic manipulation now possible, entirely new bioconversion processes may be feasible.

Osmoregulation in Methanogens
Abs. 10 Boston College *M.F. Roberts*

Genetics in Methylotrophic Bacteria
Abs. 19 California Institute of Technology *M.E. Lidstrom*

Molecular Biology and Genetics of the Acetate-Utilizing Methanogenic Bacteria
Abs. 34 University of California - Los Angeles *R.P. Gunsalus*

Conversion of Acetic Acid to Methane by Thermophiles
Abs. 51 Cornell University *S.H. Zinder*

Microbiology and Physiology of Anaerobic Fermentations of Cellulose
Abs. 70 University of Georgia *H.D. Peck, Jr., L.G. Ljungdahl. L.E. Mortenson and J.K.W. Wiegel*

Biochemistry and Genetics of Autotrophy in *Methanococcus*
Abs. 73 University of Georgia *W.B. Whitman*

Genetics of the Methanogenic Bacterium, *Methanococcus voltae* With Attention to Genetic Expression Mechanisms
Abs. 84 University of Illinois *J. Konisky*

Exploratory Studies on the Bacterial Formation of Methane
Abs. 86 University of Illinois *R.S. Wolfe*

The Role of Purine Degradation in Methane Biosynthesis and Energy Production in *Methanococcus vannielii*
Abs. 93 University of Kentucky *E. DeMoll*

Carbon Metabolism in Methylotrophic Bacteria
Abs. 102 Los Alamos National Laboratory *C.J. Unkefer*

Genetics of Bacteria that Utilize One-Carbon Compounds
Abs. 130 University of Minnesota - Navarre *R.S. Hanson*

Mechanistic Enzymology of CO Dehydrogenase from *Clostridium thermoaceticum*
Abs. 138 University of Nebraska *S.W. Ragsdale*

Transmethylation Reactions During Methanogenesis from Acetate in *Methanosarcina barkeri*
Abs. 142 Ohio State University *J.A. Krzycki*

Structure and Regulation of Methanogen Genes
Abs. 144 Ohio State University *J.N. Reeve*

Enzymological Studies of One-Carbon Reactions in the Pathway of Acetate Utilization by Methanogenic Bacteria
Abs. 181 Virginia Polytechnic Institute and State University *J.G. Ferry*

Carbon Monoxide Metabolism by Photosynthetic Bacteria
Abs. 196 University of Wisconsin *P.W. Ludden and G.P. Roberts*

13. EXTREMOPHILIC MICROBES

The mechanisms by which microorganisms cope with exceedingly high temperatures, alkalinity, salinity and other factors is an intriguing series of questions. Ordinary life succumbs to such conditions. What mechanisms are there that afford the microbes the ability not only to survive, but also to grow under harsh conditions? This is the theme of this category in which a variety of approaches are being employed. The potential benefit of such information generated could be in designing new biosystems and biologically-derived system enzymes that are capable of activity under harsh industrial process conditions as part of new biotechnological developments.

Membrane Bioenergetics of Salt Tolerant Organisms
Abs. 31 University of California - Irvine *J.K. Lanyi*

Effect of Growth Temperature on Enzyme Folding
Abs. 59 Georgia State University *A.T. Abdelal*

The Metabolism of Hydrogen by Extremely Thermophilic Bacteria
Abs. 60 University of Georgia *M.W.W. Adams*

Hemicellulases from Anaerobic Thermophiles
Abs. 74 University of Georgia *J. Wiegel*

Phylogenetic Analysis of Hyperthermophilic Natural Populations Using Ribosomal RNA Sequences
Abs. 88 Indiana University *N.R. Pace*

Analysis of Thermally Stable Electron Transport components in the Hyperthermophilic Bacterium, *Pyrodictium brockii*
Abs. 90 Johns Hopkins University *R.J. Maier*

Structure and Regulation of L-Glutamate Dehydrogenase in Hyperthermophilic *Archaea* (Archaebacteria)
Abs. 103 University of Maryland *F.T. Robb*

The Respiratory Chain of Alkaliphilic Bacteria
Abs. 134 Mount Sinai School of Medicine *T.A. Krulwich*

Structure and Regulation of an Archaebacterial Promoter: An In Vivo Study
Abs. 141 Ohio State University *C.J. Daniels*

14. MICROBIAL RESPIRATION, NUTRITION AND METAL METABOLISM

In this category some of the unusual characteristics of microbes are under investigation. The ability to switch from aerobic to anaerobic respiration is one question being pursued. Others deal with how certain organisms handle heavy metal ions as part of their metabolism. This type of information could bear on a variety of microbial biotechnology developments.

Genetic Control of Nitrate Assimilation in *Klebsiella pneumoniae*
Abs. 49 Cornell University *V.J. Stewart*

Heavy Metal-lux Sensor Fusions and Gene Regulation
Abs. 77 University of Illinois - Chicago *S. Silver*

Studies on the *bo*-type Ubiquinol Oxidase From *Escherichia coli*
Abs. 82 University of Illinois *R.B. Gennis*

Genomic Plasticity and Catabolic Potential of *Pseudomonas cepacia*
Abs. 107 University of Massachusetts *T.G. Lessie*

Enzymes of Respiratory Iron Oxidation
Abs. 108 Meharry Medical College *R. Blake II*

Biochemistry of Dissimilatory Sulfur Oxidation
Abs. 109 Meharry Medical College *R. Blake II*

15. BIOMATERIALS

The prospects of developing entirely new materials using either organisms or enzymes-based processes is a prospect of great interest. In collaboration with the Materials Sciences Division of the Office of Basic Energy Sciences efforts are being put into studies that could result in the synthesis of new enzymes, polymers and other substances using biological precursors and ideas as the basis.

Enzymatic Synthesis of Materials

Abs. 95 Lawrence Berkeley Laboratory *M.D. Alper, M. Bednarski, H.W. Blanch, M. Callstrom, D. Clark, J.F. Kirsch, B. Novak, P.G. Schultz and C.-H. Wong*

The Effect of Oligosaccharides on Glycoprotein Stability

Abs. 172 Stanford University *C.F. Goochee*

Novel Biomaterials: Genetically Engineered Pores

Abs. 201 Worcester Foundation for Experimental Biology *H. Bayley*

INVESTIGATOR INDEX

UNITED STATES
DEPARTMENT OF ENERGY
WASHINGTON, D.C. 20545

ER-17