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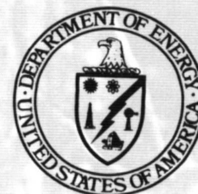

ANNUAL REPORT AND SUMMARIES OF FY 1993 ACTIVITIES

DIVISION OF ENERGY BIOSCIENCES

SEPTEMBER 1993

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

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Cover Picture: *Photo taken along Chesapeake and Ohio Canal in Maryland. Shows diversity of plant conversions among numerous species. Photo by R. Rabson.*

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WASHINGTON, D.C. 20585

Program Overview of the Division of Energy Biosciences

With contemporary society having a growing appreciation of the tremendous value of biotechnological developments in a medical context, the role of plants and non-health related microorganisms also requires increased appreciation. Historically, mankind has had vital dependence on such organisms from the very origin of humans. This has been not only in terms of food, but also as fuels, as fibers, as medicinal drugs and a vast variety of other roles. The fact of the matter is that plants and microorganisms have massive capabilities to perform biochemical conversions and other functions that have been of use to humans, but such resources have only been exploited until now to a limited extent. With recent developments in molecular biology and chemical analyses, plus a wide variety of other technological advances, entirely new and hitherto unavailable approaches have become possible that would generate knowledge of enormous value to humankind. Such opportunities will undoubtedly result in discoveries that will not only improve the supply of food, generate new pharmaceuticals, provide new polymers for fabrics and plastics, but also influence the opportunities to use renewable resources in place of non-renewables for fuels and for a wide variety of other purposes. In addition to all of these contributions, improved understandings of the functions of plants and non-health related microorganisms will most certainly contribute to the restoration of environments that in some cases had been disrupted from their natural, benign condition by mankind's somewhat unconcerned developments.

The mission of the Energy Biosciences program is to generate fundamental information about plants and non-health related microorganisms that will constitute the base for new biotechnologies as well as supply information to improve usages of such organisms in their current form. The collective aims are totally consistent with the Department of Energy's objectives of developing alternate energy sources, replacements for otherwise fossil energy derived products and providing critical fundamental information for the preservation and restoration of environmental conditions affected by energy related activities.

The EB program takes full advantage of its organizational locale in the Office of Basic Energy Sciences to directly interact with such disciplines as Materials Sciences, Chemistry, Engineering and Geosciences to promote cross-disciplinary research and planning activities. These interactions range from joint funding to the impact of biological expertise in the management and function of the numerous national facilities administered by Basic Energy Sciences. One of the major specific objectives of the EB program is to probe the enormous capabilities of the specified organisms to carry out biochemical conversions. The limitation to realization of entirely new products and processes via biotechnology is the lack of basic understanding of natural processes. Such knowledge will then afford the advantage of developing procedures to the benefit of people and their society in providing new products along with providing new employment possibilities.

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 fully understood. Ultimately, such knowledge will underpin the new biotechnologies referred to above. 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.

The scope of any program is determined by a number of factors. While the Energy Biosciences program is not especially large in budget allocations, the scientific scope is fairly broad. Not only does the program cover some areas that are important, well populated, research areas, but, in addition, serious efforts are made to generate interest in critical research areas that are quite underpopulated. Investigators are encouraged to explore ideas that are simply not given much attention for a variety of reasons including a certain amount of riskiness in being able to come up with results in a given time period. Each of the components of the scope is aimed at contributing to building the base of fundamental knowledge about biological mechanisms. Such insightful information will provide opportunities for coming forth with innovative applications in the future.

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 integrated entities.

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, that 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 (such as microbial physiology) that are important but underpopulated is also encompassed.

Please refer to pages 117-137 for additional breakdown of the program into categories.

Despite the vital importance of plants and non-medically oriented microorganisms, these organisms, for many years, have not received the basic research attention they deserve. The Energy Biosciences program, along with certain units in the National Science Foundation and the Department of Agriculture, have been attempting to fill this critical gap. Associated with this effort have been joint efforts which reflect common interests, but at the same time retain the differing individual specific objectives of the three agencies. While these activities to date have been predominantly with plant science research and training (see below), similar collaborative and coordinated efforts are currently being planned for microbial research and training that will probably include additional agencies, including the National Institutes of Health, Office of Naval Research and the Environmental Protection Agency.

One important current aspect of the Energy Biosciences program is the effort to encourage collaborations and coordination among investigators. Clearly, with the diversity of highly sophisticated research technologies becoming more available with time, and their value in enhancing the knowledge about various biological phenomena, it is important to apply them whenever feasible. However, no one individual can be expert in everything, nor can every laboratory afford to have all of the instrumentation. Therefore, collaborations among researchers with different expertises are becoming ever more important. The integration of varied approaches to problems including genetics, physiology, biochemistry and others such as computational and biophysical sciences will certainly provide deeper insights into biological functions and will be fostered where feasible. That is not to suggest that every biological problem is immediately amenable to such integrated approaches, but where appropriate the integration should be sought in order to expedite progress.

In addition to the varied scientific advances that occurred during Fiscal Year 1993 ranging from the discovery, cloning and characterization of noteworthy genes in plants and microbes to the development and application of neural network computer programs that would greatly enhance the speed and ability to identify and discern structures of biological molecules, there were a number of other notable achievements. Upon examination of the abstracts contained herein, examples of advances in diverse areas can be seen. An example of one such study includes research on enzyme regulation:

The survival of biological systems requires tight control of the metabolic pathways that are in operation at any particular time. Twenty-five to thirty years ago considerable attention was paid to regulatory mechanisms acting at the enzyme level that provided short-term control. These studies focused on the popular biochemically-defined model systems (e.g., *E. coli*, yeast, bovine liver). The recent growth in interest in plant science has paralleled the development of modern molecular biology tools which permit sophisticated analysis of developmentally and transiently controlled gene regulation. This led to a rapid advancement in our knowledge of plant gene regulation. The less popular discipline of plant biochemistry is being advanced by a number of EB-funded researchers exploring plant regulatory mechanisms directly affecting enzyme activities. One example is the studies conducted by Dr. Steven Huber and colleagues at North Carolina State University. Dr. Huber has been able to demonstrate that protein phosphorylation occurs during the control of sucrose phosphate synthetase and nitrate reductase, central enzymes in sucrose synthesis and nitrate metabolism. Studies of this type are critical to the future of biotechnological processes involving the modification of plant metabolism.

With respect to technology transfer in which fundamental information generated by the studies supported is adopted and applied in an industrial context, there are numerous instances of where Energy Biosciences supported investigators in universities and other institutions have developed connections with commercial firms. This has meant that when particular knowledge emerges, there is easy transfer of such information. A fine example of the closeness with which the investigators involved in basic research interchange easily with persons from industrial firms was illustrated in a workshop meeting held in late July on Plant Lipids in Minneapolis. The workshop was organized by the chairpersons of the Network on Plant Lipids sponsored under the DOE/NSF/USDA Joint Program on Collaborative Research in Plant Biology. The network, which was initiated about a year ago, had an attendance of over one hundred scientists of which significant representation was from industrial firms. This type of interaction encourages not only information transfer, but also potential collaborations and other activities that will expedite applications. This is evidenced occasionally by the procurement of patents on particular scientific developments. The bottom line is that unlike some scientific areas, where there are serious questions raised about the dearth of technology transfer, in most biological areas the question of technology transfer is not a very serious one. One need only examine the interactions that have occurred that have resulted in early applications of discoveries to support such a conclusion. The interest of the scientists about potential applications of basic information, combined with the corresponding interest on the part of commercial firms in such applications, results in a healthy environment for such transference. This does imply that encouragement of technology transfer related to the Energy Biosciences program is an objective.

The breakdown of how the resources available to EB were distributed in FY 1993 is indicated in the following table.

	Number of Projects	FY 93 funding (in thousands)	Percent of total funds
University and Non-Profit Institutions	182	$ 16,381	65%
Michigan State University Plant Research Laboratory	13	3,292	13%
Three-Agency Plant Science Collaboration Activities--Universities	3	1,830	7%
National Laboratories Brookhaven National Lab Lawrence Berkeley Lab Los Alamos National Lab National Renewable Energy Lab.	11	2,390	9.5%
Small Business Innovation Research (SBIR) contribution, and Miscellaneous		866	3.5%
Conferences, Educational Activities	12	421	1.5%
Databases (joint funding)	1	70	0.5%
	222	$ 25,250	

Some words of explanation are appropriate about the funding figures indicated with abstracts. Most projects are committed to three years of funding with the investigator receiving initially two years of funding with a third year following. 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 1993 was the recipient of 246 new research applications following the screening of numerous preapplications. Of the new applications received, some 12 new projects were funded (ca. 4%). 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 and certain microbiological topics of other agencies as well. All of this indicates a situation where a considerable number of investigators with quality projects are not receiving support from Federal agencies.

During a fiscal year when the Energy Biosciences program resources are seriously limited, new applications covering selected research topics are deferred for the year by not encouraging submissions after receipt of preapplications. The purpose is to avoid investing large amounts of time and effort necessary on the part of an investigator to prepare a research application and also on the part of the research community in reviewing applications. The intent is to reduce the expenditure of effort on the part of the research community in pursuing a resource that is virtually unattainable due to severe budget limitations. The deferred topic areas are changed and are again included in subsequent years of application considerations. Like the declination of any fine research application, this procedure is not gratifying, but it is an attempt to conserve scientists' valuable time.

In the course of the fiscal year, the EB program has provided partial support for the following conferences, workshops or training activities:

1. Conference on Multicellular and Interactive Behavior of Bacteria, Woods Hole, Massachusetts, March 28 - April 1, 1993

2. The Past, Present and Future of Plant Biology, A Symposium to honor Professor Joseph E. Varner, Washington University, St. Louis, Missouri, May 27-29, 1993

3. The Cyanobacterial Workshop, 1993 - The Use of Cyanobacteria to Explore Basic Biological Processes, Pacific Grove, California, May 30 - June 2, 1993

4. FASEB Conference on Ubiquitin and Intracellular Protein Degradation, Saxtons River, Vermont, June 20-25, 1993

5. American Society of Plant Physiologists Plant Biochemistry Course, 1993, University of Wisconsin, Madison, Wisconsin, June 30 - July 18, 1993

6. Summer Investigations into the Metabolic Diversity of the Microbial World, Marine Biological Laboratory, Woods Hole, Massachusetts, Summer 1993

7. Fifth International Conference on Arabidopsis Research, Ohio State University, Columbus, Ohio, August 19-22, 1993.

8. International Symposium on Cellular and Molecular Biology of Phosphate and Phosphorylated Compounds in Microorganisms, Woods, Hole, Massachusetts, September 13-17, 1993

9. Workshop on Stems and Trunks in Plant Form and Function, Oregon State University, Corvallis, Oregon, February 1994

10. Lighting in Controlled Environments Workshop, University of Wisconsin, Madison, Wisconsin, March 27-30, 1994

In addition, funding was provided for three new Life Sciences Research Foundation post-doctoral fellows. These include:

Paul Blount (University of Wisconsin)
David R. Lerner (University of California-San Diego)
Peter Margolis (Stanford University)

Each of these fellowships has a three-year duration.

One of the very noteworthy events of this fiscal year was the convening of a workshop to discuss the needs and opportunities relating to plant biochemistry. The workshop participants were asked to come forth with impressions of what the research and training needs were in order to advance the employment of plants for the benefit of society. In addition, examples were provided of opportunities for the development of new and valuable plant products depending on plant biochemical research. The representation included academic, industrial and governmental personnel. The principal organizer and summarizer of the workshop was Dr. Chris Somerville. A summary report of the workshop was composed using both the views of the attendees and a number of other highly knowledgeable non-attendees who were asked to make comments on an early draft of the summary report. Copies of the report are available on request to the Energy Biosciences office.

Another activity initiated during this fiscal year is an effort to encourage greater participation in plant science training research by minority students. The project, started at Michigan State University, involves having undergraduate students from Howard University, and subsequently other historically black colleges and universities, spend their summer in plant science laboratories at Michigan State University. The intent is to generate genuine interest in plant science research by the direct involvement of the student in the respective investigations. The students are to be members of teams probing plant science problems, and they are to work closely with other investigators in performing experiments. It is expected that with their participation much curiosity and interest will be generated in the students so that they will become seriously committed to seeking graduate education in science.

In Fiscal Year 1992 the second phase of the three agency (DOE/NSF/USDA) Joint Collaborative Program in Plant Sciences was initiated. The program included two components with the objectives of establishing: 1) multidisciplinary training in plant biology, and 2) networks for integrating activities in specific plant research areas. A brief meeting of the representatives of the various programs and those of the federal agencies was held in April 1993 to hear about the progress being made along with opportunities for discussions about the objectives and other aspects of the programs. Clearly, on the basis of reports to date, the three agency program is progressing very well. A number of very excellent meetings, such as the workshop on plant lipids mentioned earlier, and one termed Cytonet, covering relationships between the cytoplasm and other components of plant cells, have been held, with others in the planning stage. The multidisciplinary training activities are well underway also.

The question of how the quality of the program is maintained arose during the last couple of years. All new research applications received that fall within the scope of the program are peer reviewed. This also includes on-going projects which are subject to review on the average every three years. The funding duration for grants averages three years with extensions possible following the renewal review. The review process includes reviews by mail, reviews by site visits in certain instances, and panel reviews. In all cases efforts are made to obtain rigorous, but fair and objective evaluations upon which decisions may be made concerning the quality of the science as well as its potential importance in furthering biological understanding.

As is the case every year, **the staff of the Energy Biosciences program wishes to convey its deep gratitude to the hundreds of persons in this country and abroad who have generously given their time and effort in evaluating the very large number of applications that are received by the program.** Needless to say, without such assistance on the part of the many members of the scientific community, who graciously donate their efforts, the program would not possess the quality that it does.

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

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The staff members of the Energy Biosciences program are :

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Ms. Patricia A. Snyder
Ms. Mary Jo Martin

Division of Energy Biosciences
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U.S. Department of Energy
Washington, DC 20585

Abstracts of Projects Supported in FY 1993

U.S. Department of Agriculture

Madison, WI 53705-2398

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

R.H. Atalla, Forest Products Laboratory *$100,000*

The studies of wood cell wall structure include both a program of measurements of the range of variation of molecular composition and organization within the walls, and an effort to develop a deeper understanding of the processes of structure formation. The methods used include Raman spectroscopy and the Raman microprobe, solid state ^{13}C NMR, and a number of complementary instrumental methods which allow exploration of the states of molecular organization 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 molecular organization in cell walls has been expanded to include biomimetic aggregates incorporating cellulose, hemicelluloses, and lignin precursors polymerized within the polysaccharide matrices. Preparation and characterization of these aggregates has allowed 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, and, more recently, evidence that these interactions allow the polysaccharides to function as templates that organize the assembly of lignin; (4) photoconductivity in woody tissue suggesting unanticipated pathways for charge transport at the nanoscale level 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
$63,000

Sucrose-phosphate synthase (SPS) is a key enzyme of the sucrose formation pathway in leaves, and in many species is reversibly light activated by a mechanism known to involve protein phosphorylation. We have partially purified the protein kinase (SPS-kinase) that phosphorylates and inactivates SPS *in vitro*. The native enzyme has a molecular weight of approx. 147,000 kDa (by gel filtration) and appears to be a homodimer composed of 65 to 70-kDa subunits. We are attempting to obtain amino acid sequence information to produce oligonucleotide probes and use PCR to amplify the kinase gene. We have also sequenced the major regulatory phosphorylation site that is responsible for inactivation of SPS. By comparison with the deduced amino acid sequence of spinach leaf SPS (from R. Klein and M. Salvucci, University of Kentucky), we can identify Ser-158 as the modified residue. There are basic amino acid residues located N-terminal to the phosphorylated seryl residue which may be important recognition elements. Experiments are also underway to elucidate the basis for the apparent light-activation of SPS-protein phosphatase (SPS-PP) activity. New molecular forms of SPS-PP can be resolved by Mono Q chromatography of an enzyme preparation from light leaves compared to dark leaves, but the basis for the effect is still not clear. We have also followed up the recent observation that spinach leaf NADH:nitrate reductase (NR) is light modulated, in parallel with SPS, by a mechanism involving phosphorylation of seryl residues. Peptide mapping analysis indicated that there could be as many as three phosphorylation sites on NR; however, only one or two appear to be of regulatory significance. Phosphorylation of key proteins may be a mechanism regulating C/N balance.

Arizona State University
Tempe, AZ 85287-1604

3. Antenna Organization in Green Photosynthetic Bacteria
R.E. Blankenship, Department of Chemistry and Biochemistry *$104,000*

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 have been used to gain more information about the structure of the pigment oligomers found in chlorosomes. Time-resolved spectroscopy has given insight into the pathway and kinetics of excitation flow from the peripheral region of the chlorosome to the reaction center. New ultrafast absorbance measurements are underway to better define the early energy transfer processes. 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 appears to operate on at least two positions within the energy transfer pathway. 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

4. The Chlorophyll-Binding Protein CP47 in Photosystem II

W.F.J. Vermaas, Department of Botany *$178,010 (FY 92 Funds/2 years)*

Chlorophylls in photosynthetic systems serve to absorb light and to transfer light energy to the so-called reaction center; here this energy is used to fuel biological reactions, such as the reduction of carbon dioxide to sugars, and oxidation of water to molecular oxygen. In photosynthetic systems, chlorophylls are bound to proteins, which provide a suitable orientation and positioning of the chlorophyll molecules, making light-harvesting and energy-transfer processes highly efficient. A main question we approach in our research project is the nature of chlorophyll-protein interactions, and the effect of the protein environment on the properties of the chlorophyll and vice versa. We study the chlorophyll-binding protein CP47 of the photosystem II complex as a suitable system to address this question. By genetic engineering we have obtained a strain of the transformable cyanobacterium Synechocystis 6803 in which most, if not all, chlorophyll is associated with photosystem II. Mutations are now introduced at selected positions of the CP47 protein, and resulting mutants are studied in terms of light energy transfer properties. CP47 appears to be a multi-functional protein in the photosystem II complex: In addition to its involvement in energy transfer, the CP47 protein also participates in creating the environment of the water-splitting (and oxygen-evolving) system. We use targeted mutagenesis to identify and analyze the domains of CP47 that are involved in proper assembly and function of the water-splitting apparatus.

University of Arizona

Tucson, AZ 85721

5. 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 (FY 92 Funds/2 years)

Several factors limit photosynthetic carbon assimilation. These include the intrinsic limitations of the photosystems, the assimilation of CO_2, and metabolism, translocation and storage of carbon products. While strategies that circumvent these identified bottlenecks are being explored in many laboratories, we suggest a different approach. A significant amount of carbon in the biosphere is stored in the form of sugar alcohols, acyclic and cyclic polyols. Different steric forms of polyols are present in virtually every plant, albeit in higher plants that are usually found in small amounts. Generally, few systematic studies that list these compounds have been carried out. Using gene transformation, we have diverted carbon from sugar production into the production of polyols and are investigating effects on photosynthesis, biomass production and environmental stress tolerance in transgenic plants. The genes that we use lead to the production of either mannitol, sorbitol, or ononitol (methyl-inositol). Lines have also been obtained that accumulate several of these polyols. We use tobacco, *Arabidopsis* and tomato as models. All transgenic plants are phenotypically normal even though in some combinations the polyol or polyols accumulating are the major low molecular weight C6-carbon

compound. All polyols confer salt stress tolerance albeit to different degrees depending on the type of polyol. Present experiments include measurements of carbon flux through the engineered pathways.

University of Arizona
Tucson, AZ 85721

6. 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 & Cellular Biology

$87,000

Many plant species separate and 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 physiological, biochemical, and molecular mechanisms of border cell separation in *Pisum sativum.* Border cell separation is developmentally regulated, and varies in response to environmental signals. The process of cell separation is correlated with at least two pectin degrading enzymes, a hydrolase and a pectinesterase. The goals of the project are to characterize 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. Our results are consistent with the possibility that activity of the PE has a dramatic impact on the biology of the root cap. When PE activity is high, during the process of border cell separation, pH of the cell surface as measured by uptake of fluorescein is below 5.0. When PE activity is low, fluorescein uptake does not occur, indicating that the pH of the cell surface in the absence of PE activity increases by at least an order of magnitude. Monoclonal antibodies to pectin of differing degrees of esterification have been used to detect changes in pectin in the root cap during border cell separation. The results are consistent with the possibility that deesterification of pectin progresses linearly during border cell separation, so that when cell separation is complete all of the pectin is deesterified. In addition, the results indicate that localization of the pectin changes from being bound to cell walls of the root tip, to being extracellular. Heterologous probes have been used to select putative root cap PE clones from a root cap specific cDNA library.

University of Arizona
Tucson, AZ 85721

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

B. Larkins, Department of Plant Sciences *$186,001 (FY 92 Funds/2 years)*

We are investigating the interactions between zein proteins in maize endosperm and the role these proteins play in influencing the texture of the mature kernel. Zeins are the storage proteins of maize seed. They are synthesized on rough endoplasmic reticulum (RER) membranes and associate within the lumen of the RER into spherical structures called protein bodies. Two of these proteins, the beta- and gamma-zeins are cysteine-rich proteins that are extensively cross-linked through disulfide bonds. Several kinds of evidence suggest that these sulfur-rich proteins have important structural functions in protein body formation and endosperm hardness. First, the beta- and gamma-zeins are the first proteins to appear in protein bodies, and the way in which protein bodies develop suggests they may

play a role in organizing the alpha- and delta-zeins. Second, mutants that have a soft, starchy endosperm can be converted to a normal phenotype by a 3-fold increase in gamma-zein synthesis. Our recent evidence also suggests that enhanced synthesis of the gamma-zein protein can increase the hardness of even normal genotypes. We are using two approaches to investigate the role the beta- and gamma-zeins play in protein body formation and endosperm texture. First, we have screened a large number of soft, opaque mutants in order to identify mutations that affect the synthesis of the beta- or gamma-zeins. This past year we screened over 6000 mutant lines induced by the Mutator transposable element, as well as several EMS-induced mutants. We found 3 mutants in which gamma-zein synthesis is significantly reduced. These mutants have soft, starchy endosperms, consistent with the notion that this protein plays a role in endosperm hardness. Experiments are in progress to map these mutations and characterize the ultrastructural changes associated with the reduction in gamma-zein content. To study the interaction between zein proteins during protein body formation, we constructed expression vectors consisting of rice endosperm-specific promoters and the coding sequences of the alpha-, beta-, gamma- and delta-zeins. We have also constructed vectors containing a combination of beta- and gamma-zeins and the alpha- and delta-zeins. Transgenic tobacco plants that should produce the alpha, gamma- and delta-zeins are being analyzed. We are in the process of making transgenic plants that will simultaneously synthesize the two types of zein proteins. With the help of Dr. Bill Park at Texas A&M University, we are also testing these gene constructs in transgenic rice plants. Once plants have been identified that produce significant quantities of each type of protein, or combinations of proteins, we will make the appropriate genetic crosses and investigate the interactions between proteins when they are synthesized simultaneously.

University of Arizona
Tucson, AZ 85721

8. Molecular Characterization of the Role of a Calcium Channel in Plant Development

K.S. Schumaker, Department of Plant Sciences — *$146,000 (2 years)*

During the course of development, plants convert physical and chemical signals into specific growth responses. The pathway from environmental cue to developmental change involves a series of biochemical changes in the cell. Controlled changes in cellular calcium ion concentrations are thought to be an important component of this signal transduction pathway. In the moss *Physcomitrella patens*, addition of the phytohormone cytokinin causes a rapid, transient increase in cytoplasmic calcium resulting in vegetative bud formation. Whole cell studies suggest that calcium enters moss cells through plasma membrane calcium channels.

Our research examines the molecular nature of this calcium channel. We use moss protoplasts to establish the presence and biochemically characterize this channel in the native membrane. Preliminary studies show that moss channel activity has similar transport characteristics (voltage-dependence, antagonist and agonist sensitivity) with voltage-dependent calcium channels in animal cells. Taking advantage of the antagonist sensitivity of the channel, we are using isolated plasma membranes to identify and purify the channel polypeptide. Because there may be a specific moss homolog to the animal calcium channel, we have begun molecular studies to isolate the gene encoding the moss channel. Studies are also underway to determine the mechanisms underlying channel gating. Preliminary studies indicate that one of the cytokinin effects in the induction of bud formation is channel regulation.

We expect that access to the channel (polypeptide and gene) and understanding its functional properties will advance our knowledge of the molecular mechanisms underlying calcium regulation and calcium's role in plant development.

University of Arizona
Tucson, AZ 85721

9. Phytoalexin Detoxification Genes and Gene Products: Implication for the Evolution of Host Specific Traits for Pathogenicity
H.D. VanEtten, Department of Plant Pathology — *$84,270*

The production of antimicrobial compounds (phytoalexins) by plants is believed to function as an active mechanism for disease resistance. Our previous work with the diseases of pea and chickpea caused by the fungus *Nectria haematococca* suggest that plant pathogenic fungi may circumvent a phytoalexin-based plant resistance mechanism by detoxifying their host's phytoalexins. Whereas detoxification of the pea phytoalexin pisatin is accomplished via a substrate inducible cytochrome P-450 monooxygenase, medicarpin and maackiain, the phytoalexins from chickpea are detoxified by uncharacterized monooxygenases. This past year a gene (*MAK*1) encoding a monooxygenase that detoxifies the chickpea phytoalexins has been cloned and shown to be different from the genes (*PDA*) encoding the detoxification of pisatin. Of the six known *PDA* genes only those associated with high levels of enzymatic activity in whole cells are linked with high virulence on pea. By studying their level of expression and the biochemical properties of chimeric and native genes products, it has been demonstrated that the observed whole cell phenotypes result from differences in the level of expression of the *PDA* genes and not the biochemical properties of the enzymes encoded by the different genes.

Boston College
Chestnut Hill, MA 02167-3860

10. Osmoregulation in Methanogens
M.F. Roberts, Department of Chemistry — *$86,000*

The ultimate goal of these studies is to understand on a molecular level how methanogens deal with osmotic stress and to use these insights for increasing the salt tolerance of other cells. Three different areas are under investigation: (1) *in vitro* and *in vivo* ^{13}C, ^{1}H, ^{31}P, and ^{15}N NMR studies of *Methanococcus thermolithotrophicus* and *Methanohalophilus* strain FDF1 using soluble substrates for methanogenesis to monitor organic osmolyte production, uptake, or loss (some of these may be paralleled with HPLC studies), (2) ^{39}K and ^{23}Na relaxation studies of these two organisms plus *Methanobacterium thermoautotrophicum* to characterize environmental differences for ions in low and high NaCl cultures, and (3) characterization of lysine 2,3-aminomutase (postulated as the key enzyme for the formation of N^{Σ}-acetyl-ß-lysine), and the K^+ activation of cDPGase (the enzyme that hydrolyzes cyclic-2,3-diphosphoglycerate to 2,3-DPG). The first of these will give us an excellent handle on how the intact cells determine which of several osmolytes will be accumulated or released under different medium conditions. The second will use multiple quantum NMR experiments to estimate slow (T_{2s}) and fast (T_{2f}) components of ^{39}K and ^{23}Na T_2 to see if there are significant changes in

macromolecule/ion interactions with altered external NaCl. The protein work will provide us with at least one enzyme (LAM) whose activity is regulated by changes in external NaCl and another that is activated by intracellular K^+.

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

11. Differential Regulation of Plastid mRNA Stability
D.B. Stern *$168,000 (2 years)*

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 identified and partially purified three proteins from spinach chloroplasts that are involved in the formation of a 3' hairpin RNA:protein complex. One RNA-binding protein of 41 kd binds to a double-stranded region of the hairpin, and mutant RNAs unable to bind this protein are degraded in several types of *in vitro* assays. To understand the mechanism by which the 41 kd protein and other RNA-binding proteins stabilize RNA, we are introducing reporter genes with different 3' hairpin structures into tobacco chloroplasts by stable transformation, with the aim of using altered RNA sequences to manipulate RNA accumulation and gene expression *in vivo*. Since RNA-binding proteins appear to affect the ability of ribonucleases to degrade RNA, we have also studied an endonuclease activity that cleaves near the hairpin, and whose activity may be regulated by the presence or absence of RNA-binding proteins. Purification of the endonuclease and the hairpin-binding proteins are currently in progress.

Brookhaven National Laboratory
Upton, NY 11973

12. Plant Molecular Genetics
B. Burr and F.A. Burr, Biology Department *$350,000*

We have established two recombinant inbred families of maize that have been used to construct a high density map of the maize genome with molecular markers. These populations are being used by the maize genetics community for rapid mapping of cloned genes.

These populations are also well suited for the study of genetic factors controlling quantitative traits. Our aim has been to identify and define these loci and study their interactions at the molecular level. We have chosen three model systems for investigation. The most intensively studied one to date has been anthocyanin accumulation which was selected because many of the genes involved in its regulation and biosynthesis have been cloned. The primary lesson from this trait is that regulatory genes are candidates for quantitative trait loci. This is borne out when we examine levels of transcription. This result may be because slight changes in regulatory genes, as opposed to structural

genes, are most likely to have measurable effects on the overall pathway. A second model system being examined is carotenoid biosynthesis. Here it is possible to quantitatively measure many of the intermediate compounds in the carotenoid pathway from phytoene to the xanthophylls. We are using carotenoids as a quantitative trait to test the prediction that the most consequential loci implicated in the control of these characters will be regulatory genes. A third quantitative trait that we have investigated is telomere length. This trait demonstrates that there are clear differences in quantitative trait loci between populations and has been used to test the accuracy of our models.

Brookhaven National Laboratory
Upton, NY 11973

13. Molecular Bases of Photoadaptation in Unicellular, Eucaryotic Algae
P.G. Falkowski and J. LaRoche, Department of Applied Science *$70,000*

In contrast to higher plants, the photosynthetic apparatus in eucaryotic algae is remarkably plastic. The cellular level of light harvesting pigment protein complexes 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 LHCPs. 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. Nuclear run-on transcription assays have been developed to assess the importance of transcriptional control and 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

14. Regulatory Enzymes of the Thylakoid: Isolation and Characterization of Protein Kinases and a Protein Phosphatase
G. Hind, Biology Department *$355,000*

This project seeks information on the structure, function, and organization of enzymes associated with the stromal surface of the thylakoid. Of principal concern are enzymes that optimize photosynthetic efficiency.

The distribution of light energy between the two photosystems is modulated by a redox-regulated, reversible phosphorylation of a mobile pool of light-harvesting chlorophyll *a/b* protein (LHC-II). The responsible protein kinase has been sequenced from the cDNA. The deduced precursor protein contains an N-terminal chloroplast transit peptide of 101 amino acids, while the mature protein has an N-terminal domain of 358 residues, separated by a 13-residue, glutamine-rich link from a C-terminal

domain of 167 residues. The primary sequence lacks canonical protein kinase motifs but contains presumptive copper redox centers, possibly involved in control of catalytic activity. Functional sites for nucleotide binding, substrate binding, autophosphorylation, and redox control will be identified in native kinase and mutant enzyme expressed from the cDNA. Parallel studies will examine the structure and function of a 25 kDa protein kinase which we have also isolated from thylakoids and have partially purified.

The corresponding dephosphorylation of LHC-II is catalyzed by a constitutively active, fluoride-sensitive phosphatase with pH 8.0 optimum. We have isolated this enzyme from pea thylakoids as a 51.5 kDa, catalytically active monomer with an inhibitor-sensitivity profile resembling that of mitochondrial and prokaryotic phosphoprotein phosphatases. The primary sequence of thylakoid phosphatase will be determined from the cDNA. Post-translational modifications that provide for its attachment to the membrane will be examined in detail.

Brookhaven National Laboratory
Upton, NY 11973

15. Characterization of Stearoyl-ACP Desaturase
J. Shanklin, Biology Department *$270,000*

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

16. δ-Aminolevulinate Biosynthesis in Oxygenic Prokaryotes
S. Beale, Division of Biology and Medicine *$97,000*

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

17. Genetics in Methylotrophic Bacteria
M.E. Lidstrom, Environmental Engineering Science *$194,000 (2 years)*

The purpose of this project is to use genetic techniques to study the transcriptional regulation of C-1 specific functions in methylotrophic bacteria. Part of this effort involves the study of promoters, and we have identified promoter regions upstream of *moxF* genes (genes encoding the large subunit of methanol dehydrogenase) from two different Type I methanotrophs and the facultative serine pathway methanol-utilizer, *Methylobacterium extorquens* AM1. Transcriptional start sites have been mapped, and the sequences upstream are not similar to each other, nor to any known promoters. Further work is in progress to characterize the promoter regions, but the sequences suggest that novel sigma factors must be necessary for their transcription. In order to investigate this idea, we have purified the RNA polymerase from *M. extorquens* AM1, and have isolated a putative sigma factor that directs specific transcription of *moxF in vitro.* We are currently cloning the gene encoding this polypeptide. The rest of the project has focused on understanding the regulatory network for methanol oxidation in *M. extorquens* AM1. A number of methanol oxidation genes (*mox* genes) have been identified that are required for transcription of the structural genes for methanol dehydrogenase. The function of these genes and their relationship to each other is currently under study.

California Institute of Technology
Pasadena, CA 91125

18. Molecular and Genetic Analysis of LEAFY, a Gene Controlling Floral Induction and Flower Development in Arabidopsis thaliana
E. Meyerowitz, Division of Biology *$124,000*

We are studying the LEAFY gene of *Arabidopsis thaliana* as a way of understanding two related processes: floral induction, and floral organ differentiation. Plants mutant for LEAFY show, in several ways, a partial conversion of each flower to an inflorescence stem, and in addition an absence of petals and stamens. LEAFY is thus necessary for lateral meristems to assume a fully floral identity, and also for proper development of floral organs. To study the effect of LEAFY on floral induction, we have cloned the gene, shown it to be the earliest known gene expressed in developing floral primordia, and shown that it acts in concert with at least two other meristem identity genes, APETALA1 and CAULIFLOWER, in specifying that lateral meristems be flowers. To study the action of LEAFY in specifying petals and stamens, we have analyzed the expression of a variety of the organ identity genes of *Arabidopsis* flowers in plants mutant for LEAFY (with or without additional mutations in APETALA1 and CAULIFLOWER), and shown that LEAFY, with the other meristem identity genes, is necessary for the induction of the later-acting organ identity genes. LEAFY thus plays a key role in the translation of environmental signals into patterns of gene induction in developing flowers. That

LEAFY may be a key regulator of reproductive development in plants other than *Arabidopsis* is indicated by our having cloned parts of homologous genes from very distant relatives of *Arabidopsis*, including the nonflowering plant *Ginkgo biloba.*

University of California
Berkeley, CA 94720

19. Genetic Analysis of Adh1 Regulation
M. Freeling, Department of Plant Biology *$94,000*

There are two *Alcohol dehydrogenase* genes in maize. Both are intricately connected to the development and to the environment of the plant. This project aims to better understand the "language of gene regulation" used to instruct the *Adh* genes; our approach is to obtain mutants that are altered in regulatory behaviors and then to elucidate the mechanism by which misinformation was delivered or interpreted. We have been most successful in characterizing several transposon-directed, small deletions and insertions that altered organ specificities of expression, especially in pollen. Deletions of the TATA box alter organ specificities by starting messages at different points in different organs, and some messages are translated better than others (Kloeckener-Gruissem et al., 1992). Suspecting surrogate TATA binding functions, we cloned two functional, duplicate *Tbp* genes in maize, found that both function in yeast and both are expressed nonidentically in plants (Vogel et al., 1993), but there was not a special pollen *Tbp* as we had suspected. Dawe and Freeling (1992) found that tiny insertions in a specific region of the DNA encoding untranslated leader generated over- or underexpression of ADH1 message specifically in pollen. Further, we found that wild-type alleles of *Adh2* naturally variegate in specific epidermal cells just below the root meristem. -- Various selection procedures allowed us to select hundreds of *Adh* mutants and revertants over the years. Unfortunately, we have been unable to select mutants genes necessary to turn *Adh* "on" anywhere in the plant. We have sequenced an anaerobic protein and have preliminary evidence that it is regulatory, and ADH is an anaerobic protein, but this genetics laboratory cannot continue with a system that will not deliver upstream **mutants.** For that reason, we have used other funding sources to develop a replacement system that can better address our questions as to the "language" of developmental gene regulation: the gene pathway that induces the ligule.

The leaf of maize is in two parts, the sheath that wraps around the stem, and the blade that lays out in the sun. Between the sheath and blade lies the ligule (i.e., epidermal ligule fringe and two auricles). Several mutants alter the shape or position of the ligule; four are cloned. Recessive mutants in two genes remove the ligule: *lg1* is cloned and *lg2* is transposon-tagged. From genetic experiments, we know that Lg2 precedes LG1 in function, and that the R-type helix-loop-helix transcriptional factor that turns on anthocyanine genes in the ligule is downstream from LG1; certain lines of maize only color the ligule of the leaf. Finally, we have mutants that alter only parts of the ligule, and must certainly lie even further downstream. See Freeling (1992). We are preparing to renew this DOE grant using the genes in the ligule induction cascade as our experimental system. The questions of gene regulation asked of *Adh* genes and of ligule induction genes are actually the same.

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Dawe RK, Lachmansingh AR and M Freeling (1992). Transposon-mediated mutations in the untranslated leader of maize *Adh1* that increase and decrease pollen-specific gene expression. *The*

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Vogel, JM, Roth B, Cigan M and M Freeling (1993). Expression of the two maize TATA-binding protein genes, and function of the encoded TBP proteins by complementation in yeast. *The Plant Cell*, in press.
Freeling, M. 1992. A conceptual framework for maize leaf development. *Develop. Biol.* 153:44-58.

University of California
Berkeley, CA 94720

20. Regulation of Tomato Fruit Growth by MVA and GTP-Binding Proteins
W. Gruissem, Department of Plant Biology *$214,500 (FY 92 Funds/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

21. Determination of Genes and Phenotypes of Bacteria Necessary for Epiphytic Colonization and Survival on Plants
S.E. Lindow, Department of Plant Pathology *$80,000*

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 that allow them to survive the stress of the hostile leaf surface environment. We have applied a random mutagenesis approach to identify genes in *Pseudomonas syringae* that are involved in epiphytic fitness, thereby directly identifying genes which are required for epiphytic growth or survival, and avoiding the limitations of investigating only predetermined phenotypes exhibited by bacteria in culture. Of 42 mutants that were decreased in their tolerance of stresses associated with dry leaf surfaces, most did not show deficiencies in stress tolerance phenotypes that were expressed in culture. Four mutants with the highest sensitivity to

stress on leaves grew normally on wet leaves but exhibited multiple phenotypic alterations resulting from single Tn5 insertions. One locus encodes proteins of 46 and 21 kD whose function is still unknown but may condition the expression of other genes which presumably mediate tolerance to environmental stress. Further characterization of two other distinct environmental stress tolerance loci is proceeding and the "downstream" genes regulated by one or both of these loci are being identified and their pattern of regulation characterized using fusions with a promoterless ice nucleation gene.

University of California
Berkeley, CA 94720

22. Phytochrome from Green Plants: Assay, Purification and Characterization
P.H. Quail, Department of Plant Biology *$194,000 (FY 92 Funds/2 years)*

The regulatory photoreceptor, phytochrome, is encoded by a family of five divergent genes, *phyA, B, C, D* and *E*. Using photomorphogenic mutants and transgenic plants overexpressing different phytochromes, we have obtained initial evidence that individual family members perform discrete photosensory functions in regulating plant responses to light. Hypocotyl elongation growth in transgenic *Arabidopsis* seedlings overexpressing phytochrome B is substantially more sensitive to inhibition by continuous red light (Rc) than in wild type (WT). Conversely, the phytochrome B-deficient *hy3* mutant of *Arabidopsis* is unresponsive to Rc. However, neither phytochrome B-overexpressors nor the *hy3* mutant are different from WT in responsiveness to continuous far-red light (FRc). These data indicate that phytochrome B mediates the effects of Rc but not FRc. By contrast, transgenic phytochrome A-overexpressors are hypersensitive to FRc, and phytochrome A-deficient *hy8_* mutants are unresponsive to FRc but normal in responsiveness to Rc. These data indicate that in WT seedlings phytochrome A mediates the effects of FRc but not Rc. Taken together, the evidence supports the conclusion that phytochromes A and B have reciprocal photosensory roles in mediating photomorphogenic responses in etiolated seedlings to the red/far-red region of the spectrum.

University of California
Berkeley, CA 94720

23. Cloning and Characterization of Genes Determining Disease Resistance in Arabidopsis-Pseudomonas Interactions
B.J. Staskawicz, Department of Plant Pathology *$101,000*

We are currently genetically dissecting the disease resistance pathway in *Arabidopsis-Pseudomonas* interactions. Specifically, we have screened diepoxybutane mutagenized M2 seed and naturally occurring ecotypes of *Arabidopsis* for disease susceptible plants that no longer mount a defense reaction in response to strains of *Pseudomonas syringae* containing the avrRpt2 avirulence gene. Four diepoxybutane-induced mutants and one natural occurring ecotype (Wu-0) have been identified and genetically characterized. Genetic complementation analyses have revealed that all the Col-0 mutants and the Wu-0 ecotype map at or very close to the RPS2 locus.

Fine structure RFLP mapping has allowed us to determine that this locus maps on chromosome 4 closely linked the M600 RFLP marker. This information has allowed us to construct a YAC contig that spans this locus. Furthermore we have localized the locus to a cosmid contig that spans approximately 100 kB. We are currently examining these mutants for alterations at the DNA and RNA

level in hopes of correlating susceptible phenotypes with specific nucleic acid alterations. Finally, binary cosmids containing this region are currently being introduced into the disease susceptible mutants by Agrobacterium-mediated transformation to identify cosmids that contain the RPS2 locus.

University of California
Berkeley, CA 94720

24. Analysis of Genes Essential for Floral Development in Arabidopsis
P. Zambryski, Department of Plant Biology *$91,000*

The *tousled* mutation of *Arabidopsis thaliana* exhibits a complex phenotype. The leaves of the mutant are abnormal, displaying deeper serrations on their margins than wild type leaves. Flowers of homozygous *tsl* plants each contain a random set of floral organs (sepals, petals, stamens and a bicarpellate gynoecium); their positions are correct, but their number is reduced. Scanning electron microscopy of *tsl* floral meristems indicates that the *TSL* gene acts early during flower development, and affects the number and positioning of the primordia in the floral meristem. A *tsl* mutant line has been generated which contains a T-DNA insertion element that cosegregates with the mutant phenotype. The T-DNA has been used to identify and characterize the *TSL* locus. A fragment of wild-type DNA from the region has been transformed into *tsl* plants and rescues the mutant phenotype. The *TSL* gene encodes a 78 kDa protein with three structural domains. The C-terminal half of the protein shares the conserved residues found in the catalytic domain of known protein kinases, and is most homologous to the serine/threonine class. It does not fall into any known subgrouping of protein kinases, and may be the first representative of a new family. The N-terminal half of the protein consists of a glutamine-rich domain followed by an α-helical domain which contains two segments predicted to participate in coiled-coil structures. The *TSL* gene is most abundantly expressed in developing floral meristems, but the transcript can also be detected at lower levels in both roots and leaves. The *TSL* gene product may participate in a signalling pathway acting during several stages of normal plant development.

University of California
Davis, CA 95616

25. Cellulose Binding Proteins of Clostridium cellulovorans Cellulase
R.H. Doi, Department of Biochemistry and Biophysics
$212,000 (FY 92 Funds/2 years)

The overall goal of this project is to determine the structure and function of the multi-subunit *C. cellulovorans* cellulase which is capable of degrading crystalline cellulose. The enzyme complex (cellulosome) is comprised of at least 10 different enzymatic and non-enzymatic subunits. As part of this specific proposal we have cloned and sequenced a major non-enzymatic subunit that has been designated as cellulose binding protein A (CbpA). CbpA is essential for the degradation of crystalline cellulose and is a large 190 kDa protein. From its amino acid sequence homology to other cellulases, a putative cellulose binding domain (CBD) was recognized. By subcloning and expression of this part of the cbpA gene, a polypeptide of about 20,000 daltons was obtained that was capable of binding to crystalline, but not soluble forms of cellulose. Furthermore an investigation of one of the 8 hydrophic repeats of CbpA revealed that the hydrophic repeat polypeptide was capable of binding endoglucanases. We have designated this as the endoglucanase binding domain (EBD). These

results indicate that the entire CbpA molecule is not required for the analysis of its functions. Current studies are determining the specific amino acid residues responsible for the function of the CBD and EBD domains.

University of California
Davis, CA 95616

26. 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 (FY 92 Funds/2 years)*

In the urbanized societies, hydroelectric power plants, urban populations and agriculture often compete for high-quality water. Therefore, a technology facilitating the use of low-quality water or reuse of water reduces dependence on this limited resource. Since low-quality water or reused agricultural water is usually enriched in toxic elements and salts, increased tolerance of toxic elements and salts is an important breeding objective for 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 incorporate that competence into crops. The focus of this phase of the project is on genetic and physiological characterization of salt tolerance in halophytic *Lophopyrum elongatum.* The entire genome and each of the seven chromosomes of this species have been incorporated into wheat. The entire genome dramatically enhances the salt tolerance of wheat. While each of the seven chromosomes was shown to be involved in this enhancement, chromosome 3E was shown to play a major role. The same chromosome was shown to play a major role in the exclusion of toxic Na^+ and the accumulation of compatible K^+, resulting in high K^+/Na^+ ratios in leaves under salt stress. Other *L. elongatum* chromosomes controlling the K^+/Na^+ ratio and the accumulation of Cl^-, Ca^{2+}, and Mg^{2+} were identified and it was shown that K^+/Na^+ accumulation highly correlates with grain yield under salt stress in wheat and *L. elongatum.*

University of California
Davis, CA 95616

27. Protein Translocation and Assembly in Chloroplasts

S.M. Theg, Section of Plant Biology *$182,000 (2 years)*

The objective of this project is to investigate a number of aspects relating to the biogenesis and operation of the oxygen-evolving complex (OEC) in chloroplasts. This enzyme complex, which is responsible for most of the oxygen in the atmosphere, is extrinsically associated with the inner surface of the chloroplast thylakoid membrane, and is formed from three nuclear-encoded subunits. These subunits must be translocated across the two chloroplast envelopes and the thylakoid membrane before they can be assembled into the enzyme complex. The experiments undertaken during this project will (i) create site-directed mutations in each of the three extrinsic subunits, and evaluate their effects on the ability of the subunits to assemble correctly into the OEC; (ii) use those mutated subunits that retain the ability to assemble as site-directed probes of OEC function; and (iii) determine the absolute energy requirements for the translocation of OEC subunits across the envelope and thylakoid membranes. These experiments will lead to identification of the amino acid residues

responsible for the binding of the subunits to one another in the complex, and perhaps for binding the required cofactors Mn, Ca^{2+} and Cl^-. In addition, this study will provide the first estimate of the true energy costs to eukaryotic cells of their considerable protein trafficking activities.

University of California
Davis, CA 95616-8515

28. Vacuole Biogenesis in Differentiating Plant Cells
T.A. Wilkins, Department of Agronomy and Range Science
$236,000 (FY 92 Funds/2 years)

During plant cell differentiation, the complex interaction between internal cellular forces and cell wall structure governs the direction of cell expansion and ultimately determines the shape of the cell. As a focal point to study the crucial role of vacuoles in turgor-driven cell expansion, our efforts focus on the regulation of vacuolar H^+-ATPase (V-ATPase) gene expression in rapidly elongating cotton seed trichomes. The expression of V-ATPase subunit A mRNAs are temporally regulated during trichome elongation in two successive waves that accompany the onset of elongation and the developmental switch from isotropic to anisotropic cell expansion. The sudden decrease of V-ATPase mRNAs to basal levels of expression presages termination of trichome elongation. Subunit A mRNAs cannot be detected in a glabrous ("fiberless") genetic mutant in developing ovules corresponding to the stage of peak elongation in the wild-type, suggesting the possibility of a trichome-specific isoform. Two cotton V-ATPase subunit A cDNA clones have been shown to belong to a superfamily, comprised of two discrete multigene families in both diploid and tetraploid cotton (*Gossypium*) species. This finding indicates an ancient origin of the families that predates polyploidization of tetraploid species and is the first molecular evidence of secondary polyploidy in the cotton family. Efforts are continuing to investigate the regulation of V-ATPases at the RNA and protein levels in wild-type and genetic trichome mutants.

University of California
Irvine, CA 92717

29. Membrane Bioenergetics of Salt Tolerant Organisms
J.K. Lanyi, Department of Physiology and Biophysics *$306,000 (2 years)*

Chemiosmotic energy transduction is part of the strategy for dealing with high salt concentrations in the extremely halophilic halobacteria. The proton and chloride transporting bacterial rhodopsins and the proton transporting ATPase are described. Studies of the first two systems, bacteriorhodopsin and halorhodopsin, respectively, are focussed on the thermodynamics of the reaction cycles, the role of the immediate environment of the retinal Schiff base in initiating ion transfer, and on single residues that are identified as participants in the transport. Site-specific mutagenesis with gene expression in *Halobacterium halobium*, and optical multichannel spectroscopy are special methods developed for this work. Studies of the third system, the membrane ATPase of *Halobacterium saccharovorum*, concentrate on describing the catalytic site, the nature and causes of non-linear hydrolytic kinetics and its relationship to energy coupling, and the shared features with eubacterial and eukaryotic ATPases.

University of California
La Jolla, CA 92093-0116

30. Structure, Biosynthesis and Role of Complex Protein-bound Glycans
M.J. Chrispeels, Department of Biology *$91,000*

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 have isolated a mutant of *Arabidopsis thaliana* that is blocked in the pathway of glycan modification. Glycans accumulate in the $Man_5GlcNAc_2$ form, and the mutant is deficient in GlcNAc-transferase I. Further studies on this mutant plant will include the complementation with mammalian GlcNAc transferase I. If complementation is successful then we will be in a good position to study the targeting of this Golgi enzyme. It would furthermore be the first demonstration that a human enzyme can complement a plant mutant.

The glycoproteins that are being studied are in the arcelin/α-amylase inhibitor family. 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 are underway. Our next objective will be to transform other grain legumes (mung bean, adzuki bean, chickpea) with the α-amylase inhibitor gene to make the seeds resistant to *Callosobruchus maculatus* and *C. sinenis.* 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 and have evolved.

University of California
Los Angeles, CA 90024-1569

31. Energy Capture and Use in Plants and Bacteria
P.D. Boyer, Molecular Biology Institute *$73,537*

Present research focuses on property of the F_1-type ATPases from spinach chloroplasts and beef heart mitochondria. A principal aim is to assess the hypothesis that during ATP hydrolysis three catalytic sites function equivalently and sequentially with strong catalytic cooperativity. Evidence indicates that with a trinitrophenol derivative of ATP (TNP-ATP) unusual preference for binding to a third catalytic site with weak affinity for ATP may be uncovered. Other evidence indicates a previously overlooked participation of Mg^{2+} in the onset of a potent inhibition by TNP-ADP. This has led to erroneous literature reports and their clarification promises to give useful insights into mechanism. In another approach, ^{18}O exchange techniques are being used to probe whether the action of specific inhibitors aurovertin and tentoxin interfere with catalytic cooperativity and change catalytic characteristics. Preliminary findings indicate that aurovertin at low concentrations partially frees catalytic sites from cooperativity and, because identical sequential reactions with three sites are not feasible with one bound inhibitor present, give rise to multiple catalytic pathways revealed by ^{18}O isotopomer distributions. A goal is to bring these and related studies to the publication state and close out the project during the year.

University of California
Los Angeles, CA 90024

32. Molecular Biology and Genetics of the Acetate-Utilizing Methanogenic Bacteria
R.P. Gunsalus, Department of Microbiology and Molecular Genetics
$227,000 (FY 92 Funds/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, designated *cdhA*, have been cloned from *Methanosarcina thermophila* TM-1 and their transcriptional regulation is being characterized in response to acetate availability. We are also examining post-transcriptional processing of *cdhA* mRNA upon shift to alternative methanogenic substrates. In companion studies, the molecular and physiological basis of osmoregulation in methanogens is being examined. When external salinity or dissolved solute levels increase, the cells produce N-acetyl-ß-lysine and ß-glutamine as osmoprotectants. We are establishing the biosynthetic pathways for their production and studying their physiological regulation. Uptake studies with the alternative osmolytes, α-glutamate and glycine-betaine are also in progress to determine when and how each is transported into the cell. These projects offer useful models to elucidate regulatory mechanisms employed in acetotrophic methanogens. The aim of these studies is to better understand how the methanogens adapt to changing environments and how they ultimately contribute to anaerobic biodecomposition reactions in nature.

University of California
Los Angeles, CA 90024

33. The Gibberellin A_{20} 3ß-hydroxylase: Isolation of the Enzyme and Its Molecular Biology
B.O. Phinney and J. MacMillan, Department of Biology *$142,000 (2 years)*

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 GA_{20}-3ß-hydroxylase that catalyzes this step. We have previously incubated tissue preparations with $[17\text{-}^{13}C,^{3}H]GA_{20}$ as substrate and have found by GC-MS studies that the metabolic products are GA_1 and GA_{29}, the highest GA_{20}-3ß-hydroxylase activity was obtained from nodal tissues. We have been unsuccessful in an attempt to isolate a maize cDNA for the GA_{20}-3ß-hydroxylase by screening a maize cDNA library with a monoclonal antibody raised against a partially purified bean GA_{20}-3ß-hydroxylase. In addition, we have continued and expanded our search for transposon (both Ac and Mu) induced dwarf mutants of maize. To date we have isolated three non-allelic GA-responding dwarfs which are currently being examined by Southern blots for the presence of transposon hybridizing bands which segregate with the dwarf phenotypes.

University of California
Los Angeles, CA 90024-1606

34. Sensory Transduction of the CO_2 Response of Guard Cells
E. Zeiger, Department of Biology *$182,000 (FY 92 Funds/2 years)*

Carbon dioxide plays a key role in the regulation of stomatal movements and in the coupling between stomata and the photosynthesizing mesophyll. This research project is aimed at the characterization of sensory-transducing mechanisms of the CO_2 response of guard cells. Two metabolic reactions are potential biochemical sensors for the CO_2 signal: the carboxylation of phosphoenolpyruvate by PEP carboxylase, and the carboxylation of ribulose bisphosphate by Rubisco. Metabolism of CO_2 via these reactions results in the accumulation of two distinct osmoregulatory products: malate and sucrose. We analyze the organic acid and sugar content of isolated guard cells by HPLC to determine which osmoregulatory pathway(s) is modulated when stomatal apertures change. We study the osmoregulation of the CO_2 response in growth chamber-grown *Vicia* leaves in which stomatal apertures are primarily controlled by ambient CO_2 concentrations. Recent results indicate that guard cells from stomata opened in response to lower CO_2 concentrations accumulate malate in the early stages of opening. Malate content declines later and osmoregulation by sucrose becomes substantial. These data substantiate periodic reports on the role of sucrose in guard cell osmoregulation. We are currently conducting the HPLC analysis of malate and sucrose in conjunction with potassium and chloride measurements, and we are developing radiotracer methods aimed at the characterization of key regulatory steps in the sensory transduction of the CO_2 response.

University of California
Santa Cruz, CA 95064

35. Tonoplast Transport and Salt Tolerance in Plants
L. Taiz, Biology Department *$92,000*

We have been studying the mechanism of the key primary active transport process at the tonoplast: ATP-driven proton transport carried out by the vacuolar ATPase (V-ATPase) in plants as well as in yeast. In yeast we have shown that Cys 261 in the catalytic domain is the site of binding by N-ethylmaleimide, although it is not required for catalytic activity. Cys 284, however, is required for activity, although its function remains to be determined. We have also been studying the kinetics of sulfite activation of the yeast V-ATPase. The V-ATPase exhibits nonlinear hydrolysis kinetics caused by tightly-bound ADP at the catalytic site. Sulfite promotes the reactivation of inactive enzyme by causing a 5-fold increase in the Km for nucleotide. Since reactivation of hydrolysis is associated with a decrease in proton transport, sulfite appears to induce "slip". The increase in Km results in sulfite inhibition at low substrate concentrations. In collaboration with Dr. Thomas Rausch at the University of Frankfurt we have investigated the effect of salinity on the expression of the gene for the A subunit of the V-ATPase of carrot suspension-cultured cells. NaCl induced a 2-fold increase in proton transport activity, without, however, significantly altering ATPase activity, immunologically-detectable protein synthesis or gene transcription. These results suggest that NaCl may enhance proton pumping by altering the coupling efficiency between hydrolysis and proton transport.

University of Chicago
Chicago, IL 60637

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

R. Haselkorn, Department of Molecular Genetics and Cell Biology
$184,008 (2 years)

Our program has both narrowed and expanded. Focus has sharpened on the putative sigma factor required for the transcription of the *nif*A genes of *Rhodobacter*. Mutagenesis followed by screening with a *nif*A::*lacZ* fusion has not turned up any candidates, raising the possibility that the sigma factor is used for other, essential genes as well. Alternative selections based on expression of the sigma gene from a library are being constructed. Meanwhile, the search for genes regulated by *nif*R4, the *rpo*N homologue in *Rhodobacter*, has expanded to exploit the complete cosmid library constructed recently. Blots of the entire library are being hybridized differentially with RNA from wild-type cells after saturation with RNA from a *nif*R4 mutant, both induced by nitrogen step-down and anaerobiosis. Clones that map away from the already known *nif* genes will be picked up and characterized further by sequencing (a little) and by gene inactivation, using the interrupted cloned gene as mutagen.

The physical map of the *Rhodobacter* chromosome was extended to the fine-structure level. A complete set of overlapping cosmids (192 in all) covers the chromosome and the large plasmid in strain SB1003. The entire set of cosmids has been transferred to Barry Marrs at DuPont for maintenance and distribution to the community as needed. We are prepared to hybridize any cloned DNA fragment to the cosmid array for mapping purposes. Cosmids identified by this experiment should then be available from Marrs. At present we are using the cosmid set to define the rearrangements of the chromosome in a number of *Rhodobacter* strains and to create a set of deletions removing, systematically, 30-40 kb segments of the chromosome corresponding to the insert in each of the cosmids.

The University of Chicago
Chicago, IL 60637

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

D.G. Lynn, Department of Chemistry *$91,000*

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

38. The Magnesium Chelation Step in Chlorophyll Biosynthesis
J.D. Weinstein, Department of Biological Sciences — *$87,000*

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. Using an active organelle-free preparation from pea chloroplasts, we have demonstrated that magnesium insertion requires a minimum of two discrete steps. The first step is an activation step which requires a higher protein concentration and can utilize ATPγS instead of ATP. The second (catalytic) step also requires ATP, but does not work with ATPγS. The second step occurs in solutions of more dilute protein concentration. Progress has been made in obtaining a completely soluble system which will facilitate the fractionation of the two or more protein components which are required for activity. Preliminary work with a similar organelle-free preparation from greening cucumber cotyledons has demonstrated that this system also requires two different protein-containing fractions, and that the activity in each fraction increases with greening time.

Cold Spring Harbor Laboratory
Cold Spring Harbor, NY 11724

39. The Suppression of Mutations Generated by Mu Transposons in Maize
R.A. Martienssen and V. Sundaresan — *$75,000*

Mutations caused by the insertion of *Mutator (Mu)* transposable elements are frequently dependent on the presence of active autonomous elements elsewhere in the genome. Such mutations are phenotypically suppressed when *Mu* activity is lost. Recessive alleles of this class of mutation at three

different cloned loci have *Mu1* or *Mu8* elements in the promoter region of the affected gene. In the case of the pale green seedling lethal *hcf106*, phenotypic suppression is accompanied by transcript initiation within the *Mu1* element that results in the production of a functional chimeric *Mu1::Hcf106* message. This results in a normal dark green phenotype that can be used to monitor loss of *Mu* activity both germinally and in somatic sectors. Plants doubly mutant for *hcf106* and for a dominant *lesion-mimic* mutation lose both phenotypes co-ordinately resulting in sectors of wild-type tissue in double mutant plants. We are using these observations to develop (1) a simple PCR method for amplifying chimeric *Mu1*-containing transcripts from tagged suppressible mutations, and (2) a clonal analysis method based on the co-ordinate loss of suppressible phenotypes during development. We are also attempting to reconstruct the suppression phenomenon in transgenic tobacco to dissect the various *cis* and *trans* acting components.

University of Connecticut
Storrs, CT 06269

40. Sugar Transport and Metabolism in Thermotoga

A.H. Romano and K.M. Noll, Department of Molecular and Cell Biology

$168,000 (2 years)

Members of the genus *Thermotoga* represent the most extremely thermophilic members of the domain Bacteria (eubacteria), and the deepest and most slowly evolving phylogenetic lineage in this domain. *Thermotoga* species are strictly anaerobic and fermentative. The best-known species *Tt. maritima* ferments glucose to lactate, acetate, CO_2, and H^2. Nothing is known of the mechanisms by which sugars are transported into *Thermotoga* cells, and studies in other laboratories that have concentrated on a few individual enzyme systems have not yielded a coherent picture of the pathways of sugar catabolism. The goal of this project is to elucidate mechanisms of sugar transport and primary pathways of sugar catabolism in *Thermotoga* species. Uptake of isotopic non-metabolizable analogs of glucose ([^{14}C]-2-deoxy-D-glucose, [^{14}C]-methyl-α-D-glucopyranoside, [^{14}C]-6-deoxy-D-glucose) and lactose ([^{14}C]-methyl-ß-D-thiogalactopyranoside) will be measured to determine whether they are transported by simple or facilitated diffusion, active transport, or a group translocation system such as the phosphoenolpyruvate:sugar phosphotransferase system. Pathways of sugar catabolism will be determined by: a) Identification of enzyme activities that are characteristic or unique to known pathways. b) Determination of the metabolic fate of [^{14}C]-D-glucose labeled at specific carbon atoms (C1, C2, C3,4, C6) by measurement of radioactivity in individual carbon atoms of the fermentation products (lactate, acetate, CO_2). The characterization of sugar transport and metabolism in this closest descendant of the ancestral bacterium, taken together with studies in other laboratories of sugar metabolism in anaerobic hyperthermophiles of the domain Archaea (archaebacteria) should provide insight into the evolution of cell metabolism and fundamental aspects of thermophily that promise to be useful in biotechnological applications.

Cornell University
Ithaca, NY 14853

41. Anaerobic Metabolism of Aromatic Compounds by Phototrophic Bacteria: Biochemical Aspects
J. Gibson, Biochemistry Section, Division of Biological Sciences *$83,000*

Very large quantities of compounds containing benzene rings enter the environment, primarily from plant material and from man-made chemical production. These are of interest because lignin, a major plant polymer made up of aromatic substituents, constitutes the second most abundant plant product, and is therefore an important renewable resource. Man-made products are more commonly of concern because of potential toxicity if they are able to enter ground water supplies. Aerobic attack on the aromatic nucleus has been extensively characterized, and shown to require molecular oxygen. Anaerobic mineralization of aromatic compounds is carried out only by a limited range of bacterial species. A metabolic sequence radically different from the aerobic one is involved, involving reductive dearomatization of coenzyme A thioesters of model compounds such as benzoate and 4-hydroxybenzoate. Neither the enzymology nor the regulation of this reaction sequence is fully understood. This project uses the versatile phototrophic bacterium *Rhodopseudomonas palustris*, which grows well on a large number of simple and substituted aromatic acids, for detailed study of the pathway. The initial stages have been clarified by characterizing and cloning two specific coenzyme A ligases. A third enzyme, which appears to be induced by growth on the fully reduced alicyclic compound cyclohexanecarboxylate, is currently being purified. Apart from their intrinsic interest, these enzymes are proving useful for the synthesis of CoA thioesters of cyclohexadienylcarboxylates, which are potential intermediates in the stepwise reduction of the aromatic nucleus. The interconversion of these isomers is being studied in cell extracts and also in the intracellular pools of cells incubated anaerobically under different physiological conditions. In collaboration with Dr. C. S. Harwood at the University of Iowa, molecular analysis is being used to study the regulation of the reductive stages of anaerobic aromatic degradation, which appear to be rate-limiting in cells, with a view to genetic manipulation of degradation rates.

Cornell University
Ithaca, NY 14853-2703

42. Molecular and Physiological Analysis of Cytoplasmic Male Sterility
M.R. Hanson, Division of Biological Sciences *$186,000 (FY 92 Funds/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 are being analyzed with respect

to protein and RNA-level expression and targeting of the chimeric gene product; male fertility and respiratory function are being 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

43. 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 *$74,000*

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 or its F_1 or F_2, and in aberrant segregation ratios in interspecific F_2's. The interspecific barriers impede 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 have assembled a unique combination of plant materials, including interspecific layer chimeras, isocytoplasmic lines and transformants with a pollen-specific reporter gene. We are studying the interspecific barriers using these materials and a combination of cytological, genetic and molecular techniques. Our goals are to determine: 1) the functional basis of unilateral incongruity including the time and developmental step(s) interrupted and the tissue and genomes involved, 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 on 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

44. Characterization of a Putative Receptor Protein Kinase and its Role in Self-Incompatibility
J.B. Nasrallah and M.E. Nasrallah, Section of Plant Biology, Division of Biological Sciences
$91,000

Self-pollination in Brassica is prevented by the action of genes that reside at the highly polymorphic self-incompatibility (*S*) locus. Self/nonself recognition is attributed to the activity of identical "*S* alleles" in pollen and stigma, and results in the inhibition of self-pollen at the surface of stigmatic papillar cells. We have shown that the *S* locus is complex and consists of at least two genes that are expressed specifically in reproductive tissues. Of these, the *SLG* gene encodes a secreted glycoprotein, and the *SRK* gene encodes a transmembrane protein kinase. The structure of *SRK* gene indicates that pollen-stigma signalling in crucifers is similar in basic outline to animal signalling systems. Our hypothesis is that contact with self-pollen activates the SRK protein which, by phosphorylating intracellular substrates, couples the initial molecular recognition events at the papillar cell-protein interface to the

signal transduction chain that leads to pollen rejection. Our research is aimed at understanding the role of SRK in specific pollen recognition. We have shown that SRK has intrinsic serine/threonine protein kinase activity. We have determined the sequence of SRK genes isolated from different self-incompatibility genotypes, and are carrying out comparisons of SRK and SLG sequences within and between S haplotypes to identify domains that may be important in self recognition. Future experiments will aim at characterizing the SRK protein in stigmas, identifying the cell types of the stigma and anther in which the protein is expressed, and investigating its subcellular localization.

Cornell University
Ithaca, NY 14853

45. Mechanism of Inhibition of Viral Replication in Plants
P. Palukaitis, Department of Plant Pathology *$168,000 (FY 92 Funds/2 years)*

Viruses are a major class of plant pathogens that are responsible for crop losses and reductions in plant biomass. Natural resistance genes in plants can obviate the effects of such pathogens, although none of these resistance genes have been isolated. Many of these resistance genes function by blocking cell-to-cell or long-distance movement of plant viruses. To elucidate the mechanisms of inhibition of plant virus movement, we have used several approaches: (1) We have shown that a chimeric virus involving tobacco mosaic virus (TMV) expressing the cucumber mosaic virus (CMV) coat protein gene is able to move long-distance in plants resistant to the long-distance movement of TMV without its coat protein. Curiously, most of the nucleic acid encapsidated into the CMV-like particles in systemically infected leaves is of host rather than viral origin. (2) Transgenic plants expressing the CMB cell-to-cell movement gene can complement the movement of a defective CMV, but cannot complement the movement of defective TMV. Furthermore, these plants show some resistance to infection by wildtype CMV, but not wildtype TMV, indicating very specific interactions. (3) The inability of one strain of CMV to infect maize maps to the N-terminal two-thirds of the movement protein, while the inability of another strain to systemically infect *Cucurbita pepo* maps to two amino acids in the coat protein. Thus, plant interactions with different viral gene products control short- and long-distance movement.

Cornell University
Ithaca, NY 14853

46. Effects of Freezing and Cold Acclimation on the Plasma Membrane of Isolated Cereal Protoplasts
P.L. Steponkus, Department of Soil, Crop and Atmospheric Sciences *$102,000*

Over $100 million are spent annually to minimize freezing damage to agricultural crops; the majority of these expenditures are for energy-costly practices to modify the microclimate. The goals of this project are to provide a mechanistic understanding of the molecular aspects of freezing injury in winter cereals and to elucidate the mechanisms by which cold acclimation increases the cryostability of cellular membranes. Studies are in progress to elucidate the causal relationship between differences in the lipid composition of the plasma membrane and freezing tolerance of rye and oat, which represent the extremes in freezing tolerance of winter cereals. Although the freeze-induced membrane lesions are similar in rye and oat (lamellar-to-hexagonal II phase transitions and fusion of the plasma membrane with endomembranes), they occur at substantially higher temperatures in oat. This

difference in sensitivity is associated with greatly increased proportions of cerebrosides and sterylglucosides in the plasma membrane of oat, which increase the propensity for lyotropic phase transitions and inter-bilayer interactions resulting from freeze-induced dehydration. Recently, we initiated studies in collaboration with M. Thomashow (Michigan State University) to determine the cryobehavior of unique proteins that are synthesized during cold acclimation and their effects on the cryostability of lipid bilayers. These proteins minimize the occurrence of freeze-induced fusion of liposomes, which is dependent on the lipid composition of the bilayers. Studies are in progress to determine if the decreased incidence of fusion is the result of either direct protein-lipid interactions or indirect effects resulting from amelioration of the freezing stress.

Cornell University
Ithaca, NY 14853

47. Genetic Control of Nitrate Assimilation in Klebsiella pneumoniae
V.J. Stewart, Section of Microbiology, Division of Biological Sciences *$80,754*

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, cloned and sequenced the structural genes for assimilatory nitrate and nitrite reductases, *nasA* and *nasB*, respectively. The predicted *nasA* gene product shares similarity with prokaryotic respiratory nitrate reductases and other enzymes that use molybdopterin guanine dinucleotide as a cofactor. The predicted *nasB* gene product shares similarity with assimilatory nitrate reductase of *Aspergillus nidulans*. Mutational analysis shows that a third gene, *nasD*, is located upstream of *nasBA*. The *nasD* gene appears to be essential for nitrate reduction to nitrite. There may also be additional *nas* genes upstream of *nasD*. A region immediately downstream of *nasA*, provisionally termed *nasC*, may also be important for nitrate or nitrite reduction. Finally, we have begun to isolate and characterize *trans*-acting mutations that alter nitrate and nitrite controls of *nas* operon expression. Together, these studies will lead to a more detailed view of how nitrogen metabolism is coordinated and regulated.

Cornell University
Ithaca, NY 14853

48. Studies of the Genetic Regulation of the Thermomonospora fusca Cellulase Complex
D.B. Wilson, Section of Biochemistry, Molecular and Cell Biology
$172,000 (2 years)

The goals of this project are to determine the molecular mechanisms regulating cellulase synthesis in the soil bacterium *Thermomonospora fusca* and to determine the mechanism by which *T. fusca* cellulases degrade crystalline cellulose. We have refined our 3-d x-ray structure of the E2 catalytic domain (E2cd) to 1.35Å. We have also crystallized E2cd in the presence of cellobiose and localized it in the active site cleft of E2cd. There are four Asp residues in the active site cleft that might participate in catalysis and all four are being modified by site directed mutagenesis. There are also

two Trp residues that participate in binding cellulose into the active site and these are being modified to test the effect on binding and activity. We have isolated two mutations in activity site residues, one changing Glu 265 to Gly and the other changing Arg 221 to Cys. The proteins produced by these mutants are being purified and characterized. Finally the *T. fusca* protein which binds to the universal actinomycete cellulase gene regulatory sequence, TGGGAGCGCTCCCA, is being isolated.

Cornell University
Ithaca, NY 14853-8101

49. Conversion of Acetic Acid to Methane by Thermophiles
S.H. Zinder, Division of Biological Sciences *$101,000*

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 10-20 μM respectively, in agreement with ecological data indicating that *Methanothrix* is favored by low acetate concentration; 3) the demonstration that, in contrast to *Methanosarcina, Methanothrix* strain CALS-1 did not accumulate H_2 during methanogenesis from acetate, but instead accumulated CO; 4) the purification and characterization of a thermostable acetyl-CoA synthetase from cell-free extracts of *Methanothrix* strain CALS-1; 5) 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]$^{-1}$), and much lower rates were obtained with acetyl-CoA or acetyl-phosphate as substrates; 5) the demonstration that methanogenesis from acetate in *Methanothrix* was independent of H_2 and other electron donors, in contrast to *Methanosarcina*, and in agreement with results obtained in whole cells; 7) the purification of a multisubunit membrane-bound ATPase from *Methanothrix* strain CALS-1 with properties closely resembling those recently described for another thermophilic *Methanothrix* strain. Current research is centered on biochemical factors which allow thermophilic *Methanothrix* to compete with *Methanosarcina.*

Dartmouth College
Hanover, NH 03755

50. Regulation of Gene Expression in the Bradyrhizobium japonicum/soybean symbiosis
M.L. Guerinot, Department of Biological Sciences *$91,000*

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 3 to 10 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 sequences confer iron regulation on the *hemA* gene. We have also identified a *B. japonicum* gene which appears to encode a trans-acting factor which regulates *hemA* expression. 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

51. Metabolic Mechanisms of Plant Growth at Low Water Potentials
J.S. Boyer, College of Marine Studies *$93,500*

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 of 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 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. The 28kD protein was found to be an acid phosphatase having a broad range of substrates. Experiments are underway to determine the role of the phosphatase in the growth response.

Duke University
Durham NC 27708-0338

52. Molecular, Genetic and Physiological Analysis of Photoinhibition and Photosynthetic Performance
J.E. Boynton, N.W. Gillham and C.B. Osmond, Departments of Botany and Zoology
$113,000

A major goal of this project is to combine molecular genetics, biochemistry, and physiology to understand the relationship between a chloroplast's photosynthetic performance under high light and the structure, function, and turnover of the D-1 reaction center protein of Photosystem II (PSII). Exposure of plants and algae to high irradiance often causes photoinhibitory damage to D-1, resulting in reduced photosynthetic efficiency. We are using the unicellular green alga *Chlamydomonas reinhardtii* as a model system for our studies on D-1 because of its excellent genetics and ease of chloroplast transformation. Mutations altering the normal light-dependent turnover cycle of D-1 may destabilize the balance between light-driven D-1 damage and resynthesis, thus enhancing the net amount of photoinhibitory damage. Similarly, certain mutations in the chloroplast psbA gene encoding D-1 impair the function of PSII by altering its quinone/herbicide binding region. Site-directed changes

are being made in psbA and in chloroplast genes encoding selected components of the chloroplast translational apparatus to study their effects on photosynthetic function and the D-1 cycle of synthesis and degradation. We are also beginning to select and characterize intragenic and extragenic suppressor mutations which ameliorate the extreme light sensitivity of our introduced mutations. Such suppressors may identify second-site amino-acid substitutions within D-1 which can compensate for impaired function or reduce light-driven damage, as well as other gene products involved in modulating the D-1 cycle. Novel mass spectrometry techniques we have developed will be used for analysis of stable carbon and oxygen isotope fractionation to study the metabolic consequences of mutations with altered photosynthetic performance. In aggregate, these studies should yield new insights into basic processes underlying photosynthetic performance under photoinhibitory conditions that may guide future investigations in higher plants.

Duke University
Durham, NC 27706

53. Molecular Studies of Functional Aspects of Higher Plant Mitochondria
J.N. Siedow, Department of Botany *$42,542 (7 months)*

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

54. Plant, Cell and Molecular Mechanisms of Abscisic Acid Regulation of Stomatal Apertures
W.H. Outlaw, Jr., Department of Biological Sciences
$190,000 (FY 92 Funds/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 most recent DOE-sponsored research has shown the following: (1) In response to water stress, guard-cells in ABA-synthesis-impaired leaflets accumulate ABA. This observation implies that uptake or redistribution of ABA is a mechanism that controls water loss. (2) Modification of ABA at the 4' carbonyl renders it ineffective in a rapid stomatal-opening-inhibition assay that was elaborated especially for this purpose. This result, with previous studies, indicates that any modification (required for anti-idiotypic antibody production) is not permissible. Other recently completed projects include histochemical studies of sucrose-P synthase and of phosphoenolpyruvate carboxylase, and fluoridone effects on leaf development. Current projects will identify the metabolic fate of guard-cell ABA upon stress relief and the cellular "target" for "root-source" ABA.

University of Florida
Gainesville, FL 32611

55. Ethanologenic Enzymes of Zymomonas mobilis
L.O. Ingram, Department of Microbiology and Cell Science *$100,004*

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 form the ethanologenic pathway and comprise 50% of the cytoplasmic protein. This organism has one of the highest rates of glycolytic flux known, approximately 1 μmole sugar metabolized per mg cell protein. To maintain this high flux, the levels of ethanologenic enzymes in *Z. mobilis* must be coordinated to provide the near stoichiometric activities. Most of these genes have canonical ribosomal binding sites and biased codon usage for efficient translation. Enzyme levels appear to be regulated in large measure by among glycolytic operon and within glycolytic operon by message stability, i.e., message structure. Additional studies have investigated the extent to which many of these enzymes contribute to the control of glycolytic flux, and the physical interaction of glycolytic and ethanologenic enzymes informing 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

56. Gene-enzyme Relationships of Aromatic Amino Acid Biosynthesis in Higher Plants
R.A. Jensen, Department of Microbiology and Cell Science *$217,000 (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 mid-pathway (dehydroquinate dehydratase/shikimate dehydrogenase) and post-chorismate (prephenate aminotransferase, arogenate dehydrogenase, and arogenate dehydratase) 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. The nature of the general inhibitory effect of aromatic amino acids upon growth of *Nicotiana silvertris* will be elucidated, with a major goal of overcoming problems caused by this phenomenon in isolating structural-gene and regulatory-gene mutants. 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 N. silvertris, 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

57. Effect of Growth Temperature on Enzyme Folding
A.T. Abdelal, Department of Biology *$87,000*

Carbamoylphosphate synthetase from *Salmonella typhimurium* consists of two unequal subunits that are encoded by the *carAB* operon. We have previously 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. Studies on the effect of temperature on refolding of denatured enzyme support this hypothesis: 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 a glycine residue that precedes a ß-turn sequence that is conserved among microbial CPSases. This substitution could destabilize the ß-turn and thus be unfavorable in the correct folding pathway.

We have extended our studies to the thermophile, *Bacillus stearothermophilus*. A homogeneous preparation from the arginine-specific CPSase purified from cells grown at 65°C exhibited significantly higher thermal stability than that purified from cells grown at 45°C. The *carAB* operon from *B. stearothermophilus* was cloned in *E. coli* and work is in progress to express it in the thermophile in order to obtain sufficient amounts of CPSase for physical characterization.

University of Georgia
Athens, GA 30602

58. The Metabolism of Hydrogen by Extremely Thermophilic Bacteria
M.W.W. Adams, Department of Biochemistry and Center for Metalloenzyme Studies
$88,000

Extremely thermophiles or "hyperthermophiles" 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 (600 liters) and are studying their pathways of hydrogen (H_2) metabolism. The archaea (formerly 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 these organisms we have purified nickel-containing hydrogenases, ferredoxin, rubredoxin, copper-containing pyruvate ferredoxin oxidoreductases (POR), and two different types of tungsten-containing enzyme that both function as aldehyde ferredoxin oxidoreductases. One type (AOR) is thought to couple substrate oxidation to H_2 production in a new glycolytic pathway, while the other type (FOR) 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. Crystals of AOR and POR suitable for structural determinations have been obtained and their amino acid sequences are being determined by recombinant DNA techniques. From the most thermophilic bacterium currently known, *Thermotoga maritima* (T_{max}90°C), we have purified an iron-containing hydrogenase, ferredoxin and a POR which, in contrast to POR from the archaea, contains a novel Fe/S center rather than copper. 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

59. CarbBank - A Structural and Bibliographic Database for Complex Carbohydrates
P. Albersheim, Complex Carbohydrate Research Center *$70,000**

The IBM PC-compatible, computerized database (the Complex Carbohydrate Structure Database, CCSD) and database management system (CarbBank) were created by researchers at the Complex Carbohydrate Research Center (CCRC) to meet the needs of persons interested in carbohydrate science. Building the database was the initial main focus of this effort. Making CarbBank and the CCSD available for a wider range of computer environments by making our software compatible with database systems from the National Center for Biotechnology Information (NCBI) is the next major goal. Release number 5 of the CCSD was recently distributed by the CCRC CarbBank staff. Release 5 contained over 8,000 records of complex carbohydrate sequences and corresponding bibliographic information. Release 5 was distributed with the latest version of CarbBank on floppy disks to over 500 customers at 350 sites worldwide. The next release of the CCSD, number 6, is scheduled for the near future. Release 6 will contain over 20,000 records and will require over 35 mB of disk space. Growth of the CCSD file size and customer base has forced changes in the distribution methods. Beginning in June 1992, releases of the CCSD are made on CD-ROM disks through NCBI. The CCSD and

CarbBank software, bundled with other biotechnology databases on the NCBI Data Repository CD-ROM, will be sent to our present customers as well as to the larger NCBI customer base. The dramatic increase in the size of the CCSD during the last year occurred primarily because Chemical Abstracts Service (CAS) supplied us with approximately 20,000 database records that contained complex carbohydrate sequences and bibliographic data from citations published before 1991. The CarbBank staff and European curators reviewed and corrected, where necessary, over 6,000 CAS records. The validation process should be completed within the year. The CCSD also grew as the CarbBank staff and curators systematically scanned and extracted information from current releases of a number of major scientific journals. During the next few years, CAS and curators will continue to provide new records. The CCSD is expected to grow by 2,000 records per year. The CCSD will continue to grow by the addition of new records; however, our primary focus in the immediate future will be to expand the computer base for CarbBank from only IBM-PC DOS to also include Macintosh, UNIX Windows, and Japanese (NEC 980x) environments. We intend to work closely with NCBI to create CarbBank-like modules that will run in conjunction with their multi-platform Vibrant software system. Our goal is to create software that can run on a variety of computer systems. As we achieve this goal, we will enhance an already valuable resource for the biotechnology community.

* Funded collaboratively with NSF, National Library of Medicine, National Institute of General Medical Sciences, and USDA.

University of Georgia
Athens, GA 30602-4712

60. The University of Georgia Complex Carbohydrate Research Center (CCRC)
P. Albersheim and A. Darvill, Complex Carbohydrate Research Center

$1,720,000 (2 years)

This grant supports research, analytical services, and training in plant and microbial complex carbohydrates at the University of Georgia Complex Carbohydrate Research Center (CCRC). The CCRC has a multidisciplinary faculty and staff who serve as a national resource for basic research in complex carbohydrate science. There are nine tenured or tenure-track faculty and one adjunct faculty member; three members of the staff have non-tenure-track faculty appointments. Four of the regular faculty, one adjunct faculty member, and one non-tenure-track faculty member are active participants in the plant and microbial carbohydrate program supported by this grant. The activities of this center consist of research, training, and service. Educational activities involve the training of undergraduate and graduate students, postdoctoral research associates and visiting scientists. CCRC faculty teach all or part of three undergraduate and 20 graduate courses. Twenty undergraduate and 35 graduate students are currently pursuing research projects or Ph.D. degrees in the CCRC; nine undergraduate and 16 graduate students are working in plant or microbial carbohydrate science. Two week-long laboratory training courses are held annually for ~20 scientists from institutions and industries located throughout the United States. The faculty and staff of the CCRC are currently involved in more than 90 internal or external collaborative research projects of which 35 are part of the plant and microbial carbohydrate program. The plant and microbial carbohydrate program has provided service to 98 individuals by analyzing 399 samples, each at least twice, in the time that the service has been active. These analyses include determination of glycosyl-residue and glycosyl-linkage compositions, and acquisition and interpretation of one-dimensional NMR and mass spectra. The CCRC has, in one way or another, assisted more than 135 corporations during the same time period.

University of Georgia
Athens, GA 30602-4712

61. The Structures and Functions of Oligosaccharins

P. Albersheim, Complex Carbohydrate Research Center *$434,664 (30 months)*

The research supported is designed to determine whether oligosaccharins (oligosaccharides with regulatory functions) function *in planta* by studying enzymes that generate and process oligosaccharins as well as protein receptors for oligosaccharins. The research projects that we are investigating include determining: (i) whether oligogalacturonides play a role in the establishment of rhizobia-legume symbioses, (ii) the role of fungal *endo*polygalacturonases and plant polygalacturonase inhibitor proteins in pathogenesis, (iii) the role of tobacco stem *endo*polygalacturonases in the generation of oligogalacturonides that regulate morphogenesis in tobacco stem explants, (iv) whether a newly identified *endo*polygalacturonase in tomato fruit is involved in ripening, (v) the effect of oligogalacturonides on the metabolism of auxin in tobacco stem explants, (vi) the effects of oligosaccharins on morphogenesis of *Arabidopsis*, (vii) the role of *endo*glucanases in controlling growth of pea stems, and (vii) the role of *endo*xylanases and arabinosidases in the pathogenesis of rice by *Magnaporthe grisea*.

University of Georgia
Athens, GA 30602-4712

62. Structural Studies of Complex Carbohydrates of Plant Cell Walls

A. Darvill, Complex Carbohydrate Research Center *$1,016,660 (30 months)*

The plant cell wall is the major source of all biomass and dietary fiber, and, as such, is a vital natural resource. The primary plant cell wall controls several fundamental properties of plant cells. The wall provides the first barrier to pests, it controls the rate of cell growth, it is the organelle that ultimately controls the shape of plant cells, organs, and organisms, and it is the source of an important group of regulatory molecules called oligosaccharins. This grant supports research on the structure and function of plant cell walls. The structural studies emphasize the detailed analyses of two pectic polysaccharides rhamnogalacturonan I and rhamnogalacturonan II and the hemicellulosic polysaccharide xyloglucan. These are three of the five non-cellulosic polysaccharides present in all higher plant cell walls. These studies utilize chemical and enzymatic procedures to generate oligosaccharide fragments for structural analysis. Structural studies of these polysaccharides have continually challenged us to develop new, more effective methods that use less sample for analysis, e.g., we are developing an artificial neural network system to identify complex carbohydrates from their ^{1}H-NMR and mass spectra. Our studies also include: (i) generating and characterizing a library of monoclonal antibodies against plant cell wall polysaccharides/oligosaccharides to be used to locate specific polysaccharides in different cells and tissues, (ii) studying the three-dimensional conformations of cell wall polysaccharides to determine how poly- or oligosaccharide conformation affects the structural framework of the cell wall, (iii) studies on the assembly of the cell wall and its parts, and (iv) analyzing *in vitro* structures of cell wall polysaccharides extracted from *Arabidopsis* plants with mutations that affect their cell walls. These studies are designed to identify the nature of the macromolecular components of cell walls and to elucidate cell wall macromolecular interactions. Our long-term goal for this research is to define the structure of the primary cell wall and to relate this structure to the biological functions of the organelle.

University of Georgia
Athens, GA 30602

63. Molecular Biology of Lea Genes of Higher Plants
L. Dure, Department of Biochemistry *$31,540 (10 months)*

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 30602-7229

64. Mechanisms of Lignin Biosynthesis During Xylogenesis in Zinnia elegans
K-E.L. Eriksson and J.F.D. Dean, Department of Biochemistry
$204,500 (FY 92 Funds/2 years)

Lignin, a polymer of phenylpropanoid residues 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. We have used several histochemical stains to examine the developmental sequence of lignin deposition in vascular tissues from *Zinnia elegans* plants of different ages. We are now attempting to correlate the binding of monoclonal antibodies raised against synthetic lignins (DHPs) with the results from our histochemical analyses. Using light microscopy, we have examined developing *Zinnia* xylem for enzymes and metabolites associated with the final steps of lignin deposition. Laccase and peroxidase activities are both present in these tissues, and laccase activity, in particular, appears to

correlate with the earliest stages of lignification. The patterns of laccase and peroxidase isozymes extracted from *Zinnia* tissues vary with the developmental stage of the tissue, and antibodies raised against laccase or peroxidase enzymes from sycamore maple are being used to study these changes in more detail. We intend to use the anti-lignin and anti-enzyme antibodies to perform dual-label immunolocalization studies in tissues at different developmental stages. We have examined hydrogen peroxide production in *Zinnia* stem sections by following the precipitation of cerium and have been surprised at how little hydrogen peroxide the tissue seems to make unless it is damaged severely. These techniques are all being used to examine lignification in cultured *Zinnia* cells that have been induced to undergo xylogenesis.

University of Georgia
Athens, GA 30602

65. 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 (FY 92 Funds/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.

Amino acid analogs were used to study the soybean HS response because of their ability to elicit a HS-like response. Treatment with amino acid analogs induced the transcription and translation of most, but not all, hsp families. Data suggest that the normal self-regulation of hsp transcription and translation is delayed or interfered with by analog treatment and that the synthesis of functional hsps is required for the normal regulation of HS gene expression and the acquisition of thermotolerance.

In a continuing effort to understand the complexity of HS gene families of soybean, clones coding 110 kD hsps were isolated from a soybean cDNA library constructed from enriched HMW HS-induced mRNA using the yeast hsp104 gene as probe. Soybean cDNA clones were used to isolate genomic clones from a soybean genomic library. Sequencing of these clones is in progress. The function of the 110 kD gene will be tested in a yeast hsp104 deficient mutant to establish whether the soybean 110 kD gene can complement the yeast mutant and restore thermotolerance.

The role of LMW hsps in HS and recovery has been approached by identifying hsp families targeted to the endomembrane system. Over- and underexpression of these proteins driven by a strong HS promoter (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 30602-7229

66. Microbiology and Biochemistry and Anaerobic Fermentations: The Conversion of Complex Organic Materials to Simple Gases

L.G. Ljungdahl, H.D. Peck, Jr., A. Przybyla, and J. Wiegel, Departments of Biochemistry and Microbiology *$527,300 (17.5 months)*

An enormous amount of biomass is generated annually by photosynthetic processes, a large portion of which is degraded by consortia of anaerobic microorganisms to simple organic compounds and gases. Included in these consortia are anaerobic microorganisms that grow and reproduce at extreme temperatures and pH's. The overall degradation can be divided into four fundamental biochemical processes: (a) the hydrolysis of complex materials (carbohydrates and proteins) to simple monomeric or oligomeric units (amino acids, peptides, mono and disaccharides); (b) their subsequent conversions to simple organic C_1, C_2, and C_3-compounds (acids, alcohols), (c) the production from these compounds of gases (H_2, CH_4, CO_2, CO), and (d) associated electron transfer and energy conservation processes. Our work deals with these four processes. Cellulose and hemicellulose hydrolysis is investigated with enzymes from *Clostridium thermocellum, Thermoanaerobacter ethanolicus*, and rumen anaerobic fungi of the genera *Neocallimastix* and *Orpinomyces*. The architecture of the multipolypeptide enzyme complex, the cellulosome of *C. thermocellum*, is being determined through partial dissociation to map the interaction of the 25 different polypeptides of the complex. The fungi and *T. ethanolicus* efficiently hydrolyze hemicellulose, which involves phenolic esterases and xylanases. These enzymes are being characterized. Recently, we isolated anaerobic thermophiles growing optimally at pH 10 and at 55°C and higher. The isolation of this new group of bacteria demonstrates that life exists under the combination of high temperature and high pH. We will study cell wall components and elucidate properties which enables them to thrive under these conditions. The work includes homeostasis and basic energy metabolism. These organisms are a source of alkaliphilic enzymes that may be of industrial uses. A major part of our work deals with the biosynthesis and mechanisms of action of hydrogenases, formate dehydrogenases, and CO dehydrogenases of acetogenic and sulfate reducing bacteria. They are all metal enzymes. Genes encoding these proteins cloned in *E. coli* are expressed but active enzymes have not been produced. This is likely due to the inability of *E. coli* to process heterologous redox proteins and incorporate metals into active sites. Thus, systems are developed to express various enzymes from the sulfate reducers and acetogens into more "homologous" cells, and to generate deletion mutants with selective integration vectors. The generation of energy is studied in the acetogens *Clostridium thermoaceticum* and *C. thermoautotrophicum* during autotrophic growth. They have a diverse autotrophic metabolism using electron acceptors such as sulfite, dimethylsulfoxide, CO, CO_2, aldehydes and carboxylic acids. Their membranes contain menaquinones, cytochromes, flavoproteins and ATPase systems. The electron transport system, ATP generation, and H_2-cycling are studied. Similar work will involve the hydrogenosomes that are organelles for the anaerobic fungi. They evolve H_2 as a means of disposing of electrons and purportedly generate ATP during the process. Little is known about how this organelle functions in energy generation and its importance to cell growth and metabolism.

University of Georgia
Athens, GA 30602

67. Regulation of Polyamine Synthesis in Plants
R.L. Malmberg, Botany Department $97,000

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; we have discovered two post-translational mechanisms for regulating arginine decarboxylase activity. One of these is a novel protease that clips the arginine decarboxylase pre-protein to activate it. We would like to understand this activating protease better, determine its mechanism of action, and determine its importance in the overall scheme of arginine decarboxylase regulation. (2) We have begun a similar characterization of ornithine decarboxylase by purifying it from plants. (3) We are characterizing the polyamine mutant collection we have developed. (4) Finally, we have begun to characterize the evolution of arginine decarboxylase, as an additional approach that could shed light on its functions in plants. 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

68. Soybean Ribulose Bisphosphate Carboxylase Small Subunit: Mechanisms and Determinants of RNA Turnover in Higher Plants
R.B. Meagher, Department of Genetics $94,000

In order to investigate the mechanisms and determinants controlling RNA turnover in higher plants we have developed an *in vivo* degradation system from petunia and soybean polysomes which faithfully degrades soybean ribulose-1,5-bisphosphate carboxylase small subunit (rbcS) mRNA. The most stable products represent a nested constellation of fragments, shortened from their 3' ends with intact 5' ends. Exogenously added rbcS RNA tagged with novel 5' sequence were degraded into a similar constellation of products but containing the extra 5' tag nucleotides. Detailed kinetics on the appearance of these exogenous products confirmed degradation proceeds in an overall 3' to 5' direction but suggested that there are multiple pathways of degradation. To further demonstrate a precursor product relationship, *in vitro* synthesized 3' truncated transcripts were shown to degrade into the expected smaller fragments. The system is now ready to examine the degradation of other mRNAs and various trans-acting determinants of turnover.

We have isolated three *Arabidopsis* genes encoding poly(A) binding proteins (PABP), *PAB1, PAB3,* and *PAB5*. Surprisingly, *PAB1* was expressed only in roots, while *PAB3* and *PAB5* were expressed only in flowers. A *PAB5* engineered for expression in yeast suppressed a lethal mutation in the yeast PABP gene. In yeast *PAB5* controlled poly(A) tail length and polysome loading of mRNAs. In the future we will examine the proteins activity *in vitro* in a yeast poly(A) nuclease system and our own plant RNA degradation system. The PAB5 protein was over expressed in *E. coli* and is being purified.

Intensive cloning and suppression cloning techniques will be used to identify the components interacting with the plant poly(A) binding proteins.

University of Georgia
Athens, GA 30602

69. Nitrogen Control of Chloroplast Development and Differentiation
G.W. Schmidt, Department of Botany *$90,000*

Several environmental stimuli and cellular factors affect development of the photosynthetic apparatus in plants and algae. Our studies are directed toward understanding how expression of genes whose products function in chloroplasts are regulated by nitrogen availability. The limited amounts of this nutrient in the environments of plant and photosynthetic microorganisms commonly restricts growth and development, culminating in the classical chlorophyll-deficiency syndrome. We strive to understand the molecular basis and physiological consequences of adaptation to nitrogen-deficiency in a model system in which *Chlamydomonas reinhardtii* is grown in an ammonium-limited continuous culture system. The mRNAs that encode apoproteins of light-harvesting complexes of both Photosystem II and Photosystem I reaction centers are among the nuclear gene products that are strongly regulated by nitrogen availability and how this is achieved is being studied by *in vivo* and *in vitro* transcription analyses. Chloroplast gene expression is affected at the posttranscriptional level. Several more 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 undergoing characterization at the biochemical and molecular levels.

University of Georgia
Athens, GA 30602

70. Molecular Characterization of a Maize Regulatory Gene
S.R. Wessler, Botany Department *$94,000*

The R/B gene family of maize encodes a family of basic helix-loop-helix proteins that determine where and when the anthocyanin pigment pathway will be expressed in the plant. Previous studies demonstrated that allelic diversity among family members reflects differences in gene expression, specifically in transcription initiation. We have determined that one R gene, Lc, is also under translational control. The 235 nt leader region of Lc was found to repress expression 25-30 fold in an *in vivo* assay. Repression is mediated by the presence, in cis, of a 38 codon upstream open reading frame (uORF). Furthermore, the coding capacity of the uORF influences the magnitude of repression. Two models to explain how the uORF represses downstream Lc expression (peptide repressor vs. ribosome stall) are currently being tested. It is proposed that uORF does not contribute to tissue specificity because other R genes that condition dramatically different patterns of pigmentation contain the uORF. Rather, we hypothesize the overexpression of the R protein early in development is detrimental and must be avoided. Consistent with this view was an inability by several labs to isolate transgenic petunia, tobacco or rice plants containing the Lc construct lacking uORF. The molecular basis for this phenomenon is under investigation.

University of Georgia
Athens, GA 30602

71. Biochemistry and Genetics of Autotrophy in Methanococcus
W.B. Whitman, Department of Microbiology *$74,000*

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 anabolic enzyme 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 of these mutations. In addition to biochemical analysis, these studies will utilize the greatly improved transformation procedure recently developed in this laboratory to identify the genetic basis of the mutations. To characterize the POR, the enzyme has been highly purified from *M. maripaludis*. For this complex enzyme, the substrate specificity, kinetics, and quaternary structure will be determined. In addition, the anabolic and exchange reaction with carbon dioxide will be studied to determine why the enzyme functions only in the anabolic direction *in vivo*. Key factors to be addressed will be: the electron donor to the enzyme, the requirement for additional protein components for the anabolic reaction, and interactions of the enzyme with the cytoplasmic membrane.

University of Georgia
Athens, GA 30602

72. Hemicellulases from Anaerobic Thermophiles
J. Wiegel, Center for Biological Resource Recovery and Dept. of Microbiology
$166,000 (2 years)

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 xylanases, glycosidases and xylan esterases in the ethanologenic, anaerobic thermophile *Thermoanaerobacter ethanolicus*, which grows between pH 4.5 and 9.5. Although it utilizes xylans, the xylanses activity in *T. ethanolicus* is barely measurable. As the first step, we are in the process of characterizing the hemicellulolytic enzymes from this and other anaerobic thermophiles as possible enzyme donors. 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, two acetyl(xylan)esterases, and an O-methyl glucuronidase from *Thermoanaerobacterium* spec. and xylanases from slightly acidophilic, neutrophilic and slightly alkalophilic thermophiles covering together a range from below pH 3.0 up to pH 10. We study regulatory properties of these enzymes including induction of the various enzymes involved. This year we will continue the characterization of the enzymes and to clone the appropriate enzymes first into *E. coli* using probes made accordingly to our N-terminal sequences and then start to develop a shuttle vector for cloning into *T. ethanolicus*.

University of Georgia
Tifton, GA 31793

73. Development of Innovative Techniques That May Be Used as Models to Improve Plant Performance
W.W. Hanna, G.W. Burton, Department of Agronomy *$48,000*

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 second 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. Resistance to a number of the major diseases have been identified in the weedy primary and in the tertiary gene pools of *Pennisetum*. Gene transfer procedures from these gene pools to the cultivated species are being evaluated. Apomictic (up to 90%) backcross-5 plants that resemble the cultivated species have been developed. Loss in seed set after about 10 days after anthesis is being studied and approaches are being tested to overcome the problem. Genes for leafiness and dwarfness in the secondary gene pool are being evaluated for their potential contribution for producing superior forages.

University of Hawaii
Honolulu, HI 96822

74. Violaxanthin De-Epoxidase: Biogenesis and Structure
H.Y. Yamamoto, Department of Plant Molecular Physiology *$92,154*

Violaxanthin de-epoxidase (VDE) is a lumen enzyme which, in the presence of ascorbate and low lumen pH, converts violaxanthin in the chloroplast membrane to zeaxanthin. *In vivo*, zeaxanthin forms under conditions of excess light relative to the requirements for CO_2 fixation. These conditions are present when plants are under environmental stress or are suddenly exposed to light intensities higher than their growth intensity. VDE is of interest because zeaxanthin increases the rate of non-radiative (heat) dissipation energy, thereby "down regulating" PSII apparently for protection against the potentially damaging effects of excess light. VDE has been purified in a single major component on 2-D SDS PAGE by a unique lipid-affinity precipitation step using monogalactosydiacylglycerol. N-terminal and internal amino-acid sequencing of the protein have been determined. 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 60612

75. Heavy Metal-lux Sensor Fusions and Gene Regulation
S. Silver, Department of Microbiology and Immunology *$99,000*

The project is studying gene regulation of the heavy metal resistance determinants from large plasmids of *Alcaligenes eutrophus* and other bacteria, using primarily gene fusions to reporter genes *in vivo*. 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, the enzymes associated with arsenic resistance: (a) the ArsC arsenate reductase of the plasmid *ars* resistance determinants and (b) the arsenite oxidase from *Alcaligenes faecalis* are being studied. Each of the metal ion resistance systems is inducible. Most systems have been previously cloned and sequenced, frequently in our laboratory. Using gene fusions to reporter genes, mostly *lux* (luciferase), we are characterizing the trans-acting regulatory genes and the specificity of the metal response. Regulatory proteins are being identified and purified. 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. The enzymatic transformations of arsenic hold potential for microbial bioremediation of soil and water arsenic pollution.

University of Illinois
Urbana, IL 61801

76. Genetics of Solvent-Producing Clostridia
H.P. Blaschek, Department of Food Science *$106,088*

Systems for the genetic manipulation of the acetone-butanol-ethanol (ABE) fermentation microorganism, *Clostridium acetobutylicum* are being developed and used to engineer strains with enhanced extracellular cellulolytic activity. The overall objective is the development of tailor-made strains with improved biotechnological potential for the fermentative conversion of cellulose to butanol. A phage-plasmid hybrid (phagemid) designated pCAK1 was constructed by ligating the 5 Kbp pAK102 *Escherichia coli* plasmid and the 6.6 Kbp HaeIII linearized replicative form of the CAK1 viruslike particle from *C. acetobutylicum* NCIB 6444. The functionality of pCAK1 in the *E. coli* host system, especially in generating ssDNA, in the absence of impairing *E. coli* cell viability, together with the successful introduction of pCAK1 into *C. acetobutylicum* is the basis for the development of a M13-like genetic system for the genus *Clostridium.* A 3.4 Kb EcoRI fragment containing the endo-ß-1,1-glucanase gene engB from *C. cellulovorans* was subcloned into the lacZ region of the *E. coli-C. acetobutylicum* shuttle vector pMTL500E to produce a 9.3 kb plasmid, designated pAK301. Plasmid pAK301 was transformed into *C. acetobutylicum* ATCC 824 using electroporation. Heterologous expression of the *C. cellulovorans* engB gene by *C. acetobutylicum* BKW-1 was detected as zones of hydrolysis on carboxymethylcellulose plates stained with Congo Red. The extracellular cellulase preparation from *C. acetobutylicum* BKW-1 has specific activity towards CMC which is more than four-fold that present in *C. acetobutylicum* ATCC 824.

University of Illinois

Urbana, IL 61801-4798

77. Mechanism and Structure of the Plant Plasma Membrane Ca^{2+}-ATPase

D.P. Briskin, Department of Agronomy *$80,999*

The plasma membrane Ca^{2+}-ATPase couples ATP hydrolysis to the extrusion of Ca^{2+} at the plasma membrane of 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. Using amino acid modifying reagents, the involvement of essential arginine, lysine, histidine and cysteine residues in the catalytic/transport mechanism of the plasma membrane Ca^{2+}-ATPase has been shown. 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. Based upon differential detergent solubilization of the plasma membrane H^+-ATPase and Ca^{2+}-ATPase, an enriched preparation of the plasma membrane Ca^{2+}-ATPase was produced which could be reconstituted into liposomes. This enriched preparation will be utilized in further studies on the reaction/transport mechanism of this enzyme.

University of Illinois

Urbana, IL 61801

78. Photosynthesis in Intact Plants

A.R. Crofts, Department of Physiology and Biophysics *$113,000*

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 collaborative field and laboratory-based research using these instruments is underway. An important part of the project is a 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, the intermediate electron transfer chain, including the b_6/f complex and its interactions with the quinone pool, and the down-regulation of photosynthesis under high light. For photosystem II, 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, and the interface between photosystem II and the oxygen evolution enhancing proteins. We are using *Chlamydomonas reinhardtii* as a model for the higher plant system. We plan to extend this approach to studies of the light-harvesting complexes, which are involved in control of light delivery to the photosystem. We are studying the temperature and pressure 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 underway, and the collaborations established around the instrumentation developed.

University of Illinois
Urbana, IL 61801

79. Mechanism of Proton Pumping in Bacteriorhodopsin
T.G. Ebrey, Department of Physiology and Biophysics *$105,000*

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 bacteriorhodopsin, especially tyrosines, lysines, and aspartic acids, c) the ability of protons to have access to these protonable groups, and d) the control of the amino acids near the protonated Schiff base on the pK of the Schiff base.

University of Illinois
Urbana, IL 61801

80. Studies on the bo-type Ubiquinol Oxidase From Escherichia coli
R.B. Gennis, Department of Biochemistry *$232,000 (FY 92 Funds/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

81. Regulation of Cell Division in Higher Plants
T. Jacobs, Department of Plant Biology *$182,000 (2 years)*

Stringent regulation of cell division is a fundamental requirement for both the proper distribution of genetic material to the daughter cells of a mitosis, as well as the orderly progression of developmental events in higher eukaryotes. In mature angiosperms, mitotic activity is concentrated in meristems at the apices of the plant axis. This project is aimed at elucidating the essential regulatory elements that guide plant cells into, out of, and through the stages of the cell cycle, G1, S, G2, and M. Certain passages in this progression are controlled by a protein kinase, p34, and related enzymes of the cyclin-dependent kinase (CDK) family. These proteins are regulated by their phosphorylation states and their associations with regulatory subunits called cyclins. We have cloned several cDNAs encoding putative pea CDKs and cyclins. By synchronizing mitoses in the root apical meristems of hydroponically-grown peas, we have identified CDK activity peaks in S-phase and at the G2/M transition. A set of unidentified proteins is phosphorylated with parallel periodicity. Our current work is focused on: 1) completing the sequence analysis of our pea CDK and cyclin clones; 2) verifying the authenticity of these clones by functional tests in heterologous systems; 3) raising antibodies that will specifically recognize each cyclin and CDK, and, if possible, specific phosphorylation states of the latter; and, 4) determining the makeup and cell cycle-associated kinetics of the various complexes formed by these proteins in pea cells.

University of Illinois
Urbana, IL 61801

82. Genetics of the Methanogenic Archaebacterium, Methanococcus voltae with Attention to Genetic Expression Mechanisms
J. Konisky, Department of Microbiology *$182,000 (FY 92 Funds/2 years)*

The objective of this research program is to study the genetics, physiology and molecular biology of *Methanococcus voltae*, a marine archaebacterium. *M. voltae* is a strictly anaerobic microorganism that produces methane as a primary metabolic product. ATP formation in *M. voltae* is chemiosmotically-based and is mediated by a sodium current that we believe is maintained through a primary sodium pump. While cells grown at pH6.0 do not generate a transmembrane sodium gradient, cells grown at pH8.2 generate a sodium gradient in which the intracellular level of sodium ions is maintained at a concentration that is twelve fold below the concentration of sodium ions in the medium. These results suggest that there may be other facets of cell physiology that differ in cells grown at pH6.0 and 8.2.

We have recently cloned and sequenced the *M. voltae* gene that encodes a cell surface polypeptide (the S-layer protein) that covers the cell surface, but whose function has not been fully defined. We have found that when *M. voltae* is grown at low pH in a medium containing reduced sodium, this gene is not expressed. We are currently carrying out studies to characterize the promoter structure of this gene for the purpose of determining the molecular mechanism that underlies how growth conditions lead to modulation of gene expression.

It is anticipated 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

83. Genetic Studies on Cytoplasmic Male Sterility in Maize

J.R. Laughnan and S. Gabay-Laughnan, Department of Plant Biology

$85,000 (FY 92 Funds/2 years)

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. 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. 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. Studies on transposition of spontaneously-occurring restorers and standard restorers of *cms-S* initially involved the *wx*-translocation system. In order to verify the transposable-element characteristics of S restorers, we have measured rates of recombination between transposed and nontransposed versions of *Rf*s on the one hand, and visible gene markers on the other, in the absence of the *wx*-translocation system. The results are positive. 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, seven independently-occurring spontaneous *Rf-nf* genes have been identified. The allelic relationship between all newly-arisen *Rf* genes is under study. *Rf-nf* genes have been found to be functional in hybrid backgrounds and the genetic nature of this hybrid vigor effect is under study. We have observed differential behavior of the *Rf-nf* genes in hybrid backgrounds, depending on whether they were transmitted through the maternal or through the paternal parent. We are hypothesizing that this is due to imprinting. 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 designated R. The transcriptional profile of this region changes upon cytoplasmic reversion to fertility. Revertants in all nuclear backgrounds lack a 1.6 kb 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*. The R region has recently been sequenced and found to differ from the R1 sequence of N cytoplasm. The point of divergence falls within an open reading frame. No significant similarities exist between the R open reading frame and any published sequence. Experiments aimed at determining whether the R sequence encodes a polypeptide will be undertaken.

University of Illinois
Urbana, IL 61801

84. Exploratory Studies on the Bacterial Formation of Methane
R.S. Wolfe, Department of Microbiology *$95,108 (FY 92 Funds/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

85. 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 (FY 92 Funds/2 years)

This interdisciplinary research training program is designed to engage students in plant biological research in currently pressing areas pertaining to anthropogenic factors influencing the photosynthetic performance and productivity of plants. The academic curriculum, seminar series and laboratory

training acquaint students with current problems and provide a broadly-based and novel expertise required to make progress with these complex issues. Thus the interdisciplinary outlook and multidisciplinary nature of the training is especially important in the future photosynthesis research where many of the most significant issues span the range from physics to agronomy. Detailed information about mechanism, which comes for the most part from laboratory advances in basic photosynthesis research at the molecular level, is integrated with the physiology of intact plants and ultimately placed in an ecological and agronomic context. Graduate students and young postdoctoral trainees work on projects coordinated between the laboratories of at least two training program faculty who have conceptually different outlooks and approaches to the problem. The program is designed to encourage the involvement of undergraduate students by providing summer fellowships, including this summer, several outstanding recent high school graduates who will begin university in the fall. Preparations are underway for a program to be offered each of the next three summers for high school and community college teachers to gain insights into how plant biological research can address critical issues of societal concern as well as to gain expertise in research techniques and methods which they can take back to their own classrooms. Special efforts are being made to involve teachers from predominantly minority schools by coordinating our recruitment with similar programs already in operation at the University.

Indiana University
Bloomington, IN 47405

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

N.R. Pace, Department of Biology *$52,000**

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, including new and most deeply divergent lineages of Bacteria and Archaea.

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.

* Funded collaboratively with DOE's Office of Health and Environmental Research

Iowa State University
Ames, IA 50011

87. Organ-Specific Gene Expression in Maize: The P-wr Allele
T. Peterson, Department of Zoology and Genetics *$188,521 (2 years)*

The maize *P* gene encodes a Myb-homologous regulator of genes for flavonoid pigment biosynthesis. The *P-wr* allele gives a distinctive organ-specific expression pattern of colorless pericarp and red cob glumes. The aim of this research is to determine the mechanism(s) of organ-specific expression of the *P-wr* allele. Interestingly, *P-wr* transcripts are present in the colorless pericarps as well as the pigmented cob glumes. Moreover, transcripts of two structural genes for flavonoid biosynthesis are differentially expressed during development of *P-wr* pericarps. Our immediate aim is to isolate and compare full-length *P-wr* cDNA clones from pericarp and cob glumes, and to search for any differences in the RNA expressed in these two organs. Then, we will use particle gun bombardment to test the functionality of each *P-wr* cDNA in pericarp and cob glumes. Additionally, we will perform biochemical and genetic tests to further characterize the *P-wr* allele. The results should help to elucidate general mechanisms of gene regulation, especially as to how genes can be expressed in specific organs during plant development.

University of Iowa
Iowa City, IA 52242

88. Molecular Biology of Anaerobic Aromatic Biodegradation
C.S. Harwood, Department of Microbiology *$57,000*

We have been studying the molecular basis for anaerobic benzoate and 4-hydroxybenzoate degradation 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 cloned and are sequencing the genes encoding benzoate-CoA ligase and 4-hydroxybenzoate-CoA ligase, the enzymes that catalyze the initial reactions of benzoate and 4-hydroxybenzoate degradation. In addition, we have sequenced and characterized a regulatory gene (termed *aadR,* for anaerobic aromatic degradation regulator) that encodes a transcriptional activator of these two aromatic acid ligases. We hope to use these genes to identify environmental signals that regulate expression of anaerobic aromatic compound degradation. 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 degradation 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 that may be particularly well suited for specific applications in bioremediation or product formation.

Johns Hopkins University
Baltimore, MD 21218

89. Analysis of Thermally-stable Electron Transport Factors from the Hyperthermophilic Archaebacterium Pyrodictium brockii
R.J. Maier, Department of Biology *$87,639*

The mechanisms by which hyperthermophilic archaebacteria grow and carry out metabolic functions at elevated temperatures have yet to be determined. The objective of this work is to develop an understanding of the metabolic characteristics of, and the electron transport enzymes involved in, hydrogen/sulfur transformation by hyperthermophilic archaebacteria. Efforts will focus on the autotrophic H_2-oxidizing bacterium, *Pyrodictium brockii*, which has a reported optimum growth temperature of 105°C in pure culture. Biochemical and genetic characterization of enzymes involved in the hydrogen oxidizing electron transport pathway will be pursued. Characterization of a quinone and a *c*-type cytochrome, and analysis of the topology in the membrane of these electron transport proteins in net energy generation will be pursued. Determining the thermal stability of electron transport components in comparison with mesophilic counterparts in membranes is a major goal. The quinone will be biochemically analyzed, and the gene encoding the *c*-type cytochrome will be cloned and sequenced to obtain the predicted amino acid sequence. The long-term goal is to understand some of the factors contributing to the biochemical basis of extreme thermophily.

Johns Hopkins University
Baltimore, MD 21218-2685

90. Transport of Ions Across the Inner Envelope Membrane of Chloroplasts
R.E. McCarty, Department of Biology *$210,000 (FY 92 Funds/2 years)*

The metabolic diversity of the chloroplast is astonishing. In addition to photosynthetic carbon reduction, fatty acid and pyrimidine nucleotide biosynthesis, chloroplasts are the site of nitrite and sulfite reduction. Moreover, both Ca^{2+} and Cl^- are required for oxygen evolution by the oxygen evolving complex of the thylakoid membrane. How NO_2^-, SO_3^-, Cl^-, and Ca^{2+} cross the inner envelope membrane is unknown. For nitrite, diffusion of HNO_2 is a possible transport mechanism. The properties of nitrite-mediated proton accumulation by phospholipid (asolectin) vesicles were those predicated by simple HNO_2 diffusion. This behavior was not followed for NO_2^--induced acidification of the interior of vesicles of inner envelope vesicles. Thus, a transporter for NO_2^- may be present in the inner envelope vesicles.

In addition to its role in photosynthesis oxygen evolution, Ca^{2+} very likely fulfills regulatory functions in plants. Although the vacuole probably contains a Ca^{2+} channel, the chloroplast could function to buffer cytosolic Ca^{2+} concentrations. Also, how Ca^{2+} crossed the envelope for its eventual incorporation into the oxygen evolving complex is unknown. Very preliminary evidence for Ca^{2+} transport by inner envelope vesicles has been obtained.

University of Kentucky
Lexington, KY 40546-0091

91. 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 (FY 92 Funds/2 years)*

The assembly of the tetra-Mn cluster of the water-oxidizing complex (WOC) involves sequential photooxidation and ligation of individual Mn atoms in a process called photoactivation. Formation of the cluster promotes functional binding of the 17/23 kD_a extrinsic polypeptides thereby completing the assembly of catalytically active WOC's with PS2. Studies of the mechanism of photoactivation with Mn^{2+}/Ca^{2+}-depleted, 17/23 kD_a-less PS2 membranes show that Ca^{2+} is essential for the ligation of $Mn{\geq}^{3+}$ as a water oxidizing cluster. Eadie-Scatchard kinetic analyses indicate a requirement of two Ca^{2+} sites with K_m values of 90 μM and 1.65 mM, values similar to Ca^{2+}-K_m values for O_2-evolution itself by 17/23 kD-less WOC/PS2 membranes. The divalent cation specificity ($Ca^{2+}>Sr^{2+}>Cd^{2+}>Ba^{2+}>>Mg^{2+}$) for enhancing the photoassembly of the Mn-cluster and O_2 evolution are also very similar. Illumination of 17/23 kD_a-less PS2 under photoactivation conditions in the absence of Ca^{2+} leads to the ligation of EDTA nonextractable "excess" $Mn{\geq}^{3+}$ (~16 Mn/200 Chl) with the membranes and not as Mn oxides. This Mn is catalytically inactive even after prolonged incubations with Ca^{2+}; however, like the native Mn-cluster; it is dissociated by NH_2OH, Tris, and ≥40° treatments. Following its dissociation, catalytically active Mn-clusters (≤4 Mn/200 Chl) can be assembled by re-illumination of the same membranes in the presence of Mn^{2+}/Ca^{2+}. On the other hand, reconstitution of 17/23 kD_a-less PS2 membranes with the 17/23 kD_a proteins permits subsequent partial photoassembly of O_2-evolving Mn-clusters even in the absence of Ca^{2+}. In this case, post-incubation of the photoactivated membranes with Ca^{2+} in darkness is necessary for the conversion of latent to active O_2-evolving Mn-clusters. EPR and thermoluminescence analyses of these differently photoactivated membranes may yield additional insights into the chemical nature of the ligated $Mn{\geq}^{3+}$.

University of Kentucky
Lexington, KY 40546

92. Mechanism and Significance of Post-Translational Modifications in the Large Subunit of Ribulose-Bisphosphate Carboxylase/Oxygenase

R.L. Houtz, Department of Horticulture and Landscape Architecture
$179,000 (FY 92 Funds/2 years)

The methylation of Lys-14 in the large subunit (LS) of higher plant ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) is catalyzed by S-adenosylmethionine (AdoMet):rubisco LS (lysine) $^{\epsilon}$N-methyltransferase (rubisco LSMT). This enzyme is highly specific catalyzing the methylation of only rubisco and only lysyl residue 14 in the LS. Based on X-ray crystallography and enzymological studies methylation of Lys-14 may have significant functional and structural ramifications. The objective of our studies is to provide a detailed structural analysis of rubisco LSMT at the protein and DNA level, and to provide a functional analysis of the *in vitro* and *in vivo* consequences of Lys-14 methylation. Our immediate efforts have been targeted towards purification of rubisco LSMT and we have developed an affinity purification technique for the isolation of pea rubisco LSMT using des(methyl) spinach rubisco immobilized to immobilon-P^{-} membranes. This technique results in an approximate 8000-fold

purification with a 10% recovery of total rubisco LSMT activity. Purified pea rubisco LSMT is a monomeric protein that migrates with an average molecular weight of 58 kD as determined by gel-permeation chromatography, native- and SDS-PAGE. Partial amino acid sequence of pea rubisco LSMT was obtained from affinity purified preparations after electroblotting to immobilon-CD⁻ membranes, followed by *in situ* enzymatic fragmentation with pepsin, and subsequent peptide isolation by reverse-phase HPLC. Four peptic peptides were sequenced, and we are currently attempting to amplify pea rubisco LSMT cDNA with degenerate oligonucleotide probes.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

93. Enzymatic Synthesis and Biomolecular Materials
M.D. Alper, M.D. Bednarski, M. Callstrom, J.F. Kirsch, D.E. Koshland, B.Novak, P.G. Schultz and C.-H. Wong, Center for Advanced Materials — *$167,000**

The goal of this research is the use of natural biological processes and molecules in the synthesis of new materials.

One component focuses on 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; generation of catalytic antibodies for materials synthesis; characterization and processing of the polymer products of these reactions and understanding the structure/function relationships of this new class of materials.

Novel polymers have been synthesized based on biological structures. One has been bound to enzymes thus allowing the enzymes to retain activity in unbuffered water, high temperature and organic solvents. It has allowed peptide synthesis with high yield among a variety of proteases.

Work is also progressing on the synthesis of organic thin films on metal, semiconductor and other surfaces to alter interfacial and surface properties, and to fabricate sensor devices.

* Funded jointly with the Division of Materials Sciences (DOE)

Lawrence Berkeley Laboratory
Berkeley, CA 94720

94. Characterization of Carotenoid and Bacteriochlorophyll Biosynthesis Genes from a Photosynthetic and a Non-Photosynthetic Bacterium
J. Hearst, Structural Biology Division — *$284,000*

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

95. Center for the Analysis of Plant Signal Transduction
W. Gruissem and S.-H. Kim, Structural Biology Division *$63,000*

In mammalian as well as in yeast cells, several proteins involved in signal transduction and cell trafficking, e.g., the ras, Rap/Rho and Rab/Ypt family proteins, undergo protein prenylation. In an attempt to understand signal transduction in plants, we have been studying protein prenylation in the tomato plant. Genetic and enzymatic approaches were chosen to address the problem. Using the genetic approach, we cloned a putative protein farnesyl transferase beta-subunit. Currently, we are in the process of cloning the protein farnesyl transferase alpha subunit. Using the enzymatic approach we have established an assay for protein farnesyl transferase. Applying the assay we are currently trying to purify and characterize the enzyme.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

96. Mechanism of Water Splitting and Oxygen Evolution in Photosynthesis
M.P. Klein, Structural Biology Division *$226,000*

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. We suggest that oxidation of a protein residue, serving as a ligand to the Mn cluster, becomes oxidized during this transition. 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 2.72 A and 2.85 A 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 generally in the membrane plane while the 3.3Å vector is generally 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 structural model of the Mn cluster of the OEC.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

97. Photosynthetic Pigment Proteins and Photosynthetic Light Reactions
K. Sauer, Structural Biology Division *$320,000*

Excitation transfer and trapping in reaction centers of photosynthetic membranes occurs in less than one nanosecond following the absorption of visible light photons. We have investigated the kinetics and energetics of this process using wavelength-resolved transient absorption change and fluorescence decay measurements applied to the light-harvesting pigment-protein phycocyanin (C-PC). X-ray crystallographic structural information for C-PC has enabled us to carry out excitation transfer calculations using Förster inductive-resonance transfer applied to pigment arrays of known geometry. A mutant in which one of the three types of chromophores in C-PC is deleted is similar to allophycocyanin, for which we have recently completed a detailed study of excitation transfer dynamics. We have used time-resolved fluorescence relaxation for the mutant and wild-type C-PC monomers and subunits to resolve the spectroscopic properties of the different chromophore types at room temperature and at 77K. Photosynthetic reaction centers convert absorbed photon energy into charge separation between electron donors and acceptors. We have investigated 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 are extending these studies to photosynthetic bacterial systems, where detailed structural information on the reaction centers is available, to 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 useful for the design of biomimetic synthetic devices for solar energy conversion.

Lehigh University
Bethlehem, PA 18015

98. Post-Transcriptional Regulation of Chloroplast Gene Expression by Nuclear Encoded Gene Products
M.R. Kuchka, Department of Molecular Biology *$178,000 (2 years)*

Our research addresses the expression of chloroplast genes and the involvement of nuclear encoded gene products in specific stages of this expression. We have been studying this process in the eukaryotic alga *Chlamydomonas reinhardtii*, focusing on the synthesis of a single chloroplast encoded polypeptide, the D2 reaction center protein of photosystem II. At least four nuclear genes have been identified by mutation, the products of which are essential for the expression of D2 in the chloroplast. One nuclear gene product works to stabilize the D2-encoding mRNA, psbD, while three others promote the translation of this message. In strains carrying mutations in the genes encoding these latter three products, psbD mRNA is associated with thylakoid membranes and with large classes of polysomes suggesting that translation of the message is initiated normally. However, immunoprecipitation of pulse-labeled proteins with D2-specific antisera detects only trace amounts of the mature sized D2 protein in these strains. We infer from these results that nuclear gene products are required for D2 synthesis at a stage following psbD translation initiation. Nuclear suppressors have been isolated from

phenotypic revertants of the original mutant strains. One suppressor allows for wild type expression of D2 in all original mutant backgrounds. We are currently attempting to characterize the mechanism of suppression effected by this new mutation and to identify others like it in order to understand how the products of these loci work together with the original nuclear gene products in D2 protein synthesis. We are also characterizing two allelic mutants which transcribe but fail to accumulate stable psbD mRNA. Several lines of experimental evidence suggest that the 5' untranslated region (UTR) of the psbD message is the important *cis*-acting element influencing psbD message half-life. The association of proteins from both wild type and mutant cell extracts alters the electrophoretic mobility of an *in vitro* synthesized psbD 5' UTR. We are continuing to pursue this line of experimentation together with uv-crosslinking experiments to identify specific *trans*-acting factors which associate with and stabilize psbD transcripts. Finally, RFLP analysis of the original mutants and an insertional mutagenesis/gene tagging strategy are underway to identify the nuclear genes of interest.

Los Alamos National Laboratory
Los Alamos, NM 87545

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

Methylotrophs are able to grow on simple one-carbon (C_1) compounds such as methane, methylamine or methanol. These organisms derive their energy and reducing power from the oxidation of these C_1 compounds by O_2 and must condense C_1 units to form all of the macromolecular constituents of the cell. The ability of methylotrophs to grow on C_1 compounds gives them considerable potential because they could be used to produce a variety of useful compounds from inexpensive precursors such as methanol or methane. At a practical level, methylotrophs have been used for the large-scale production of single cell protein and alcohol oxidase and are potential sources for the production of many other extracellular metabolites. In addition, methylotrophs may be useful in environmental clean-up of chlorinated solvents, primary alcohols, and amines. In order for this potential to be realized, the fundamental biochemistry and physiology of their metabolism must be understood. Currently, we are examining the metabolism in two methylotrophic strains which assimilate formaldehyde by distinct pathways. Representative of the serine type methylotrophs are *Methylobacterium extorquens* AM1 which assimilate formaldehyde via serine trans-hydroxymethylase and the icl⁻ serine pathway. In addition, we are studying metabolism in the methylotrophic bacterium *Methylophylus methylotrophus* AS1 which assimilates formaldehyde via the ribulose monophosphate (RuMP) pathway. Our approach to the metabolic questions will be to incubate growing cells and cell extracts with ^{13}C-labeled substrates, the products of which can be identified by NMR. Information obtained from tracing one- and two-carbon units give insight into metabolic conversions. Using this approach we have shown that the pathway for oxidation of acetyl-CoA in *Methylobacterium extorquens* AM1, a function required for growth on methanol, involves breaking the carbon-carbon bond in acetate and scrambling the C_1 and C_2 carbon. This data cannot be explained by any known pathway for acetate oxidation. In addition, we have shown *Methylophylus methylotrophus* AS1 oxidizes glucose with flux through both FDP-aldolase and KDPG aldolase implying that both the Entner-Doudoroff pathway and the Embden-Meyerhof pathway for glycolysis are operative in this organism.

University of Maryland
Baltimore, MD 21201

100. Structure and Regulation of L-glutamate Dehydrogenase from Hyperthermophilic Archaea (Archaebacteria)
F.T. Robb, Center of Marine Biotechnology *$181,993 (FY 92 Funds/2 years)*

Our research focus is on the metabolic properties of hyperthermophiles and mechanisms of protein "hyperthermostability". Several marine Archaea that have the unusual property of being able to grow above 100°C have recently been described. We have isolated and characterized the enzyme glutamate dehydrogenase (GDH) from *P. furiosus* (maximal growth temperature 103°C), a new, unnamed isolate called ES4 (maximal growth temperature 110°C) and *Thermococcus litoralis* (maximal growth temperature 96°C). X-ray crystallography studies with these proteins are in progress. Precision scanning microcalorimetry showed that thermal activation of the GDHs from both *P. furiosus* and ES4 occur at 55°C. In both cases, irreversible denaturation takes place at 113°C. ES4 GDH, which has a half life of 3.5 hours at 105°C, is the most thermostable dehydrogenase reported so far. The gdhA genes of *P. furiosus*, ES4 and *T. litoralis* have been cloned and sequenced. Partial sequences of gdhA genes from two other hyperthermophilic isolates (GE5 and GIH) have also been obtained and this data forms the basis for a phylogenetic comparison of hyperthermophiles. GDHs from *P. furiosus* and ES4 have been expressed in an *in vitro* system using a rabbit reticulocyte lysate. We have also developed an *in vivo* expression system in *E. coli* in which the start codons of the gdhA genes of *P. furiosus* and ES4 have been modified to allow transcription from a T7 promoter. These constructs allow high level expression of GDH in *E. coli*. Site directed mutagenesis studies that will elucidate the molecular basis of the adaptations required for protein stability at high temperatures will follow in the near future. The characterization of *P. furiosus* GDH appeared in *J. Biol. Chem.* in November, 1992, and a manuscript describing the purification, cloning and *in vitro* expression of ES4 GDH has been accepted in press by the same journal. A manuscript describing the characterization of the *T. litoralis* GDH has been submitted to *J. Bacteriol.*

University of Maryland
Baltimore, MD 21202

101. Mechanisms of Transcriptional Gene Regulation in the Methanogenic Archaea
K. Sowers, Center of Marine Biotechnology *$83,061*

The goal of this project is to determine the mechanisms of transcriptional gene regulation in the methanogenic Archaea. Although the Archaea have structural gene characteristics that are similar to those of both the Bacteria and the Eucarya, preliminary evidence suggests that they employ Eucarya-like transcription factors for site-specific transcription initiation. We have shown that transcription of the gene encoding CO dehydrogenase (cdh) from *Methanosarcina thermophila* is highly regulated in response to substrate. Cell fractions containing transcription initiation factors will be identified by an *in vitro* transcription assay that employs the upstream promoter region of cdh as a template. DNA binding sequences will then be identified by footprinting or gel binding assays. Transcription factors will be purified from cell fractions by either direct screening with DNA that contains protein binding sequence or by affinity chromatography. Target regulatory sequence will be modified via site-directed mutagenesis and the effects on *in vitro* transcription will be determined. Genes encoding transcription factors will be isolated and sequenced with the goal of identifying

putative sites of protein-DNA binding for conducting future structure-function studies via site-directed mutagenesis. Results of this study will determine whether gene regulation in the Archaea functions by mechanisms that are analogous to the other two lineages or by mechanisms that are unique to this phylogenetic line. Regardless of which mechanism(s) is revealed by this investigation, the results will provide further insight into the global molecular strategies of gene regulation.

University of Maryland
College Park, MD 20742

102. Identifying Calcium Channels and Porters in Plant Membranes
H. Sze, Department of Botany *$89,439*

Calcium transport proteins are the dominant regulators of the signaling function of Ca^{2+}. Cytosolic Ca^{2+} levels are regulated by coordinating passive Ca^{2+} fluxes via channels that increase [Ca^{2+}] and by energy-dependent Ca^{2+} transport via Ca^{2+}-pumping ATPases and H^+/Ca^{2+} antiporters which lower the cytosolic [Ca^{2+}]. Our current goal is to identify selected Ca^{2+} transporters with biochemical and molecular methods as a first step to understanding their structure, function and regulation. One endoplasmic reticulum bound Ca^{2+}-ATPase, identified as Ca^{2+}-dependent phosphoenzyme of 120 kD, has characteristics resembling a calmodulin-stimulated Ca^{2+} pump. This pump is being purified from carrot suspension cultured cells. Results indicate that plants may possess several types of Ca^{2+}-ATPases in which the expression and activity may be differentially regulated depending on the cell-type, subcellular membrane and developmental stage. We are also studying an inositol-1,4,5-trisphosphate (IP_3) sensitive Ca^{2+} channel found on plant endomembranes. Since the properties of a plant IP_3-sensitive Ca^{2+} channel are similar to the animal counterpart, efforts are underway to obtain the gene encoding the plant IP_3-dependent Ca^{2+} channel. Studying the regulation of Ca^{2+} pumps and channels is an important step towards understanding how plants grow and develop.

Massachusetts Institute of Technology
Cambridge, MA 02139

103. Genetic and Biophysical Studies of the Photosynthetic Reaction Center
D.C. Youvan, Department of Chemistry *$116,000 (FY 92 Funds/14 months)*

A principal goal of this research is to engineer a "wrong-way" electron transfer mutant that uses the inactive branch of the bacterial photosynthetic reaction center. Experimental data obtained to date indicate that the directionality of electron transfer is not determined by a single amino acid residue, so it is essential to employ mutagenesis techniques that create many simultaneous mutations. We have developed "helix swap" and other symmetrization procedures wherein entire segments of the protein are deleted and replaced by other segments. We can now report on the first successful "combinatorial" mutagenesis of the reaction center, wherein the amino acid sequence is scrambled over long segments according to rules that are consistent with what we know about the protein's structure and function. These combinatorial experiments are feasible in *Rb. capsulatus* because of the high complexity of the libraries obtained, i.e., over 10^6 mutants are generated by a single experiment. The Digital Imaging Spectrophotometer (supported by a DOE URI) is essential in screening such large libraries. Several mutants have been isolated that are candidates for wrong-way electron transfer.

University of Massachusetts
Amherst, MA 01003

104. Cellulose Fermentation by Nitrogen-Fixing Anaerobic Bacteria
E. Canale-Parola, Department of Microbiology — *$92,000*

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 bacteria 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

105. Genomic Plasticity and Catabolic Potential of Pseudomonas cepacia
T.G. Lessie, Department of Microbiology — *$56,700*

An important goal of the project has been to develop a physical map of the *P. cepacia* genome that would serve as a framework for analysis of the distribution of key genes and transposable gene-activating elements in this bacterium. Our first efforts to construct such a map suggested that the *P. cepacia* genome consisted of multiple replicons. More detailed analyses of the organization of macrorestriction fragments generated by treatment of DNA from *P. cepacia* 17616 with *SwaI*, *PacI*, and *PmeI* has indicated that this strain contains three chromosomes of 3.4, 2.5, and 0.9 Mb. Resolution of unrestricted DNA by pulsed-field electrophoresis, using a CHEF gel apparatus operated at low voltages, revealed the presence of small amounts of the linearized forms of all three replicons. The overall genome size of *P. cepacia* 17616, including the 170-kb cryptic plasmid in this strain, was 7 Mb. An unusual lysine auxotroph in our collection which had suffered the loss of multiple functions, including the ability to utilize ribitol, phthalate, and penicillin was found to have a 0.7 deletion of DNA from the 2.5 Mb replicon. Another strain had a 0.25 Mb deletion from the 0.9 Mb replicon. Southern hybridization experiments indicated that the 3.4-, 2.5-, and 0.9 Mb replicons each contained r-RNA genes. To determine the distribution of other markers, we constructed a derivative of Tn*5-751* containing a *SwaI* site, and used it to insertionally inactivate and map genes related to various biosynthetic and catabolic functions.

Meharry Medical College
Nashville, TN 37208

106. Molecular Characterization of Bacterial Respiration on Minerals
R. Blake II, Biochemistry Department *$87,000*

Aerobic respiration on reduced iron is a principal metabolic activity exhibited by certain chemolithotrophic bacteria that inhabit ore-bearing geological formations exposed to the atmosphere. Recent studies have revealed that each phylogenetically distinct group of iron-oxidizing bacteria expresses one or more unique acid-stable, redox-active biomolecules in conspicuous quantities during aerobic respiration on iron. Structural and functional studies continue on 2 such novel biomolecules that have been purified to electrophoretic homogeneity, rusticyanin from *Thiobacillus ferrooxidans* and cytochrome$_{579}$ from *Leptospirillum ferrooxidans.* The aim of these studies is to determine the role of each protein in the iron respiratory chain of its respective organism. Other redox-active components present in cell-free extracts of iron-oxidizing organisms will be sought, isolated, and investigated with regard to their roles in the same respiratory chains. Another aim is to investigate the molecular principles whereby these bacteria recognize and adhere to their insoluble inorganic substrates. An assay is under development to quantify the binding of these bacteria to their particulate substrates. This assay will be exploited to investigate the mechanism(s), consequences, and principal features of bacterial absorption to insoluble metal sulfides. 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

107. Biochemistry of Dissimilatory Sulfur Oxidation
R. Blake II, Biochemistry Department *$85,972*

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, quantification, and characterization 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 denitrificans* and *Thiobacillus ferrooxidans.* Three major experimental goals are proposed: (1) to purify known sulfur-transformation enzymes to electrophoretic homogeneity; (2) to characterize the structural and functional properties of each purified enzyme; and (3) to perform immunochemical analyses of protein expression using cell-free extracts and polyclonal antibodies directed against each protein purified in goal number one. Both adenosine-5'-phosphosulfate (APS) reductase and bisulfite reductase have been purified to electrophoretic homogeneity from cell-free extracts of *T. denitrificans.* Initial velocity kinetic studies are in progress to define the steady-state kinetic mechanism of the APS reductase. 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 State University DOE Plant Research Laboratory
East Lansing, MI 48824

108. Molecular Basis of Symbiotic Plant-Microbe Interactions
F.J. de Bruijn *$231,646*

The induction of nitrogen-fixing root 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. The persistence and competitive ability of the microbes in the soil and the rhizosphere of plants are important factors in early stages of rhizobial infection. In order to increase our understanding of these early stages, we wish to understand the molecular basis of the microbial response to common environmental stresses and plant factors secreted into the rhizosphere, to develop user friendly methods for the detection and classification of soil microbes and to explore the use of specific nutritional mediators to create "biased rhizopheres". Once the infection process has been initiated, distinct sets of plant genes are induced, which are involved in nodule ontogeny and in symbiotic nitrogen fixation. In order to understand the regulatory circuits responsible for symbiotic control of the expression of these loci, we wish to elucidate the molecular basis of the signal transduction pathways responsible for nodule-(cell-)-specific expression of plant genes encoding symbiosis-specific proteins (nodulins).

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

109. Enzymatic Mechanisms and Regulation of Plant Cell Wall Biosynthesis
D. Delmer *$276,246*

The major 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. Progress in the field of cellulose synthesis has been hampered by inability to detect a convincing *in vitro* activity for the cellulose synthase of plants. However, working with extracts of developing cotton fibers and using affinity labeling techniques, we have identified putative catalytic and regulatory subunits for the enzyme. For callose synthase, a putative catalytic subunit and two non-catalytic subunits have been identified. We are purifying and sequencing these with the goal of eventually identifying and characterizing the genes which code for them. A different approach involves selection of mutants of *Arabidopsis* impaired in cell wall synthesis. One constitutive mutant impaired in secondary wall cellulose synthesis in specific cell types has been characterized; several other putative temperature-sensitive mutants impaired in cellulose, callose, or lignin synthesis are currently being characterized. The task is also beginning to explore aspects of regulation of cytoskeletal organization with studies involving the role of an annexin and a small GTP-binding protein of the *ras* superfamily in regulating organization of actin and/or localization of callose synthase. In addition, preliminary studies indicate that regulatory tyrosine phosphorylation of proteins occurs during wounding or hormone treatment of plants, and we propose to examine the relationship, if any, of these to the regulation of cytoskeletal re-organization.

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

110. Molecular Mechanisms That Regulate the Expression of Genes in Plants
P. Green *$237,646*

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. We have recently developed a system to measure mRNA decay rates in stably transformed tobacco cells grown in suspension cultures. Using this system we have shown that DST sequences, highly conserved among plant SAUR genes, target reporter transcripts for rapid decay in tobacco. Studies are now underway to elucidate the mechanisms by which DST sequences and other instability determinants mediate selective mRNA decay. 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 characterize 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

111. Biogenesis of Plant-specific Cell Organelles
K. Keegstra *$260,647*

We propose to initiate investigations on cell wall biosynthesis. Our objective will be to study the glycosyltransferases responsible for the synthesis of noncellulosic wall polysaccharides and glycoproteins. We propose two distinct, but complementary, approaches. The first is a biochemical approach to assay, purify and characterize specific glycosyltransferases; we will begin by studying the fucosyltransferase and the galactosyltransferase involved in xyloglucan biosynthesis. These enzymes were chosen because both acceptor and donor substrates are available and because the structure of the product, i.e. xyloglucan, is well defined. Our second approach will be to employ molecular biology techniques to isolate cDNA clones for any glycosyltransferases within the endoplasmic reticulum and Golgi apparatus of plant cells. Several strategies will be pursued in our efforts to isolate such clones. All of these strategies will attempt to take advantage of recent advances in the cloning and characterization of glycosyltransferases in animal and yeast systems. Employing a combination of biochemical and molecular biological approaches increases the probability that we will make rapid progress in this important, but understudied, area.

At the same time that we initiate this new research program, we intend to continue our studies on the biogenesis of chloroplasts; focusing especially on the import of cytoplasmically-synthesized proteins. Our major objectives in this project are to identify and characterize the components of the transport apparatus, especially those that reside in the chloroplastic envelope membranes.

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

112. Action and Synthesis of Plant Hormones
H. Kende *$258,646*

The objective of this project is to gain knowledge on the synthesis and mode of action of the plant hormones ethylene and cytokinin. Our research on ethylene biosynthesis and action combines physiological, biochemical and molecular approaches. We are interested in two problems that receive relatively little attention, namely the mechanisms of positive and negative feedback regulation of ethylene biosynthesis and the role of ethylene in vegetative growth. We are investigating the effect of ethylene on the expression of genes encoding both enzymes of the ethylene biosynthetic pathway, 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase and are localizing the expression of these genes by tissue printing and *in situ* hybridization. We are also examining the role of a protein in *Arabidopsis* which appears to regulate ethylene biosynthesis. Our work on growth of deepwater rice deals with the control of ethylene synthesis by low partial pressures of oxygen and the regulation of cell cycle genes in the intercalary meristem. We have resumed work on a hormonal response that we have studied twenty years ago, namely the control of nitrate reductase (NR) activity by cytokinins in *Agrostemma githago*. Progress in research on cytokinins has been slow relative to that with other plant hormones. After having described the system at the physiological and biochemical levels, we have cloned cDNA probes for three NR genes of *Agrostemma* to investigate some of the important mechanistic questions regarding the induction of NR, e.g. whether nitrate and cytokinins regulate the same or different NR genes.

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

113. Interaction of Nuclear and Organelle Genomes
L. McIntosh *$258,646*

It is possible to unravel the molecular events surrounding energy balance - and thus growth and yield - in plants through isolation and manipulation of genes encoding critical protein components of photosynthesis and oxidative phosphorylation.

Possibly the most striking functional difference between plant and animal mitochondria is the presence of two terminal oxidases in higher plants. Plants contain a "normal" cytochrome *c* oxidase along with an "alternative" oxidase which was first characterized by its resistance to cyanide. The electrons flowing through this alternative pathway are not linked to the production of a transmembrane potential and thus are lost, or "wasted", for the production of ATP. We wish to understand how respiration is controlled in higher plants and, in particular, how the distribution of electron flow between the two terminal oxidases is affected by the environment and is reflected in altered carbon utilization.

The cyanobacterium *Synechocystis* sp. PCC 6803 is used 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 the "splitting" of water and generation of oxygen by PSII. The reaction center of PSI is now also being investigated through alteration of specific amino acid residues. Recently, the amino acids ligands for the core [4Fe-4S] center F_x have been confirmed using this approach.

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

114. Sensory Transduction in Plants
K.L. Poff *$237,646*

The primary objective of this project is to understand the mechanisms for the acquisition of environmental information via light reception. In particular, we are studying the blue light photoreceptor pigment system(s) which control(s) numerous light responses such as phototropism in flowering plants. 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. 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. Therefore, an understanding of this process of adaptation is necessary to understand the plant's response to the long term irradiations that are important for the plant under field conditions. Our approach to the study of the pathways for phototropism and phototropic adaptation 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

115. Molecular Mechanisms of Trafficking in the Plant Cell
N.V. Raikhel *$237,646*

Maintenance of separate subcellular compartments in eukaryotic cells depends on the correct sorting and targeting of newly synthesized proteins. Thus, mechanisms must exist in the cell to assure that these proteins are targeted to, and subsequently translocated across, the correct intracellular membranes. We are working with proteins destined for different compartments: vacuole, cell wall and nucleus. We are interested in understanding the molecular determinants of differential protein compartmentalization and identifying the components of the molecular machinery which carry out the sorting process. We have recently analyzed and characterized sequences responsible for protein sorting to the vacuole and to nucleus. We are now concentrating our efforts on the identification and isolation of receptors which recognize these sorting sequences and subsequently mediate protein transport to the vacuole and nucleus.

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

116. Physiological and Molecular Genetics of Arabidopsis
C.R. Somerville *$320,646*

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

117. Molecular Basis of Disease Resistance in Barley
S.C. Somerville *$235,646*

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 pathogen *Xanthomonas campestris* pv. *campestris.* With both projects, our objective is to recover genes encoding disease resistance. The characterization of resistance genes will be an important step in determining a key biochemical component of disease resistance. In addition, understanding the genetic mechanism responsible for the highly polymorphic nature of the barley *M1-a* powdery mildew resistance locus is of interest, as 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.

We have taken two strategies to clone the barley *M1-a* powdery mildew resistance locus. One is to identify closely-linked and flanking randomly amplified polymorphic DNA (RAPD) markers that can be used to initiate a chromosome walk to the *M1-a* locus. The second strategy is to utilize substractive hybridization to isolate cDNA clones unique to resistant barley lines but absent in susceptible mutants. Because chromosome walking has been demonstrated to be a successful technique for recovering genes from *Arabidopsis,* we will employ this method to isolate clones for the *RXC1* locus, which confers resistance to the black rot disease caused by *X. c. campestris.*

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

118. Biochemical and Molecular Aspects of Plant Pathogenesis
J.D. Walton *$237,646*

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

119. Developmental Biology of Nitrogen-Fixing Cyanobacteria
C.P. Wolk *$258,646*

Anabaena and related cyanobacteria utilize solar energy to fix nitrogen gas within cells called heterocysts. We used derivatives of transposon Tn5 to mutagenize the *Anabaena* genome. Mutants unable to fix nitrogen in the presence of oxygen (Fox^- phenotype) were characterized, with an emphasis on two mutants (sites: *hetP*, and the N10 gene) in which heterocyst formation is blocked at a very early stage, and one (M7 gene) in which blockage occurs later. The *hetP* gene shares with one other the property that multiple copies of the wild type gene induce strings of heterocysts; N10 and neighboring genes share homology with components of polyketide synthases; and M7 shows homology with permeases. Aldehyde-biosynthetic genes *luxC, D*, and *E* are expressed in *Anabaena* sufficiently strongly to permit use of *luxAB* (encoding luciferase) to localize transcription to single cells, and without the toxicity resulting from use of exogenous aldehyde. Certain mutations affecting heterocyst formation were found also to influence akinete formation, providing evidence of a biochemical, and probably an evolutionary, relationship between those alternative differentiation processes. To illustrate the potential use of cyanobacteria as agents of bioremediation, appropriate genes, including genes encoding 4-chlorobenzoate dehalogenase, were expressed in *Anabaena*, and a simple colorimetric

assay developed for dehalogenases. 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

120. Environmental Control of Plant Development and Its Relation to Plant Hormones
J.A.D. Zeevaart *$240,647*

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, how these hormones are distributed in the plant, and how they act. Spinach plants treated with the growth retardant BX-112 had reduced levels of GA_1 (3ß-OH) and GA_8, and accumulated GA_{53}, GA_{44}, GA_{19}, and GA_{20}. The levels of four additional dihydroxy-GAs, 3-*epi*-GA_1 (3α-OH), GA_{29} (2ß-OH), GA_{50}(1ßOH), and GA_{81} (2α-OH), that are metabolites of GA_{20}, were not reduced in plants treated with BX-112. This indicates that this chemical specifically blocks 3ß-hydroxylation. Transfer of spinach from short to long days caused an increase in all identified GAs of the early 13-hydroxylation pathway, with GA_{20}, GA_1, and GA_8 showing the largest increases. These findings support the position that of the GAs belonging to the early-13-hydroxylation pathway, GA_1 is the primary GA active *per se* for stem elongation in spinach. The rise in endogenous GA_1 in plants in LD is most likely the primary factor for stem elongation.

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 48824-1319

121. A Structural Assessment of the Role of Cell Surface Carbohydrates of Rhizobium in the Rhizobium/Legume Symbiosis
R.W. Hollingsworth, Department of Biochemistry

$155,000 (FY 92 Funds/2 years)
$40,000 (FY 93 Funds - Supplement for equipment)

In our laboratory, we are interested in the roles of cell surface carbohydrates of *Rhizobium* in determining the initiation and final outcome of the interaction between these bacteria and plants. The

strategy we employ is to chemically characterize cell surface components from wild type strains in pure culture and develop detection methods for determining their relevance in symbiosis. These methods include *in situ* localization in nodules or infection threads by specific antibodies raised to purify, characterized antigens and chemical detection by mass spectrometry using new derivatives which we develop. The impact of environmental factors such as oxygen tension, pH and the presence of plant metabolites on the surface chemistry of bacteria grown in pure culture is also evaluated. The results are correlated with structural modification in surface chemistry observed in bacterial mutants which are defective in infection.

Michigan State University
East Lansing, MI 48824-1312

122. Control of Triacylglycerol Biosynthesis in Plants
J. Ohlrogge, Department of Botany and Plant Pathology

$86,000

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

123. A National Cooperative for Genetic Engineering of Plant Lipids
J. Ohlrogge, Department of Botany and Plant Pathology *$110,000 (2 years)*

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 48824-1319

124. Structure-Function Relationships of ADP-Glucose Pyrophosphorylase: Manipulation of the Plant & Cyanobacterial Genes for Increased Production of Starch in Plants

J. Preiss, Department of Biochemistry *$162,000 (2 years)*

Structure-function relationships of the ADPglucose pyrophosphorylase from higher plants (potato tuber and spinach leaf) and from the cyanobacterium *Anabaena* PCC 7120 will be studied. This research entails the use of amino acid residue chemical modifying reagents (e.g., pyridoxal-5-P, phenylglyoxal, adenosine diphosphate pyridoxal, 8-azido-ATP and 8-azido-ADPglucose) in experiments designed to determine the nature of involvement and the location of the various amino acids at the catalytic and regulatory (allosteric) sites. The structural gene of the *Anabaena* PCC 7120 ADPglucose pyrophosphorylase has been isolated and expressed in *Escherichia coli.* Likewise, the cDNA clones of the large and small subunits of the potato ADPglucose pyrophosphorylase have been expressed in *E. coli.* The expression of these genes and prior chemical modification studies on the purified spinach leaf ADPglucose pyrophosphorylase enables us to do site-directed mutagenesis at various regions of the cyanobacterial and plant enzymes to gain more insight on the nature of the catalytic and effector sites. Attempts will be made to construct "mutant" active plant enzymes that may either be less sensitive to allosteric inhibition by phosphate or that may not require the allosteric activator, 3-phosphoglycerate, for activity. These could be used to produce transgenic plants having increased amounts of starch. Attempts will also be made to understand why the higher plant enzyme (an heterotetramer) requires two different subunits for high activity while the native cyanobacterial enzyme (an homotetramer) with similar regulatory properties, requires only one.

Michigan State University

East Lansing, MI 48824-1101

125. Physiology and Molecular Biology of Ligninolytic Enzyme Systems in Selected Wood-Rotting Fungi

C.A. Reddy, Department of Microbiology *$166,000 (2 years)*

Phanerochaete chrysosporium produces two key families of extracellular peroxidases designated lignin peroxidases (LIPs) and manganese-dependent peroxidases (MNPs) which play a key role in various degradative activities catalyzed by this organism. Several environmental factors such as carbon, nitrogen and manganese have been shown to regulate LIP and MNP production. However, much of this knowledge has been obtained using controlled cultivation in chemically defined media with glucose as the carbon source. Furthermore, little is known about the lignin-degrading systems of other white-rot fungi as well as those of brown-rot fungi. Our recent studies on the distribution of *lip* genes in other

white-rot fungi indicate that the H2-encoding gene appears to be widely distributed among white-rot fungi. In the proposed study, we will initiate an investigation on the regulation of LIP and MNP production and *lip* and *mnp* gene expression during the growth of *P. chrysosporium* with wood as the primary substrate. We will also continue our studies, based on our preliminary results, on the analysis of the lignin-degrading systems of various white-rot fungi and compare these systems with that of *P. chrysosporium.* Additionally, the isolation and characterization of genomic DNA sequences homologous to the H2-encoding cDNA (*CLG4*) will be done in selected white-rot fungi. We will also continue our studies on the characterization of the enzymes involved in lignin-degradation by various brown-rot fungi.

Michigan State University
East Lansing, MI 48824-1319

126. One Carbon Metabolism in Anaerobic Bacteria: Regulation of Carbon and Electron Flow During Organic Acid Fermentations
J.G. Zeikus, Department of Biochemistry *$211,997 (2 years)*

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 *Butyribacterium methylotrophicum, Anaerobiospirillum succiniproducens,* and *Methanosarcina barkeri* are used as model systems. In the conversion of H_2 + CO_2, glucose, or CO to butyrate or butanol by *B. methylotrophicum*, the effect of pH on C & E flow is being studied and is found to have an effect on end products as well as the expression levels of key oxidoreductases. In *A. succiniproducens*, CO_2 services as a catabolic electron acceptor and its addition converts glucose metabolism from a lactate fermentative process into a succinate respiratory process. Molecular cloning and sequencing of the gene for PEP carboxykinase, which has been recently purified in our laboratory, is underway to gain more understanding of this key enzyme. In degradation of acetic acid to the 1-C end products CO_2 and CH_4 by *Methanosarcina barkeri*, we are studying the function of hydrogenases in electron transfer. We have identified a ferredoxin-dependent route of electron coupling to membrane-bound redox centers which uses molecular hydrogen as an intermediate. Also, we are studying the significance of formate versus hydrogen in interspecies electron transfer during syntropic degradation of organic acids by a defined triculture. Studies will define the effect of thermodynamics and bicarbonate concentration on this effect.

University of Michigan
Ann Arbor, MI 48109-1048

127. Molecular Genetics of Myosin Motors in Plants
J. Schiefelbein, Department of Biology *$163,000 (FY 92 Funds/2 years)*

The normal growth and development of plants is dependent on a variety of intracellular transport systems which serve to organize the contents of the cytoplasm, regulate organelle trafficking, and influence cell shape. The molecular motor that drives intracellular transport along actin filaments is myosin, an actin-activated ATPase. The goal of this project is to use a molecular genetic approach to identify and characterize myosin from higher plants. Using polymerase chain reaction methods,

DNA clones representing seven different myosin-like genes have been isolated from *Arabidopsis thaliana*. One of these genes (*MYA1*) encodes a myosin that is structurally similar to the yeast *MYO2*, mouse *Dilute*, and chicken p190 proteins. These myosins appear to play an important role in vesicle trafficking. Current research is focused on defining the intracellular location and function of the *MYA1* product. Other myosin DNA clones from *Arabidopsis* are being used to: (1) determine the predicted 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 the myosins by immunolocalization methods, and (4) inhibit the expression of specific myosin genes by antisense RNA methods. These studies are focused on the role of myosins during morphogenesis of the *Arabidopsis* root, because of the numerous advantages that the root possesses as a model for post-embryonic plant development. These studies will lead to a better understanding of the structure of plant myosins and their role in actin-dependent transport processes.

University of Minnesota
Minneapolis, MN 55455-0312

128. The Mechanism of Switching from an Acidogenic to a Butanol-Acetone Fermentation by Clostridium acetobutylicum
P. Rogers, Department of Microbiology *$92,000*

The objective of this project is to elucidate the detailed mechanism by which the solvent-forming bacterium, *Clostridium acetobutylicum*, regulates 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.

By delivering transposon, Tn916, into *C. acetobutylicum* during conjugation with *Enterococcus faecalis*, we have selected a panel of 42 strains with single Tn916 inserts that are both granulose-negative and solvent-negative mutants. Some of these mutants are also asporogenic. Suspected specific regulatory genes will be identified using dioxygenin-labeled probes containing cell DNA's flanking the Tn916 inserts. The probes are used to select the desired wild-type DNA regions of *C. acetobutylicum* from a DNA library prepared in *E. coli*/pBluescript. We have prepared *E. coli-C. acetobutylicum* shuttle plasmids that effectively electrotransform *C. acetobutylicum* strain 824. Shuttle plasmids with clones of suspected regulatory genes will be electroporated into a panel of mutant strains to attempt physiologic complimentation.

University of Minnesota
Navarre, MN 55392

129. Genetics of Bacteria that Utilize One-Carbon Compounds
R.S. Hanson, Gray Freshwater Biological Institute *$84,000*

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 utilize methanol synthesize up to 20% of their soluble protein as methanol

dehydrogenase (MDH). This enzyme is found in the periplasm of gram negative methylotrophs. At least 20 genes are known to be required for the expression of methanol dehydrogenase activity. We have recently constructed a mobilizable cosmid vector containing a promoterless *xyl* E gene. We cloned the regulatory sequences upstream of the MDH gene from *M. organophilum* strain XX. The vector containing the MDH promoter-regulatory sequences was used to identify and characterize 6 genes required for transcription of the MDH large subunit structural gene (*mox* F). A regulatory protein binding site upstream of the *mox* F gene and other nearby sequences important to regulation of MDH synthesis have also been identified.

Several genes whose expression is induced by methanol have been shown to contain conserved septamers upstream from their promoters.

The vector containing the promoterless *xyl* E gene has also been used to clone sequences required for the synthesis of a soluble methane monooxygenase (sMMO). The transcription of the genes encoding the five proteins required for sMMO activity occurs during copper limited growth. We are studying the mechanisms of copper regulation of sMMO synthesis.

University of Missouri
Columbia, MO 65211

130. Dosage Analysis of Gene Expression in Maize

J. Birchler, Division of Biology *$186,000 (FY 92 Funds/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

131. Position Effect as a Determinant of Variegated Pigmentation in Maize
K.C. Cone, Division of Biological Sciences *$122,000 (2 years)*

DNA methylation plays an important role in the regulation of gene expression in many eukaryotes, usually by silencing gene expression. Although many patterns of gene methylation are stably inherited, the mechanisms governing the establishment and maintenance of methylation are largely unknown. The objective of the proposed research is to gain insight into the molecular signals that control gene methylation. The project will focus on the genetic and molecular analysis of *Pl-Bh*, a gene that controls the synthesis of purple anthocyanin pigments in the maize plant. In contrast to the uniform purple pigmentation associated with vegetative and floral organs of plants carrying a wild-type *Pl* allele, plants carrying *Pl-Bh* exhibit an unusual variegated pattern of pigmentation in nearly all organs of the plant, including the kernel. The variegation is characterized by clonal arrangement of pigmented and unpigmented cells. At the nucleotide level, the *Pl-Bh* gene is virtually identical to the wild-type *Pl* gene. However, unlike the DNA of the wild-type *Pl* allele, the DNA of *Pl-Bh* is heavily methylated. Our experimental approach is to test the hypothesis that the variegated pattern of *Pl-Bh* expression and its altered tissue-specificity are due to a position effect. We propose that DNA methylation of *Pl-Bh* initiates at a *cis*-acting site some distance from the gene, is propagated stochastically toward the gene, and results in the inactivation of gene expression in random clones of cells. To test this hypothesis, we are performing genetic experiments to uncouple by recombination the *Pl-Bh* gene from its *cis*-linked regulatory element. The results of these experiments should serve to enhance our understanding of the *cis*-acting sites in DNA that can serve as initiation points for DNA methylation events. In addition, these experiments should address the question of how the expression of an essentially wild-type gene can be changed by its chromosomal context.

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 *$92,000*

Mitochondria have a central role in the production of cellular energy. 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 that have certain maize nuclear genotypes in combination with cytoplasmic genomes from more distantly-related teosintes 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 grant. We are investigating the effects of two maize nuclear genes, *Rcm1* and *Mct*, on mitochondrial function and gene expression. Plants with the teosinte cytoplasms and homozygous for the recessive *rcm* allele are small and slow-growing and the kernels are reduced in size. We are attempting to clone this locus by transposon tagging (using *Spm* and *Mutator*) so that we can study its action. The product of the *Mct* allele, present in certain maize lines, specifically alters the cytochrome oxidase subunit 2 transcript

pattern in the mitochondria from the perennial teosintes. It appears to be a gene-specific transcription factor that can activate transcription from a "cryptic" mitochondrial promoter.

University of Missouri
Columbia, MO 65211

133. Genetics of the Sulfate-Reducing Bacteria
J.D. Wall and B.J. Rapp-Giles, Biochemistry Department *$85,000*

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 developed shuttle vectors for *D. desulfuricans* and *Desulfovibrio fructosovorans* based on the small endogenous plasmid, pBG1, that we have characterized. These vectors are fully compatible with IncQ plasmids, the only other plasmid incompatibility group that is stable in the SRB. We have also found that ß-galactosidase is expressed in the SRB but the chromogenic substrate X-gal is either not permeable or the chromogen is decolorized by sulfide. In addition, we have found that Tn7 transposes into a preferred site with good efficiency in *D. desulfuricans* G200. A mini Tn7 carrying resistance to kanamycin also hops well into the chromosome making it feasible to construct single-copy insertions of cloned genes for studies of regulatory phenomenon. Finally we have also documented the transposition of two derivatives of Tn5 which opens the possibility of random mutagenesis, albeit at a low frequency at present.

Mount Sinai School of Medicine
New York, NY 10029

134. The Respiratory Chain of Alkaliphilic Bacteria
T.A. Krulwich, Department of Biochemistry *$202,999 (FY 92 Funds/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 upregulated during growth at highly alkaline pH, as shown both by mRNA abundance and by content of the complex in the membranes of pH 10.5- vs. pH 7.5-grown cells. Current efforts are focused on: parallel investigations of an *o*-type terminal oxidase; characterization of mutants that no longer can grow at pH 10.5 and have altered membrane cytochrome patterns; and on use of the purified, suitably modified, and reconstituted caa_3 complex to deliver fluorescent probes of interest to membrane vesicles.

National Renewable Energy Laboratory
Golden, CO 80401

135. The Water-Splitting Apparatus of Photosynthesis
M. Seibert, Photoconversion Branch *$150,000*

A bound tetrameric Mn cluster catalyzes photosynthetic water-splitting function by facilitating electron donation to photosystem II (PSII) and releasing O_2 as a byproduct. We have observed "anomalous"

O_2 production in chemically stressed spinach PSII membranes containing the Mn cluster and suggest that it results from oxidation of H_2O_2 that can gain access to lower S states. The kinetics of O_2 release under these conditions was 5-20 times slower than the normal 3-5 ms release time observed for O_2 from water. This could be a PSII protective mechanism. In the absence of functional Mn, exogenous Mn^{+2} or diphenylcarbazide (DPC) can act as artificial donors to PSII. When both are present, light-driven DPC donation to PSII is inhibited by micromolar concentrations of $MnCl_2$, and this phenomenon has been used to identify amino acids that contribute ligands to Mn. In order to understand the interaction between DPC and Mn^{+2} in spinach, we developed a kinetic model involving the non-competitive inhibition of DPC donation by Mn^{+2} at both high and low affinity binding sites. The former was predicted by previous work. The K_ms used in the model raise questions about the nature of various Mn-binding sites reported in the literature. Preliminary results with *Synechocystis* indicate that the K_ms for DPC donation are quite different from those of spinach and affect the interpretation of the interaction between DPC and Mn in the cyanobacterium. Investigation of Mn-ligands in *Synechocystis* site-directed mutants is in progress.

University of Nebraska
Lincoln, NE 68588-0118

136. Control of Sugar Transport and Metabolism in Zymomonas mobilis
T. Conway, School of Biological Sciences *$192,000 (2 years)*

The role of gene expression in regulating glycolytic enzyme synthesis in a balance that allows proper control over metabolic flux is being elucidated in *Zymomonas mobilis*. Glucose transport in this bacterium is via facilitated diffusion and the corresponding gene, *glf*, is co-transcribed with the genes encoding the first three intracellular steps of glucose metabolism. Regulation of the *glf-zwf-edd-glk* operon is intriguingly complex, being subject to transcriptional control and extensive post-transcriptional mRNA processing. These controls may provide a means for adjusting synthesis of the glucose facilitator and metabolic enzymes in response to metabolic demands. Ongoing experiments are designed to elucidate facilitator expression, function, and communication with the glycolytic enzymes. Molecular characterization of the facilitator is expected to add details to the current model of transmembrane transporter structure, implicating residues that determine the distinct function of non-energy-dependent transport.

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 and A.O. Jackson, University of California, Berkeley
$99,517

Our research continues to emphasize identification of viral sequences affecting viral pathogenicity by characterization of a unique class of RNA called defective interfering RNAs. DIs are linear deletion mutants of viral genomes that interfere with helper virus and reduce disease severity. These studies are providing insight into basic mechanisms important in RNA replication and RNA recombination. 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 permitted the identification of essential

regions of viral sequence in natural DIs necessary for replication. We are continuing to investigate cis-sequences important in DI encapsidation, selective competition and symptom attenuation in several host species. Study of the biogenesis of DI RNAs has conclusively shown that they arise *de novo* from the viral genome and that larger DI species evolve into smaller, more competitive forms with passage. We have also shown that DI RNAs can recombine among themselves evolving into larger species, as well as with defective viral genomes to produce infectious progeny. Deletion mutagenesis of TBSV genome has established a functional role for each of the genes encoded by the virus. The insertion of reporter genes (GUS and CAT) into regions of the viral genome has permitted study of the movement of the virus in whole plants. These studies, along with continued study of the expression of DI sequences from transgenic plants, should provide basic approaches for developing rational strategies of virus disease control.

University of Nebraska
Lincoln, NE 68583

138. Mechanistic Enzymology of CO Dehydrogenase from Clostridium thermoaceticum
S.W. Ragsdale, Department of Biochemistry *$94,000*

Acetogenic bacteria perform CO_2 and CO fixation by the reductive acetyl-CoA pathway. Carbon monoxide dehydrogenase (CODH) plays a key role in this pathway by reacting with a methyl group (donated from the methylated corrinoid/iron-sulfur protein, C/Fe-SP), with CO, and with CoA to form enzyme-bound intermediates. These intermediates are then condensed by CODH to form acetyl-CoA. The site of assembly appears to be a mixed metal center which has been described as a [Ni-X-Fe_{3-4}-S_4] complex, where X is a ligand bridge whose identity is as yet undetermined. The rate limiting step in the synthesis of acetyl-CoA from methyltetrahydrofolate, CO, and CoA has been determined to be methylation of CODH, which involves nucleophilic attack of CODH on the methyl group of the methylated C/Fe-SP, forming methyl-CODH and Co^{1+}. Our goals for the upcoming year are to use a combination of presteady-state and steady-state kinetic methods and spectroscopy to determine: (1) the mechanism of methylation of CODH including which microscopic step(s) is primarily rate determining, (2) the rates of formation and decay of each of the enzyme-bound intermediates, and (3) the identity of the metal (i.e., nickel or iron) that forms the metal-carbon bond in the CODH-CO complex.

New York University
New York, NY 10003

139. Asparagine Synthetase Gene Expression and Plant Nitrogen Metabolism
G. Coruzzi, Department of Biology *$198,000 (FY 92 Funds/2 years)*

We have been using a molecular-genetic approach to study the role of asparagine synthetase (AS) in plant nitrogen metabolism. The cDNAs encoding pea AS1 and AS2 have been used to understand more about the encoded enzymes and the factors which regulate gene expression. In particular, these pea AS cDNAs have been expressed in an *E. coli* asparagine auxotroph to test whether each AS polypeptide may assemble into a functional homodimer *in vivo*. We have deleted the glutamine binding domain of AS1 and will also attempt these complementation experiments. This latter experiment should determine whether the AS1 polypeptide can utilize glutamine and/or ammonia as a nitrogen donor in the biosynthesis of asparagine. We have shown that both AS1 and AS2 genes

of pea are negatively regulated by light. More recently we have characterized a single AS gene from *Arabidopsis* and shown a similar pattern of light regulation. We are in the process of identifying cis-elements and trans-acting factors which regulate AS gene expression in plants. We have also begun to manipulate the expression of AS in transgenic plants to test the effects on plant nitrogen assimilation. The results of our studies on AS gene regulation suggest that the manipulation of AS gene expression in transgenic plants may have implications for improving nitrogen use in plants.

North Carolina State University
Raleigh, NC 27695-7905

140. Bioenergetic and Physiological Studies of Hyperthermophilic Archaea
R.M. Kelly, Department of Chemical Engineering *$182,456 (2 years)*

The bioenergetics and growth physiology of hyperthermophilic archaea (optimal growth temperature above 90°C) have yet to be examined in sufficient detail to facilitate mechanistic studies of central metabolic pathways. This is not because these issues are unimportant. Rather, working with microorganisms at elevated temperatures, and sometimes elevated pressures, presents numerous difficulties. This work will draw together stoichiometric, kinetic and regulatory information for several hyperthermophiles to provide a basis for comparison to each other, as well as to less thermophilic organisms with similar metabolic patterns. Based on the present understanding of a putative energetic pathway in *Pyrococcus furiosus* and several amylolytic and proteolytic enzymes, which apparently are related to this pathway, we will follow metabolic patterns in this bacterium for a variety of growth conditions. The bioenergetic significance of sulfur reduction, the importance of tungsten and the relative utilization of peptides/amino acids and saccharides as carbon/energy sources are among the issues to be examined. The physiological response to thermal and nutritional stress will also be studied. This effort will then be extended to other hyperthermophiles so that the similarities and differences between high temperature heterotrophic physiologies can be determined. The results from this study will be important in further scientific and technological study of this novel group of microorganisms.

North Carolina State University
Raleigh, NC 27695-8008

141. Transcription Factors Regulating Lignin Biosynthesis in Xylem
R. Sederoff, D. O'Malley, and Ross Whetten, Department of Forestry
$194,910 (FY 92 Funds/2 years)

Lignin forms part of the embedding matrix which is essential for reinforcing cellulose microfibrils and imparting rigidity to the plant cell wall. In woody plants, lignin is about one fourth of the total plant biomass. In spite of its importance, the enzymatic pathway for lignin biosynthesis is not fully established and the mechanisms regulating lignification are not known. To this end, we have purified and characterized four enzymes in the pathway for lignin biosynthesis from loblolly pine: phenylalanine ammonia-lyase (PAL), 4-coumarate:coenzyme A ligase (4CL), cinnamyl alcohol dehydrogenase (CAD), and a laccase (LAC). We intend to identify the specific genes coding for these enzymes involved in wood formation. Within the past year, 4CL and LAC were purified and characterized. Work is in progress to further characterize genomic clones of both PAL and CAD. A full length cDNA clone of CAD has been isolated and sequenced. Partial cDNA clones for 4CL and LAC have been identified

and are being characterized. The xylem laccase is a new enzyme, associated with lignification at the tissue and subcellular level. The laccase is likely to be directly involved in polymerization of monolignols. We now have four genes in the lignin biosynthetic pathway to use for the analysis of promoters and to identify transcription factors. Our experiments should lead to a greater understanding of lignin formation, essential for genetic engineering of lignin in forest trees.

Ohio State University
Columbus, OH 43210

142. Structure and Regulation of an Archaebacterial Promoter: An In Vivo Study
C.J. Daniels, Department of Microbiology *$88,960*

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

143. Biosynthesis of Hydrocarbons
P.E. Kolattukudy, Biotechnology Center *$186,000 (2 years)*

Nonisoprenoid hydrocarbons are ubiquitous in nature and some algae produce large amounts of hydrocarbons. Biosynthesis of these hydrocarbons involves elongation of a fatty acid, reduction of the elongated acyl-CoA to the corresponding aldehyde, followed by decarbonylation of the aldehyde. The novel enzyme, decarbonylase, was purified from *Botryococcus braunii.* This enzyme appears to contain a cobalt porphyrin. Investigation on this enzyme includes determination of subunit composition, substrate specificity, retention of aldehydic H, and reversibility of the reaction as well as the mechanism of participation of the metal in the decarbonylation reaction. The acyl-CoA reductase that generates the substrate fatty aldehyde for this decarbonylase in *B. braunii* was solubilized and

purified. This reductase requires NADH as cofactor and generates free fatty aldehyde as the product. This cofactor specificity agrees with the previous finding that conversion of acyl-CoA to alkane by the cell-free preparation from *B. braunii* required specifically NADH. This reductase showed homology to the bacterial reductase.

Ohio State University
Columbus, OH 43210

144. Transmethylation Reactions During Methanogenesis from Acetate in Methanosarcina barkeri
J.A. Krzycki, Department of Microbiology *$83,000*

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. We have identified two novel abundant, easily solubilized, corrinoid proteins with sizes of 480 and 29 kDa which are methylated by acetate derived methyl groups when the terminal enzyme of the pathway is inhibited in cell extracts, and demethylated by the addition of coenzyme M. Methyl-groups from both purified proteins are converted to methane. Both proteins may participate in transmethylation reactions during methanogenesis from acetate. The isolated 480 kDa has an a_6b_6 configuration, 6 methylated corrinoids, and is transiently methylated during methanogenesis from acetate. The 29 kDa is a monomer with 1 methyl-corrinoid. Neither possesses iron-sulfur clusters, and both have base-on methylated corrinoid. The 480 kDa is methylated 100 fold less in extracts of cells grown on methanol than on acetate. Acetate grown cells possess an abundance of the 480 kDa protein, but the protein is significantly down regulated in cells grown on methanol, H_2/CO_2, or trimethylamine. This suggests the 480 kDa protein plays a unique role during methane formation from acetate. Neither corrinoid protein resembles any corrinoid protein previously identified in *Methanosarcina* including the corrinoid/iron sulfur protein associated with carbon monoxide dehydrogenase which catalyzes cleavage of acetyl-CoA. This 1.6 MDa complex was identified by alkylation with radiolabeled ethyl iodide in extracts under hydrogen. It possessed 3 subunits in addition to those of carbon monoxide dehydrogenase. Neither the 480 or 29 kDa corrinoid proteins were associated with the complex. We are continuing to characterize both proteins, and define their roles in this methanogen using antibodies 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. Transfer to coenzyme M occurs with retention of configuration, suggesting an even number of methylations in the pathway.

Ohio State University
Columbus, OH 43210

145. The Molecular Characterization of the Lignin-Forming Peroxidase: Growth, Development, and Response to Stress
L.M. Lagrimini, Department of Horticulture *$131,770*

Transgenic tobacco plants are being studied which over-express the anionic peroxidase isoenzyme under control of the CaMV 35S promoter with regard to their phenotypic characteristics. 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. A ten-fold increase of this enzyme throughout the plant resulted in 2-3 fold higher lignin levels in leaf and stem tissue and 9-10 fold higher lignin levels in root tissue. These same tissues were found to contain 200-300% higher soluble phenols which may serve as substrate for the enzyme. These observations indicate that peroxidase may be limiting in lignin formation for many tissues. 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. Roots had the same overall length as control plants, however, the branching was reduced. The root tissue was found to have low levels of auxin, and root explants were unresponsive to exogenous auxin. Using antisense RNA produced by the 35S promoter, endogenous peroxidase activity was reduced by 20-fold. These plants were shown to have elevated infertility and severely epinastic leaves. Currently, transgenic plants are being constructed with sense and antisense RNA being produced from a xylem-specific, root specific, or leaf specific promoter.

Ohio State University
Columbus, OH 43210-1292

146. Structure and Regulation of Methanogen Genes
J.N. Reeve, Department of Microbiology *$319,629 (FY 92 Funds/2 years)*

The goals of this project are to characterize the genetic organization and mechanisms of regulation of gene expression in methanogens, to use the techniques of molecular biology to dissect and understand the biosynthesis of methane and to develop genetic exchange systems for methanogens. Regulation of transcription of the *mcr* operon that encodes methyl coenzyme M reductase I (MRI), the enzyme that syntheses methane, is being studied in *Methanococcus vannielii* and *Methanobacterium thermoautotrophicum*. The numbers of transcripts per cell, their half-lives and their fate under a variety of growth conditions are being determined. The *mrt* operon, that encodes a second methyl reductase, designated MRII, in *M. thermoautotrophicum*, has been cloned so that the molecular basis for the growth dependent differential synthesis of the two isozymes, MRI and MRII, can now be investigated. The polyferredoxin-encoding *mvh*B gene of *M. thermoautotrophicum* has been expressed at high levels in *E. coli* which has facilitated the purification of sufficiently large amounts of the polyferredoxin for detailed biochemical and biophysical analyses. The role of this unusual protein in electron transport during methanogenesis is being investigated. Several novel methanogen plasmids have been obtained and are being developed by the incorporation of selectable genes, methanogen promoters and transcription terminators for use as donor DNAs and cloning vectors in transformation protocols for *M. thermoautotrophicum.*

Ohio State University
Columbus, OH 43210

147. Photosynthetic Electron Transport in Genetically Altered Chloroplasts
R.T. Sayre, Departments of Biochemistry and Plant Biology
$208,000 (FY 92 Funds/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, OH 43210-1292

148. Regulation of Alternative CO_2 Fixation Pathways in Procaryotic and Eucaryotic Photosynthetic Organisms

F.R. Tabita, Department of Microbiology *$93,000*

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 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 16PHC; this strain was capable of photoheterotrophic growth with CO_2 as electron acceptor. Subsequent studies showed that strain 16PHC catalyzed substantial rates of whole-cell CO_2 fixation, but retained the inability to synthesize RubisCO. Strain 16PHC thus possessed the same phenotype as a RubisCO deletion mutant of *Rhodospirillum rubrum*, strain I-19. Strains I-19 and 16PHC are the focus for further genetic and enzymological studies designed to determine the mechanism and regulation of the alternative CO_2 assimilatory path. Transposon mutants of 16PHC and I-19 which lose the ability to use CO_2 as electron acceptor are being used to isolate the genes required for the alternative CO_2 fixation reactions. 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

149. The Structure of Pectins from Cotton Suspension Culture Cell Walls
A. Mort, Department of Biochemistry $116,877

In this project we use cotton suspension culture cells as a model system to understand the structure and subsequently, function of pectins in plant cell walls. At present we focus on structural studies. We use anhydrous liquid HF, purified enzymes, and concentrated imidazale buffer to extract each subsection of the pectins in high yields from the walls. We find four distinct types of pectin structures: 1) Rhamnogalacturonan I (RGI), 2) Rhamnogalacturonan II, 3) 50% methyl esterified homogalacturonan, and 4) ~15% methyl esterified homogalacturonan. The RGI contains a ratio of ~1 acetate ester per rhamnose. There is partial acetylation of the galacturonic acid residue adjacent to each rhamnose. However, results suggest some may be elsewhere. It has proven rather difficult to locate all of the acetate residues, but we will continue to try. We are determining the pattern of esterification of the 50% methyl esterified homogalacturonan by methods we have developed involving conversion of methyl esterified GalA residues to galactose and subsequent specific fragmentations and analysis of the resulting mixed galactogalacturonan. The pattern appears to be an alternation of esterified and non-esterified residues, which is very different from the almost random pattern of the commercially available fruit pectins. The methyl esters in the low ester homogalacturonan appear to be clustered.

We will attempt to determine how, and if, the sections of pectin are associated with each other by using the minimal number of degradative procedures possible that still allows substantial amounts of pectins to be extracted from the walls.

Because tissue culture cells may not represent many of the cell types in real plants, we will extend our structural characterization of pectins to cotton cotyledons, probably the most homogeneous (but by no means completely homogeneous) tissue available in quantity from cotton plants.

University of Oklahoma
Norman, OK 73019-0245

150. Effect of Community Structure on Anaerobic Aromatic Degradation
M.J. McInerney, Department of Botany and Microbiology $82,000

Factors affecting the rate and extent of benzoate degradation by an anaerobic syntrophic bacterium morphologically similar to *Syntrophus buswellii* was studied in coculture with *Desulfovibrio* strain G11. In the presence of 20 mM acetate or more, benzoate degradation reached a threshold value of 2 to 29 μM, the magnitude of which depended on the initial acetate concentration. Hydrogen partial pressures were lower in cocultures that had reached a threshold for benzoate consumption, compared to cocultures where no benzoate threshold was observed. Thus, the observed threshold values were not due to hydrogen inhibition, but rather result from a thermodynamic constrain. Growth and benzoate degradation were observed when the syntrophic benzoate degrader was grown in coculture with *Desulfoarculus baarsii* that uses formate, but not hydrogen, directly demonstrating that formate can serve as the sole intermediate involved in interspecies electron transfer in syntrophic associations.

The biochemistry of fatty acid degradation, and poly-ß-hydroxyalkanoate (PHA) synthesis in *Syntrophomonas wolfei* was studied. The purified acetoacetyl-CoA thiolase had a homotetrameric subunit composition with a native molecular weight of 160,000. The reaction mechanism was Ping Pong Bi Bi, and both the condensation and thiolytic cleavage reactions were product inhibited, with a K_i^{app} of 3.2 µM for coenzyme A (CoA), and of 500 µM for acetyl-CoA. The acetoacetyl-CoA thiolase is similar in its structural, kinetic, and apparent regulatory properties to other biosynthetic acetoacetyl-CoA thiolases from phylogenetically distinct bacteria that synthesize PHA.

Oregon Graduate Institute of Science & Technology

Portland, OR 97291-1000

151. Biochemical Genetics of Lignin Degradation by Phanerochaete chrysosporium

M.H. Gold, Department of Chemistry, Biochemistry and Molecular Biology

$251,998 (FY 92 Funds/2 years)

The objective of this research is to further our understanding of the lignin degradative system of the basidiomycete *Phanerochaete chrysosporium*. We are using a variety of biochemical and molecular biological approaches to characterize the enzymes and genes involved in this process.

We are studying the structure and mechanism of lignin peroxidase (LiP) and manganese peroxidase (MnP) via spectroscopic, kinetic and bioorganic mechanistic methods. As part of this project, in collaboration with Prof. T. Poulos (University of California, Irvine) we recently published the first LiP crystal structure. We also are examining the oxidation of a variety of model dimers and polymers with these enzymes, including the oxidation of cytochrome c by lignin peroxidase. Finally, we are characterizing an intracellular quinone reductase and a dioxygenase which are involved in the further degradation of monomeric lignin degradation products.

Using our DNA transformation system for *P. chrysosporium*, we have developed an efficient homologous expression system for MnP. This system allows the expression and secretion of recombinant MnP in *P. chrysosporium* under the control of a primary metabolic gene promoter. This expression system will facilitate our structure/function studies of MnP via site-directed mutagenesis. A similar system is being developed for LiP. We also have developed the first gene replacement system for *P. chrysosporium*. This system, based on the resistance of some Ura^- mutants to the drug 5-fluoroorotate, will enable us to utilize reverse genetics in this organism, via the targeted disruption of key components of the lignin degradation system.

Oregon Graduate Institute of Science and Technology

Portland, OR 97291-1000

152. Oxidative Enzymes Involved in Fungal Cellulose Degradation

V. Renganathan, Department of Chemistry, Biochemistry and Molecular Biolgy

$166,000 (FY 92 Funds/2 years)

Several cellulolytic fungi produce extracellular cellobiose-oxidizing enzymes in addition to cellulases. These enzymes oxidize cellobiose to cellobionolactone in the presence of suitable electron acceptors such as cytochrome c or quinones. Cellobiose dehydrogenase (CDH) is an extracellular hemoflavoenzyme produced by the cellulose-degrading cultures of *Phanerochaete chrysosporium,*

Sporotrichum thermophile, and *Coniophora puteana*, a brown-rot fungus. Though CDH has been identified in a number of cellulose-degrading fungi, its role in cellulose degradation has not been understood. The aim of this project is to provide an understanding of the contribution of CDH to fungal cellulose degradation utilizing the CDH from *P. chrysosporium* as a model.

We have established that CDH from *P. chrysosporium* enhances the crystalline cellulose degradation by cellulases. In this project, the biochemical mechanisms responsible for the enhancement in cellulose hydrolysis will be probed. Our findings will be useful in enhancing the rate of crystalline cellulose hydrolysis by cellulases. Also, CDH is a unique enzyme in that it contains both a heme and a flavin on a monomeric subunit. We plan to examine the heme structure of CDH by resonance Raman spectroscopy and the mechanism of electron transfer between the heme and the flavin by spectro-electrochemical methods. CDH and ß-glucosidase compete for cellobiose formed from cellulose degradation. To understand the relative contributions of CDH and ß-glucosidase to cellobiose degradation, ß-glucosidase will be purified and its cellobiose hydrolysis kinetics will be compared to the kinetics of cellobiose dehydrogenation by CDH.

Oregon State University
Corvallis, OR 97331

153. Catalytic Mechanism of Hydrogenase from Aerobic N_2-Fixing Microorganisms
D.J. Arp, Laboratory for Nitrogen Fixation Research *$74,000*

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

154. Analysis of Potyviral Processing: A Basis for Pathogen Derived Resistance?
W.G. Dougherty, Department of Microbiology *$192,000 (FY 92 Funds/2 years)*

Tobacco etch virus (TEV) is a member of the potyviridae, a group of viruses placed in the picornavirus super-family. TEV has a single-stranded, plus sense RNA genome that is expressed initially as a large polyprotein. This genome-derived translation product is then processed by three virus-encoded proteolytic activities. One of these proteinases, referred to as the NIa proteinase, is responsible for six processing events. We have developed a prokaryotic expression system which produces high levels of functional proteinase that can be readily purified. This enzyme is being used in biochemical studies to understand the kinetics of TEV NIa proteolysis. We have also adapted our expression system and have generated methodology that has been extremely useful in the production of protein and long peptides (20 to 100 amino acids in length) that are difficult to chemically synthesize. The system consists of an expression plasmid vector into which sequences coding for a peptide or protein are inserted in-frame with the gene for glutathione S-transferase fused with the coding sequence for a TEV NIa cleavage site. This fusion protein is expressed in *E. coli* and readily purified from *E. coli* proteins using glutathione-agarose beads in an affinity chromatography procedure. The peptide or protein is selectively removed from the column by cleaving it from the glutathione S-transferase using a histidine tagged TEV NIa proteinase. The proteinase can be selectively removed from the protein or peptide using Nickel-agarose affinity chromatography which retains the TEV NIa proteinase on the column. This expression system will have wide applicability to other researchers.

University of Oregon
Eugene, OR 97403-1229

155. Transposon-Induced Nuclear Mutations that Alter Chloroplast Gene Expression
A. Barkan, Institute of Molecular Biology *$180,000 (2 years)*

The long term goal of this project is to use mutant phenotypes as a guide to nuclear genes that control the timing and cell-type specificity of chloroplast gene expression. Studies are being conducted with nuclear mutants of maize that are defective in the biogenesis or translation of chloroplast mRNAs. Currently, studies are focused on two nuclear mutants with specific and unique lesions in chloroplast RNA processing (*crp* mutants). *crp1* mutants (formerly called *hcf136*) fail to accumulate the cytochrome f/b6 complex. The protein loss is due to a defect in the metabolism of transcripts encoding the *petB* and *petD* gene products, two subunits of the missing complex. Mutant seedlings lack the monocistronic *petB* and *petD* mRNAs, which both arise in normal plants by endonucleolytic cleavage of the polycistronic primary transcript of the *psbB* gene cluster. Precursor mRNAs accumulate normally in *crp1*, indicating that its defect is due either to a failure to cleave the precursors, or a failure to stabilize the fully processed mRNAs. We are interested in both the biochemistry of this site-specific RNA processing and in the role of the processing in generating translatable mRNAs. To address the latter, we are quantifying the rates of synthesis of the *petB* and *petD* gene products with the goal of determining whether the missing transcripts are more efficiently translated than their precursors. To address the biochemistry of the defect in RNA metabolism, the *crp1* gene is being cloned via the transposon tag.

crp2 (formerly called *hcf142*) lacks the predominant mRNA encoding *petA*, but appears to be otherwise unimpaired in chloroplast RNA metabolism. The precise role of *crp2* in synthesizing or stabilizing the *petA* mRNA is being investigated through biochemical studies. A molecular clone of the *crp2* gene will be obtained by transposon-facilitated cloning and will be used to more precisely define the role of the *crp2* gene product.

Pennsylvania State University
University Park, PA 16802

156. The Characterization of Psychrophilic Microorganisms and Their Potentially Useful Cold-Active Glycosidases
J.E. Brenchley, Department of Molecular & Cell Biology *$172,000 (2 years)*

Although the dominant environment of our earth is cold, we know surprisingly little about the microorganisms inhabiting these environments. Of special interest is the characterization of microbial enzymes with high activities at low temperatures. Our objective is to physiologically characterize psychrophilic and psychrotropic microorganisms and to purify, study and genetically engineer their cold-active glycosidases. These microorganisms and enzymes can be used for low-temperature conversions of plant biomass, whey, etc., into non-polluting, low-cost carbon sources useful for fermentation by biotechnology companies and for the synthesis of chemical fuels. The specific goals are to: 1) examine isolates for glucosidases and/or galactosidases that are highly active at low temperatures, 2) characterize the physiology of these strains to determine whether the enzymes are intercellular or extracellular, which carbon sources and inducers give the best cell yields and highest enzyme activities, and whether isozymes with distinct temperature optima are synthesized during growth at different temperatures, 3) purify the most cold-active glycosidases and analyze their temperature profiles, thermolabilities, substrate specificities, pH profiles, ion requirements, etc., and 4) clone and sequence the genes of selected enzymes and compare their structures with corresponding enzymes from organisms having higher growth temperatures. In addition to yielding useful microorganisms and cold-active enzymes, this work is fundamental to our understanding of the microbial diversity in the cold environments dominating our biosphere.

Pennsylvania State University
University Park, PA 16802

157. Rapid Regulatory Control of Plant Cell Expansion and Wall Relaxation
D.J. Cosgrove, Department of Biology *$94,000*

Plant cell enlargement is controlled by biochemical processes that loosen the cell wall, induce stress relaxation, and consequently give rise to cell water uptake and wall expansion. This project is aimed at elucidating the physical, cellular and molecular mechanisms controlling wall loosening and relaxation. High-resolution analyses of cell expansion and wall relaxation indicate that both processes exhibit complex dynamics, indicative of a rapid feedback-controlled process. By stimulating plants with low-amplitude pressure sinusoids, we have discovered complex dynamics indicative of growth rate feedback. Plants display resonance, harmonics, noise attenuation and period doublings in their growth responses to these perturbations. Simultaneous electrical measurements show parallel dynamic behaviors in membrane potentials. Our results require two surprising additions to the conventional model of plant cell growth, namely that there must be a growth-rate sensor which integrates growth

over 1 min or less, and also there must be a means to alter wall relaxation rapidly - within 2-3 min in some plants - in response to the signal from the growth-rate sensor. Current work is directed at identifying the molecular nature of the growth rate sensor, its signal, and the means of altering wall relaxation. 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. We are testing for alterations in wall pH, wall redox potential, and wall enzyme activities as potential means for rapid regulation of wall relaxation. The results of these studies will further our understanding of basic growth processes in plants and their control by hormones, light, drought and other agents.

Pennsylvania State University
University Park, PA 16802

158. Role of Ca^{++}/calmodulin in the Regulation of Microtubules in Higher Plants
R. Cyr, Department of Biology *$81,000*

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 have 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 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 in conjunction with immunocytochemical and biochemical methodologies to dissect how Ca^{++}/calmodulin interacts with Mts to affect their function. 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

159. Characterization of Lignin and Mn Peroxidases from Phanerochaete chrysosporium
M. Tien, Department of Molecular and Cell Biology *$102,000*

Lignin and Mn peroxidases are found in many wood destroying white-rot fungi. They are extracellular hemeproteins capable of catalyzing the oxidation of numerous compounds, most notably lignin, its natural substrate. Lignin peroxidase catalyzes the oxidation of recalcitrant nonphenolic aromatic substrates by one electron. Mn peroxidase catalyzes the oxidation of Mn (II) to Mn (III). *P. chrysosporium* produces both of these enzymes in multiple forms. The function of these isozymes in lignin biodegradation is the major focus of our research. We are utilizing steady state and transient state kinetic methods to characterize the mechanism of these enzymes. We have also developed a

heterologous expression system for these peroxidases. Site-directed mutagenesis is presently being used to study structure and function relationships. Particular detail is being given to structural aspects which confer these enzymes with their unique catalytic activity.

University of Pennsylvania
Philadelphia, PA 19104

160. Circadian Rhythms in CAB Gene Expression
A.R. Cashmore, Plant Science Institute, Department of Biology *$125,000*

We are studying circadian rhythms in CAB gene expression in *Arabidopsis thaliana*. In these studies we are using two distinct approaches. Firstly, we are developing a transient assay system which we hope to use in evaluating the role of promoter elements in modulating the oscillations in transcription exhibited by the Arabidopsis CAB genes. We have recently cloned from Arabidopsis two additional genes, called *Lhb1B1* and *Lhb1B2*, encoding photosystem II type I CAB proteins. We have made constructs from these genes in which the promoters are fused to the reporter luciferase and by biolistic delivery we have introduced the constructs into tobacco and Arabidopsis tissue. Studies so far have demonstrated that these CAB promoter fusion constructs exhibit relatively high levels of transient light regulated expression; experiments will be performed to determine if these genes are expressed in a circadian manner. These studies will be complemented by genetically transforming Arabidopsis with appropriate constructs.

The second approach that we have used is a genetic approach. Here we are screening for mutants that contain lesions in some component of the circadian timing mechanism. We are taking advantage of the fact that the time of flowering in Arabidopsis is influenced by the photoperiod. Arabidopsis plants grown under short-day photoperiod exhibit a delay in flowering time. Here, as demonstrated for other plants, this effect on flowering reflects the ability of the plant to measure the night length; various experiments indicate that this timing mechanism is a component of a circadian clock. In our screen we have selected for mutants that flower early when grown under long nights; a secondary screen eliminated those mutants that also flowered early when grown under constant light. By these criteria we have isolated 3 putative mutants from a pool of 50,000 F2 DEB-mutagenized Arabidopsis seeds, and a further 4 putative mutants from a pool of 200,000 EMS-mutagenized seed. We are presently backcrossing these mutants to establish allelism and dominance/recessiveness to wild-type and seed are being bulked to investigate oscillations in CAB gene expression. With a view to eventually cloning some of the genes responsible for these mutants, we are also screening T-DNA tagged Arabidopsis lines for such mutants.

University of Pennsylvania
Philadelphia, PA 19104-6018

161. Membrane-Attached Electron Carriers in Photosynthesis and Respiration
F. Daldal, Department of Biology *$214,000 (2 years)*

The overall aim of this project is to identify and characterize at the molecular level various membrane-associated cytochromes that operate as electron carriers in photosynthesis and respiration. The facultative photosynthetic bacterium *Rhodobacter capsulatus* provides an excellent model system for dissecting these energy transducing pathways using multi-disciplinary approaches. The presence of

a branched photosynthetic electron transfer pathway between the cyt bc_1 complex and the reaction center was first indicated by our photosynthesis-proficient mutant of *R. capsulatus* lacking the soluble carrier cyt c_2. We have now revealed that a novel membrane-associated cytochrome, cyt c_y, structurally distinct from, and yet functionally similar to, cyt c_2 operates as an electron carrier in this soluble carrier-independent pathway. Cyt c_y is unusual in that it has two distinct domains, a hydrophobic NH_2-terminal region with a signal sequence followed by an alanine-proline rich region, and a COOH-terminal region with strong homology to cyt c mainly from mitochondria of plants and animals. A cyt c_y^- mutant of *R. capsulatus* remains photosynthesis-proficient as does the cyt c_2^- mutant, but a mutant lacking both cyts c_2 and c_y is non-photosynthetic, and it can be complemented by either cyt c_2 or cyt c_y. Thus, two distinct electron pathways, unrecognized until now, operate during photosynthesis in *R. capsulatus*, and possibly in other organisms. Genetic, physiological and biochemical approaches are currently being used to characterize the properties of cyt c_y, its association with the membrane, and the role of other membrane-associated *c*-type cytochromes distinct from cyts c_1 and c_y.

University of Pennsylvania
Philadelphia, PA 19104-6018

162. Molecular and Genetic Analysis of CTR1; A Negative Regulator in the Ethylene Signal Pathway

J. Ecker, Department of Biology *$95,000*

Mutations that affect ethylene production, perception and response have been identified. The availability of such mutants offers the potential to use genetics as a means to establish biochemical pathways, to identify substrates and to study the mechanisms that regulate hormone activity. Molecular cloning of the first of these genes, *CTR1*, has been accomplished. This project concerns the molecular, genetic and biochemical characterization of a negative regulatory gene that controls ethylene responses in *Arabidopsis thaliana*. Characterization of the CRT1 gene product and analysis of its role in the ethylene action pathway is the focus of the project. More specially, the goal of this project includes the following aims. The temporal and spatial patterns of expression of the CTR1 gene will be established using methods to detect, *in situ*, CTR1 RNA and protein. In addition, the *E. coli* ß-glucuronidase reporter gene will be linked to CRT1 regulatory sequences. These studies will provide detailed information about developmental and hormone-regulated expression characteristics of a critical component of the ethylene signal transduction pathway. Ectopic expression of the full length CTR1 protein and amino-terminal truncated forms of the protein will be carried out using transgenic plants. Anti-CTR1 antibodies will be used for identification of *in vivo* expression of CTR1 under various conditions and in the presence of different mutations. These studies will allow for testing of a model of the function of CTR1 and should provide significant additional biochemical and genetic information on the role of CTR1 in the regulation of the ethylene response. In order to identify interacting components of the ethylene response pathway, second-site suppressor mutations of the *ctr1* mutation will be isolated. Extragenic suppressor analysis may identify important components of the ethylene production and signal transduction pathways that would not be possible to recognize using other approaches.

University of Pennsylvania
Philadelphia, PA 19104-6018

163. Transport Function and Reaction Mechanism of Vacuolar H^+-Translocating Inorganic Pyrophosphatase
P.A. Rea, Department of Biology — *$88,998*

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

Our research program is directed at defining the reaction mechanism and transport capabilities of the V-PPase. Work accomplished to date includes the development of procedures for purification of the enzyme from *Vigna radiata*, reconstitution of its capacity for Mg_2PPi-energized H^+-translocation in artificial liposomes, derivation of a minimal three-state model for the ligand requirements of the enzyme and the generation of pYES2 transformants of *Saccharomyces* which express cDNAs encoding the M_r 66,000 subunit of the V-PPase from *Arabidopsis*. Thus, our immediate objectives are the application of parallel protein chemical and site-directed mutagenesis studies to the reconstituted and heterologously expressed enzyme to examine the structural requirements for pump operation on the basis of deduced sequence and topological data derived from our recent molecular studies and the kinetic data acquired from analyses of the enzymes ligand requirements. Current mechanistic studies center on the identification of sequence motifs implicated in substrate binding through the application of strategies for selective labeling and cleavage of the M_r 66,000 polypeptide and the mapping of peptide fragments subject to substrate-protectable covalent modification.

In view of the V-PPase's membership of a new category of ion translocase, its apparent ubiquity in the vacuolar membranes 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 significance.

Purdue University
West Lafayette, IN 47907

164. Crystallographic Studies of Nitrogenase and Hydrogenase
J.T. Bolin, Department of Biological Sciences — *$202,761 (FY 92 Funds/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. We have achieved the first goal of this project in that we have determined the crystal

structure of MoFe-protein, the catalytic component of nitrogenase, at atomic resolution. We are now engaged in the early stages of studies of the structures of mutant proteins that demonstrate altered functional properties. 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. We have obtained crystals of the protein and are in the process of analyzing diffraction data from these crystals. We are also attempting to extend the scope of the project to include Ni-dependent hydrogenases.

Purdue University
West Lafayette, IN 47907-1155

165. Structure and Biosynthesis of the Mixed-linkage ß-D-Glucan of Grasses
N.C. Carpita, Department of Botany and Plant Pathology *$86,000*

We investigate the chemical structure and biosynthesis *in vitro* of (1→3),(1→4)-ß-D-glucans (MGs), cell wall polymers unique to Poaceae. Membranes of the Golgi apparatus from maize (*Zea mays* L.) are used to synthesize MG *in vitro*. The MG synthesized *in vitro* is about 250 kDa and is separated from a much larger (1→3)-ß-D-glucan (callose) by gel-permeation chromatography. Diagnostic oligosaccharides, released by a sequence-dependent endoglucanase from *Bacillus subtilis*, are separated by HPLC and GLC and, coupled with gas-proportional counting and liquid flow counting, provide a specific assay for MG synthesis *in vitro*. The trisaccharide ß-D-Glc*p*-(1→4)-ß-D-Glc*p*-(1→3)-D-Glc, the tetrasaccharide $[$ß-D-Glc*p*-(1→4)$]_2$-ß-D-Glcp-(1→3)-D-Glc, and longer cellodextrin-(1→3)-D-Glc oligosaccharides are synthesized in proportions similar to those found in purified MG. Activated charcoal, added during homogenization, enhances synthesis of MG presumably by removing inhibitory compounds. Synthesis is localized to the Golgi apparatus by a combination of downward and flotation centrifugations on sucrose step gradients. The rate of synthesis does not saturate at up to 10 mM UDP-Glc. Chelators completely abolish synthesis, but synthase activity is restored by $MgCl_2$ or, to a lesser extent, $MnCl_2$. Synthesis continues well over one hour, but addition of KOH to raise the pH from 7.2 to 8.0 during reactions increases the rate, indicating that a transmembrane pH gradient may facilitate synthesis of MG. We are testing this hypothesis by manipulating pH gradients in Golgi membranes empirically and assaying the impact on the rate of synthesis of MG and a Golgi-associated callose synthase. We are presently using photoaffinity-labeled probes to identify polypeptides associated MG and callose synthesis localized in the Golgi apparatus and will use other affinity probes to purify detergent solubilized peptides associated with MG synthesis.

Purdue University
West Lafayette, IN 47907

166. Analysis of the PSII Proteins MSP and CP43'
L.A. Sherman, Department of Biological Sciences *$178,000 (FY 92 Funds/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 are analyzing various mutations in MSP to analyze O_2-evolution and the involvement of Ca^{2+} and Cl^- in the regulation of O_2-evolution. We are using these mutants to determine other proteins that are involved in the binding of these ions. Thermoluminescence and O_2-flash yield studies are performed to determine the impact of these mutations on charge recombination states $S_2Q_A^-$ and $S_3Q_B^-$.

We are analyzing the assembly of chlorophyll-protein complexes using iron deficiency and mutations we have produced in either CP43 or CP43' (a similar protein induced upon iron deficiency). In order to perform these studies, we have modified a high-resolution green gel system for analyzing chlorophyll-protein complexes on acrylamide gels. We are using this system, which can resolve over 20 Chl-protein complexes, to watch how wild-type and mutations in the *psbC'* gene assemble the major chlorophyll-protein complexes upon the re-addition of iron. We have produced a deletion mutant of the *psbC'* gene which cannot grow in iron-deficient medium, but which can grow in 1/1000 Fe. This strain recovers very slowly upon the re-addition of iron, implying that CP43' is important for Chl-protein assembly.

Rice University
Houston, TX 77251

167. Genetic and Biochemical Analysis of Solvent Formation in Clostridium acetobutylicum
G.N. Bennett and F.B. Rudolph, Department of Biochemistry and Cell Biology
$180,000 (FY 92 Funds/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. Current knowledge concerning the molecular genetics, cell regulation and metabolic engineering of this organism is still rather limited. The objectives are to improve our knowledge of the molecular genetics and enzymology of Clostridia in order to make genetic alterations which more effectively channel cell metabolism. 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. Currently strains are being evaluated to analyze the molecular mechanism of degeneration 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 are beginning analysis of oxidation reduction systems related to this process.

Rutgers University
New Brunswick, NJ 08903

168. The Role of Alternative Respiration in Plants
I. Raskin, AgBiotech Center *$154,000 (FY 92 Funds/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

169. Corn Storage Protein: A Molecular Genetic Model
J. Messing, Waksman Institute *$212,000 (FY 92 Funds/2 years)*

Our studies of regulation of gene expression are focused on raising the methionine storage in the maize kernel. Most of its methionine accumulates in the endosperm of the kernel due to the expression of the ß and δ zeins. However, in natural variants the δ zeins are the deciding factor. For instance, in the maize inbred lines BSSS53 and Mo17, alleles of a *trans*-acting factor on chromosome 4, previously called *Zpr*10/(22) and now renamed *dzr1* for δ zein regulator, can either cause overexpression in BSSS53 or repression in Mo17 of δ zeins. Interestingly, some alleles of *dzr1* show dosage, while others are dominant or recessive dependent on parentage. We were able to show by the use of B-A translocation of *dzr1* that the Mo17 allele is silenced when passed through the pollen regardless of the dosage of the allele. Silencing depends only on the immediate parentage and can be switched in the next generation indicating genomic imprinting of the Mo17 allele. From nuclear run-off experiments it appears that *dzr1* acts at the post-transcriptional level, making it an interesting locus to study from its regulation and its function. These studies will be aided by the isolation of a new δ zein gene that is not regulated by *dzr1*. Although it shares homology with the 10 kDa δ zein gene, besides higher numbers of methionine codons, it also contains lysine and tryptophan codons, which were previously believed to be absent in zein genes.

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

170. Signal Transduction Pathways that Regulate CAB Gene Expression
J. Chory, Plant Biology Laboratory *$211,000 (FY 92 Funds/2 years)*

The process of greening, or chloroplast differentiation, involves the coordinate regulation of many nuclear- and chloroplast-encoded 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. We have utilized *cab3* promoter-marker gene chimeras to select for mutants in which the *cab3* promoter is

aberrantly expressed in the dark or in response to signals from the chloroplast. *doc* (**d**ark **o**verexpression of **c**ab) mutants have elevated levels of *cab* gene expression in etiolated seedlings. The *doc* mutations currently define greater than 4 complementation groups. Some of the mutations also affect the levels of *rbcS* mRNAs in dark-grown mutant seedlings. Others affect the accumulation of *cab*, but not *rbcS*, mRNA. Thus, the *doc* mutations identify a branch point in which the control of *cab* gene expression can be genetically separated from *rbcS* expression. In *gun* (**g**enomes **un**coupled) mutants, the expression of *cab* and *rbcS* genes has become uncoupled from the expression of photosynthetic chloroplast genes. Detailed biochemical and genetical characterization of these signal transduction 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.

The Scripps Research Institute
La Jolla, CA 92037

171. Genetic Engineering with a Gene Encoding a Soybean Storage Protein
R.N. Beachy, Department of Cell Biology *$95,016*

The ß-conglycinins are soybean storage proteins encoded by genes that are tightly regulated both spatially and temporally. We have studied the Soybean Embryo Factors that bind to the *cis* elements that are presumably involved in regulating the expression of these gene promoters using both *in vitro* binding assays and *in vivo* expression assays in transgenic plants. The results obtained to date have made it evident that there are no clear correlations between the *in vivo* and the *in vitro* results, i.e., changes in single nucleotides that alter protein:DNA interactions can have little or no impact on expression of the promoters *in vivo*. In contrast, the CATGCAT (RY element) sequence, for which no binding proteins have been identified, appear to be very important for controlling gene expression. Although we have been attempting to isolate and characterize the SEF 3 and SEF 4 proteins we have to date not been successful using protein expression libraries derived from embryo cDNAs. We are continuing experiments of this type, as well as more standard protein purification procedures.

We have constructed a number of chimeric promoters with different upstream and downstream regulatory sequences in an attempt to identify, by expression assays in transgenic plants, those sequences that are uniquely responsible for the temporal and spatially regulated expression of the ß-conglycinin genes. Because of the results of previously published work from this laboratory, we concluded that the core promoters themselves may be responsible for the regulation. Therefore, we have focused our efforts on these sequences, the RY element, and the SEF 3 binding sequences. In a follow-up to our studies to modify the expression of genes in seeds, we will express several types of human and other animal genes in seeds of transgenic plants.

The Scripps Research Institute
La Jolla, CA 92037

172. Nuclear Genes Regulating Translation of Organelle mRNAs
S. Mayfield, Department of Cell Biology *$182,000 (2 years)*

The general aims of this project are to study the interactions of the nuclear and chloroplast genomes in coordinate photosynthetic gene expression. Specifically we will identify the factors and mechanism

used to regulate the translation of chloroplastic mRNAs. These factors have been identified by genetic analysis of photosynthetic mutants in *Chlamydomonas reinhardtii*, as nuclear encoded proteins that interact with the 5' untranslated region (UTR) of chloroplastic mRNAs. We have isolated a complex of four proteins that specifically bind to the 5'-UTR of the *psbA* mRNA. Binding of these proteins to the *psbA* mRNA *in vivo* is light activated and correlates with the translation level of the *psbA* mRNA. Binding of these proteins to the *psbA* mRNA can be modulated *in vitro* by phosphorylation of the protein complex. We have raised antisera and cloned a cDNA for one member of the complex (47 kDa protein), and will generate antisera and cDNA clones for the other psbA binding proteins. We will use these reagents to characterize the molecular mechanism utilized to activate protein binding to the *psbA* mRNA, and to identify the mechanism by which binding of these proteins activates translation. These studies will help us understand basic principles in nuclear/organelle interaction in coordinate gene expression, and provide insight into translational regulation, an important, but poorly understood aspect of gene regulation in plants.

University of South Carolina

Columbia, SC 29208

173. Exploration of New Perspectives and Limitations in Agrobacterium Mediated Gene Transfer Technology

L. Marton, Department of Biological Sciences *$200,000 (FY 92 Funds/2 years)*

Genetic manipulation of plants often involves introducing homologous or partly homologous genes. Ectopic introduction of homologous sequences into plant genomes may trigger epigenetic changes, such as cosupression, making transgene expression unpredictable. Problems caused by the nonallelic interaction of homologous sequences could be avoided by homologous gene targeting (HGT).

The objective of this project is to test the feasibility of HGT technology in plants using Agrobacterium mediated gene transfer (AMGT). Development of a binary Agrobacterium targeting vector and corresponding plant recipient system is in progress. The aim is to analyze the ratios of gene replacement, random insertion and other mutations occurring within the target locus simultaneously. The HGT vector is based on the nia2 nitrate reductase (NR) gene of *Arabidopsis*. The recipient is a G5 x wt F1 plant (G5 is a NR deficient mutant with a major deletion in the nia2 locus); therefore there is only a single target gene.

We recently introduced a transformation and regeneration system based on *Arabidopsis* sustained root cultures and developed two negative selection markers. Genetic changes affecting the nia2 locus can be scored from a nearly unlimited amount of transformed material by direct selection for NR deficiency on chlorate. Clones can be further classified for gene replacement, random insertion or mutation events by the presence of the positive selectable marker (within the homologous region) and the absence of the negative markers (outside of the homologous region) in the donor construct.

Southern Illinois University

Carbondale, IL 62901

174. Regulation of Alcohol Fermentation by Escherichia coli
D.P. Clark, Department of Microbiology *$89,000*

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 fusions. The build up of reduced NADH during anaerobic fermentative conditions appears to play a major role in regulating the *adh* gene. 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. The *adhE* gene of *Salmonella* has nearly been completely sequenced and although clearly homologous to that of *E. coli*, shows significant differences. Corresponding differences in kinetic behavior of the ADH enzyme activity are being investigated. The *ldh* gene, encoding the fermentative lactate dehydrogenase has also been cloned and sequenced. We are presently completing the sequence of the regulatory region and have started to construct gene fusions between *ldh* and the *cat* gene, specifying resistance to chloramphenicol. 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

175. The Effect of Oligosaccharides on Glycoprotein Stability
C.F. Goochee, Department of Chemical Engineering
$187,000 (FY 92 Funds/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

176. Nodulation Genes and Factors in Rhizobium-Legume Symbiosis
S.R. Long, Department of Biological Sciences *$246,000*

Our work concerns the association of *Rhizobium meliloti* and its legume host, alfalfa, which results in symbiotic nitrogen fixation. Understanding how the association works will help improve this and other symbioses for the purpose of diminishing energy-intensive fertilizer use. We are characterizing the bacterially synthesized signals that cause the plant host to form symbiotic root nodules, and are examining the initial period of the host response. The signals, called Nod factors, are modified short oligomers of N-acetyl glucosamine, with an N-acyl substitution on the non-reducing end residue and a C-6-sulfate substitution at the reducing end residue. We developed a new method for isolation of these morphogenic Nod factors from bacterial culture supernatants. We have shown the involvement of several sets of genes by *in vitro* enzymatic synthesis. In particular, we have found that *Rhizobium meliloti* has a complex array of genes and enzymes for the purpose of activating sulfate into the nucleotide form, PAPS. We have sequenced the second of these genes ($nodP_2Q_2$) and cloned the third (*saa*) and a possible fourth. We have also shown *in vitro* that the NodH enzyme catalyzes transfer of sulfates to the Nod factor, and that this requires a dimeric or larger N-acetylglucosamine-containing molecule as acceptor. We have established other *in vitro* reactions for analyzing the pathway of Nod factor synthesis. Some of the enzymes discovered in this work may have uses in industrial processes for sulfation of novel molecules. We are studying the early responses of the alfalfa host plant to Nod factors. In particular, we are examining the root hairs of plants by electrophysiology and dye and protein injection. We are currently attempting to modify these techniques to extend the length of time during which we can observe live root hair reactions.

Stanford University
Stanford, CA 94305

177. Enzymology and Molecular Biology of Cell Wall Biosynthesis
P.M. Ray, Department of Biological Sciences *$97,000*

Our objective is to identify plant cell wall polysaccharide synthases and clone the corresponding genes, so that the control mechanisms for wall synthesis, mechanisms that could be useful for modifying plant polysaccharide biomass production as an energy source, can be established. 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 (a wound response polysaccharide), are currently targeted.

We have been able to extensively purify pea GS-II down to 2-polypeptides of 55 and 70 kDa, by a combination of product entrapment (wherein a synthase becomes trapped within polysaccharide micelles that are produced by its synthase reaction and can easily be isolated by centrifugation), followed by isoelectric focussing and density gradient centrifugation. Peptide fragments obtained from each of these polypeptides by digestion with trypsin will be sequenced, and synthetic oligonucleotides corresponding to these sequences will be prepared and used to screen a pea gene sequence library to isolate the coding sequences for these polypeptides.

Except for density gradient centrifugation, the methods that have enabled us to purify GS-II have not worked for GS-I, and since at least 6 different polypeptides correlate with GS-I activity in density gradient fractionation, we are looking for additional methods that might be effective for fractionating Golgi membrane proteins. By affinity chromatography we have been able to purify a Golgi-localized 40 kDa doublet (2 closely related polypeptides) that may be involved in the GS-I system because these polypeptides reversibly accept glucose units from UDP-glucose (the GS-I substrate) under GS-I assay conditions. The isolated polypeptides will be used in experiments to test the participation of the doublet in polysaccharide synthesis. Tryptic peptides obtained from the doublet have been sequenced, yielding unique sequences not found in the known protein sequence database. The nucleotide equivalents of these sequences will be used, as above, as screens to detect and clone the gene(s) for the doublet polypeptides.

University of Tennessee
Knoxville, TN 37996

178. Plant Recognition of Bradyrhizobium japonicum Nod Factors
G. Stacey, Microbiology Department *$172,000 (FY 92 Funds/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. For example, nanomolar amounts of the purified molecule will induce *de novo* cortical cell division in the plant root. Recently we have completed the chemical characterization of over 10 different nodulation signals produced by three strains of *B. japonicum*. These molecules differ by the length of the chitose backbone and by the substitutions on the reducing or non-reducing terminal sugar. Most interestingly, we have identified derivatives in which the reducing hydroxyl group is blocked by a glycerol residue. We believe that these derivatives may represent intermediates in the biosynthetic pathway for the production of these lipo-oligosaccharide nodulation signals. We are currently exploring the biosynthetic pathway used to synthesize these molecules. In addition, we have obtained evidence that the specific profile of nodulation signals produced by a given *B. japonicum* strain is an important determinant of host range. We are further exploring this finding using our purified nodulation factors. We have also been collaborating with synthetic chemists and now have synthetic nodulation factors of identical structure to those purified from bacterial cultures. We are testing the biological activity of these synthetic molecules. Future work will focus on the

recognition and response to these molecules by the plant host. 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

179. Regulation of Chloroplast Number and DNA Synthesis in Higher Plants
J.E. Mullet, Department of Biochemistry and Biophysics
$155,332 (FY 92 Funds/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

180. Characterization of a 1,4-ß-D-Glucan Synthase from Dictyostellum discoideum
R.L. Blanton, Department of Biological Sciences *$69,354 (FY 92 Funds/2 years)*

Progress in the study of the eukaryotic cellulose synthase continues to be slow. Using the cellular slime mold *Dictyostellum discoideum* as a model organism for eukaryotic cellulose synthesis has several advantages: there exists an *in vitro* assay for the cellulose synthase in which the product has been extensively characterized, the cellulose synthase is inducible, and molecular methods such as gene disruption can be applied to the cellulose synthase problem.

In the past year, we have concluded an exhaustive study of cofactor requirements for maximal activity in the *in vitro* assay. The effects of mono-, di-, and tri-phosphate nucleotides has been studied. We have shown that the *Acetobacter* activator, cyclic di-guanylic acid, does not affect the activity of the *Dictyostellum* enzyme. Experiments with various disaccharides and with cellobiose, -triose, -tetraose and -pentose indicated that the cellobiose is not serving as a primer but perhaps mimics a native activator. We continue to be intrigued by the relatively high specific activity of the *Dictyostellum* enzyme and by our inability to deplete membrane preparations of activity by washing, as occurs with *Acetobacter*. We have been testing the hypothesis that the *Dictyostellum* enzyme is irreversibly activated, perhaps by covalent modification or proteolytic processing.

In collaboration with Dr. R.R. Drake (University of Arkansas School for Medical Sciences), we have begun to use a UDPG photoaffinity probe to identify UDPG-binding proteins. We have continued our genetic efforts, attempting to identify the cellulose synthase gene by complementation and beginning to apply the new method of restriction-enzyme mediated insertional mutagenesis to generate cellulose-deficient mutants.

Texas Tech University
Lubbock, TX 79409

181. The Interaction of Ferredoxin:NADP$^+$ Oxidoreductase (FNR) and Ferredoxin:Thioredoxin Reductase with Substrates
D.B. Knaff, Department of Chemistry and Biochemistry *$172,000 (2 years)*

Chemical modification studies and computer modeling of electrostatic fields were used to investigate the interaction domains involved in complex formation between ferredoxin:NADP$^+$ oxidoreductase (FNR) and ferredoxin. Complex formation protected FNR lysines K18, K33, K35 and K153 from modification by either an activated biotin ester and/or S-DABITC. These lysines are located in a domain of strong positive potential on the surface of FNR surrounding the positive end of the molecular dipole moment. Complex formation protected several negatively charged residues on ferredoxin, E29, E30, D65 and D66, which belong to two domains of negative electrostatic surface potential on either side of the [2Fe-2S] cluster of ferredoxin. The complementary shapes of these negative domains on ferredoxin and positive domains on FNR have allowed construction of a model for the FNR:ferredoxin complex. Chemical modification studies on two additional chloroplast enzymes that utilize ferredoxin as an electron donor, nitrite reductase and glutamate synthase, have provided evidence that the ferredoxin-binding sites on these enzymes, like that of FNR, are positively charged. Treatment of nitrite reductase and glutamate synthase with either the arginine-modifying reagent phenylglyoxal or the lysine-modifying agent N-acetylsuccinmide inhibited enzyme activity with reduced ferredoxin as the electron donor but had no effect on activity with the non-physiological electron donor, reduced methyl viologen. This observation, and the demonstrated inhibition of ferredoxin binding resulting from treatment of the enzymes with these reagents, has been interpreted in terms of ferredoxin-binding sites on nitrite reductase and glutamate synthase that contain at least one arginine and one lysine.

University of Utah
Salt Lake City, UT 84112

182. The Plant Mitochondrial mat-r Gene/nad1 Gene Complex
D.R. Wolstenholme, Department of Biology *$185,000 (FY 92 Funds/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 (A-E). Exons B and C are joined 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 therefore postulated to involve both *trans*-splicing and *cis*-splicing. A gene (*mat-r*) for a maturase-related protein is located in the partial group II intron upstream from the E exon; in soybean the *mat-r* gene-containing intron is continuous between exons D and E. In maize and soybean *mat-r* gene transcripts, there occur C

to U changes (edits) that increase amino acid sequence similarity between the predicted MAT-R proteins. At least one C to U edit occurs in all introns that are *trans*-spliced. Also, such introns are excised slower than are continuous introns. Further examination of intron cDNAs is being carried out to determine the extent to which editing might be essential for intron excision, and to elucidate the nature of intron component association during *trans*-splicing. Runoff transcripts of cDNAs will be employed to determine whether, *in vitro*, the continuous soybean *mat-r* gene-containing intron can self-*cis*-splice, and the two separately transcribed segments of the maize *mat-r* gene-containing intron can self-*trans*-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

183. Enzymology of Acetone-Butanol-Isopropanol Formation
J.-S. Chen, Department of Anaerobic Microbiology *$91,239*

Several anaerobic bacteria within the genus *Clostridium* produce acetone, butanol, ethanol and isopropanol, which are better known as solvents but are also chemical feedstocks and fuel additives. The goal of the project is to understand the fundamental properties of the solvent-forming process so that it will be possible to prevent degeneration of strains, to control the solventogenic switch, and to regulate the product ratio. Our approach is to determine first the molecular properties of solvent-forming enzymes. The biochemical information serves as a base toward establishing mechanisms and strategy for regulating the expression of solvent-production genes and the flow of metabolites. Our research primarily involves *Clostridium beijerinckii*, which has the distinctive ability to produce isopropanol in addition to the other solvents. We also use the facultative anaerobe *Bacillus macerans*, which produces acetone and ethanol but not butyrate or butanol. *B. macerans* provides a non-clostridial system for the study of the conversion of acetoacetyl-CoA into acetoacetate and the solventogenic switch. In clostridia, there is a multiplicity of solvent-forming enzymes that catalyze the same or similar reactions, and there are significant variations in the properties of enzymes from different strains. We now focus on alcohol dehydrogenases (ADHs) and the acetoacetyl-CoA reacting enzymes. The acetoacetyl-CoA:acetate/butyrate CoA-transferase has been purified from *C. beijerinckii* NRRL B593, and its K_m values for acetate and butyrate are significantly lower than those of the CoA-transferase from *C. acetobutylicum* ATCC 824. We have now identified four distinct primary ADHs. The primary ADH that is reactive toward either NADH and NADPH has been resolved into three species with the apparent subunit composition of α_2, $\alpha\beta$, and β_2. The properties of the resolved species will be determined next. We have identified a putative gene (*stc*) for a signal-transducing transcription regulator directly upstream to the gene (*adh*) for the primary/secondary ADH. The relationship of the *stc* gene to solvent production is being studied.

Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

184. Enzymological Studies of One-Carbon Reactions in the Pathway of Acetate Utilization by Methanogenic Bacteria
J.G. Ferry, Department of Anaerobic Microbiology *$92,000*

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 have been determined through cloning and sequencing of the genes. Both enzymes have been hyperexpressed and site-directed mutagenesis studies will be done to identify active site residues. The enzyme 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 the cobalt/iron-sulfur component is in progress. Ferredoxin is an electron acceptor for the nickel/iron-sulfur component which cleaves acetyl-CoA and oxidizes the carbonyl group to CO_2. The genes encoding this and a second ferredoxin have been studied. The function of the second ferredoxin is unknown but the gene is transcribed only when cells are grown on methanol. The gene encoding carbonic anhydrase has been cloned and sequenced. The results suggest that the enzyme may be transported outside of the cell membrane, a result which supports a role for carbonic anhydrase in transport of acetate into the cell.

Washington State University
Pullman, WA 99164-6340

185. Membrane Function in Lipid Mutants of Arabidopsis
J. Browse, Institute of Biological Chemistry *$203,830 (FY 92 Funds/2 years)*

Acyl lipids are components of the membrane barriers that delineate the compartments of plant cells and of seed oils which are an important renewable resource. My research uses 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. The *fad2* mutants contain reduced levels of polyunsaturated fatty acids but show growth characteristics at 22°C that are similar to wild type. By contrast, at 12°C, the mutants fail to undergo stem elongation during reproductive growth although they produce normal flowers and fertile seeds. After transfer to 6°C, rosette leaves of the mutants gradually die and the plants are inviable. These different responses of the mutant plants at 12°C and 6°C suggest that distinct functions may be affected at these two temperatures. The gradual development of symptoms at 6°C and other lines of evidence argue against a general collapse of membrane integrity as the cause of the lethal phenotype. Rather, they indicate that the decrease in polyunsaturated membrane lipids may initially have relatively limited effects in disrupting cellular function.

A second class of mutants that contain elevated levels of the saturated fatty acid, stearate, are extreme dwarfs. We established that the lipid defect causes the dwarf growth by isolating suppressor mutations that corevert both phenotypes. A role for membrane structure is indicated because growth

of the mutant at 36°C substantially reverses the dwarf habit without changing the lipid composition. To our knowledge, this is the first example of a mutation altering a specific cell structural component that has broad effects on plant development.

Washington State University
Pullman, WA 99164-6340

186. Regulation of Terpene Metabolism
R. Croteau, Institute of Biological Chemistry *$95,000*

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 synthases and the catabolic enzymes responsible for the synthesis of terpenyl glycoside transport derivatives. Synthases 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 changes in the levels of these enzymes. 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 and a newly developed HPLC method for the separation and quantitation of all of the relevant metabolites are being used to examine the regulation of flux through the various branches of the pathway, and to measure the intracellular concentrations of key intermediates. 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

187. Carbon Metabolism in Symbiotic Nitrogen Fixation
M.L. Kahn, Institute of Biological Chemistry *$162,000 (2 years)*

Combined nitrogen is the nutrient that most often limits plant growth. For this reason, nitrogen fertilizer is an important input used to increase crop production. However, producing nitrogen fertilizer is expensive and energy intensive and excess fertilizer use can also increase groundwater nitrate and soil acidity. Some plants, like legumes, are able to obtain nitrogen through symbiotic associations with nitrogen-fixing bacteria. The exchange of plant derived carbon compounds for bacterial nitrogen is the engine that drives these symbioses. Enzymes of the plant and bacterial tricarboxylic acid (TCA) cycles are at the center of this exchange, generating energy, reductant and biosynthetic intermediates from the catabolism of photosynthate. Recent work indicates that TCA cycle enzymes may be integrated into nodule function and development in unexpected ways. We are investigating the genetics of symbiotic carbon metabolism in both *Rhizobium meliloti* and its host, alfalfa. Specifically, our goal is to define the role of the decarboxylating leg of the TCA cycle, a series of reactions needed to synthesize amino acid precursors that may also be required to generate energy and reductant for

nitrogen fixation. We will investigate why *R. meliloti* citrate synthase mutants are unable to form nodules and use TCA cycle mutants to separate metabolic effects of mutations that affect intermediary metabolism from the effects these mutations have on development. We will also investigate the molecular genetics of plant isocitrate dehydrogenases by further studying a soybean NADP-isocitrate dehydrogenase cDNA we have cloned and by cloning cDNAs that encode other plant isocitrate dehydrogenases.

Washington State University
Pullman, WA 99164-6340

188. Towards a Detailed Understanding of Structural Variability in Lignins
N.G. Lewis, Institute of Biological Chemistry — *$161,000*

The ability of plant cells to differentiate into various tissues, organs, etc., is in large part due to alterations in cell wall synthesis. In this reporting period, the process of lignification (initiation, deposition, etc.) was comprehensively investigated as a function of wall maturation/thickening, using the woody plants *Pinus taeda* (cell cultures) and *Forsythia* sp. as experimental models. It was established that *P. taeda* cell suspension cultures, when grown on NAA-containing medium underwent lignification, this being established at the ultrastructural, chemical, biochemical and gene level; this system permits delineation of the initiation of lignin monomer synthesis, and mechanism of monomer transport to the cell walls. We have also purified to homogeneity a cell-wall bound monolignol coupling enzyme with laccase-like activity in preparation to define its true role in lignin synthesis. Additionally, factors controlling lignin heterogeneity, using both model systems, are now being defined in response to "normal" growth and development, wounding and pathogen attack, and abnormal physiological stress.

Washington State University
Pullman, WA 99164-4660

189. Isocitrate Lyase and the Glyoxylate Cycle
B.A. McFadden, Department of Biochemistry and Biophysics — *$80,524*

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, mutagenesis of the gene is being directed towards replacing *his*-266 and -306 as well as *ser*-319 and -321, all of which have been placed at the active site by chemical modification, 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* [*J.Mol.Biol.* 220, 13 (1991)]. Recently, we have completed the replacement of *lys*-193 by *his, arg, glu* and *leu*. The *his* and *arg* variants showed reduced activity and an elevated K_m for isocitrate and the other variants were inactive. Both active variant enzymes supported slow growth on acetate when expressed in an *icl*-minus strain of *E. coli*. Thus *lys*-193 is essential to the normal function of *icl* both *in vitro* and *in vivo* (*J.Bacteriol.*, in press).

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

Washington State University
Pullman, WA 99164-6340

190. Enhancement of Photoassimilate Utilization by Manipulation of the ADPglucose Pyrophosphorylase Genes
T.W. Okita, Institute of Biological Chemistry *$85,000*

The goal of this project is to increase the conversion of photosynthate into starch via manipulation of ADPglucose pyrophosphorylase (ADPG-PP), a key enzyme in starch biosynthesis. To gain insight into the structure/function relationship of the plant ADPG-PP we have elucidated the heterotetrameric structure of the potato tuber enzyme using 2-D gel electrophoresis, isolated cDNA clones encoding each subunit, and over-expressed the enzyme in *Escherichia coli* (in collaboration with G. Barry, Monsanto Co). This bacterial expression system, which yields ample amounts of active enzyme, is currently being utilized in both enzymological and mutagenesis studies. Thus far a number of mutants with altered catalytic and possibly allosteric function have been identified. Temporal and spatial expression of the genes encoding each subunit as well as their correlation to starch biosynthesis have been elucidated. Results from these studies indicate that a post-transcriptional event may play a role in the regulation of ADPG-PP expression. As a step towards understanding of the metabolic signals influencing ADPG-PP expression we have also analyzed changes in its expression at the transcript, antigen, and enzyme activity levels as a response to sucrose. Genomic clones encoding the small subunit have been isolated and the gene structure determined. Spatial studies conducted with small subunit promoter-GUS fusions indicated that the same gene may be expressed in both photosynthetic and non-photosynthetic tissues. Currently, cis acting elements and their involvement in spatial and temporal expression are under investigation.

Washington University
St. Louis, MO 63130

191. Processing and Targeting of the Thiol Protease, Aleurain
J.C. Rogers, Department of Biology *$45,756 (6 monts)*

We are studying the targeting and processing of a barley vacuolar thiol protease, aleurain. Aleurain is synthesized as a proenzyme that is cleaved in two steps after it reaches an acidified post-Golgi compartment. The sequence of aleurain is 65% identical to that of mammalian cathepsin H, a lysosomal enzyme whose natural substrates are unknown. We purified aleurain to homogeneity and found that its Km for certain synthetic substrates was essentially the same as that of cathepsin H; both enzymes are primarily aminopeptidases. By expressing barley proteins in tobacco suspension culture protoplasts, we found that proaleurain was targeted to the vacuole while another barley thiol protease, proEP-B, was quantitatively secreted. Fusions between the two different proteins showed that the N-terminal 12 amino acids of proaleurain were necessary and sufficient to direct it to the plant vacuole. This targeting determinant could be divided into two smaller determinants, SSSSFADS and SNPIR, each of which was sufficient to target proEP-B chimeras to the plant vacuole, but with lower efficiency.

We now have used a synthetic peptide containing the proaleurain targeting determinants on an affinity column to purify to homogeneity a 70 kD integral membrane protein from lysates of pea clathrin-coated vesicles. This protein has a binding affinity of 15 nM for the proaleurain peptide, but does not bind to the corresponding sequence from proEP-B. We hypothesize that this 70 kD protein is a vacuolar sorting receptor and ongoing work is designed to test that hypothesis.

Washington University
St. Louis, MO 63130

192. Plant Cell Wall Architecture
J.E. Varner, Biology Department *$48,641*

Our previous work showed that cell wall proline-rich proteins (PRPs) and glycine-rich proteins (GRPs) accumulate in the cells which are lignified in several dicot species. Since xylem tissue includes the main cell types which are lignified, we are interested in gene expression of GRPs and PRPs, and other proteins which are involved in secondary cell wall thickening during xylogenesis. Xylogenesis is a dynamic process which involves hormonal induction, microtubule reorganization, microtubule-oriented secondary cell wall thickening, lignin deposition and finally, breakdown of cytoplasm. Since the main feature of xylogenesis is the deposition of additional wall components, study of the mechanism of xylogenesis will greatly advance our knowledge of the synthesis and assembly of wall macromolecules.

We are using the *in vitro* xylogenesis system from isolated *Zinnia* mesophyll cells. Our first goal was to isolate genes which are specifically expressed during xylogenesis. We have used subtractive hybridization methods to isolate a number of cDNA clones for differentially regulated genes from the cells after hormonal induction. So far, we have partially characterized 18 different cDNA clones from 239 positive clones. These differentially regulated genes can be divided into three sets according to the characteristics of gene expression in the induction medium and the control medium. The first set is induced in both the induction medium and the control medium without hormones. The second set is induced mainly in the induction medium and in the control medium with the addition of NAA alone. Two of these genes are exclusively induced by auxin. The third set of genes is induced mainly in the induction medium. Since these genes are not induced by either auxin or cytokinin alone, they may be directly involved in the process of xylogenesis.

Our experiments on the localization of H_2O_2 production reinforce the earlier ideas of others that H_2O_2 is involved in normal lignification.

Wayne State University
Detroit, MI 48201

193. Site-directed Mutagenesis of an Energy Transducing Membrane Protein-Bacteriorhodopsin
R. Needleman, Dept of Biochemistry *$166,461 (FY 92 Funds/2 years)*

Our goal is to understand the mechanism of proton transport in the light-driven membrane protein, bacteriorhodopsin. To achieve this we have evaluated the role of particular amino acids in bacteriorhodopsin by site-directed mutation and expression of the protein in the natural host, *H.*

halobium. During this last year we have concentrated on developing improved vectors for the expression of bacteriorhodopsin and, in collaboration with Janos Lanyi, using mutants to define the amino acids involved in the proton uptake and release. In particular, this work has led to the discovery of two pathways: At a pH>6, the proton release occurs before proton uptake; at a pH<6 proton release occurs later in the photocycle, and is delayed until after proton uptake. Proton uptake occurs in both cases by aspartate-96 during the N--->O reaction, while proton release is probably from D85 at low pH, and from a so-far unidentified group at high pH. In addition to these observations, we have used single and double mutants to identify major events of the photocycle, including the factors that are involved in the deprotonation of aspartate-96. This deprotonation is observed in mutant D212N even under conditions where the Schiff remains protonated, suggesting that the immediate environment of D96 directly affects its pK. We are continuing this work with the hope of identifying the amino acids involved in proton release at high pH and those amino acids involved in determining the pK of the Schiff base.

University of Wisconsin
Madison, WI 53706-1598

194. Regulation of Extracellular Polygalacturonase Production in Pseudomonas solanacearum

C. Allen, Department of Plant Pathology *$142337 (FY 92 Funds/2 years)*

Pseudomonas solanacearum causes bacterial wilt of many economically important crop plants including potato, banana, and peanut. The bacterium produces at least three extracellular polygalacturonases (PGs) that contribute to plant pathogenesis. These enzymes degrade pectin, a major component of plant cells. The general objective of this research is to investigate the genetic and biochemical mechanisms regulating PG production. Bacterial PG activity is increased 100-fold by growth in plant tissue, suggesting that a specific signal or condition in the plant host induces expression of the PG genes. Determining how host plants induce bacterial virulence genes is central to understanding the plant-pathogen interaction. 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*) produce about 10% of wildtype levels of *endo*-PG and about 50% of wildtype level of *exo*-PG. They are substantially reduced in virulence. *pehR-gus* reporter gene fusions are expressed at tenfold higher levels when bacteria grow in plant tissue rather than in rich medium. In addition, expression of *pehR* is repressed by *phcA*, a previously described global regulator of virulence functions, since *pehR* expression increases four- to eightfold in a *phcA* mutant background. These data suggest that regulation of PG and other virulence factors occurs through a regulatory cascade that responds to a plant signal. To determine the mechanism by which *pehR* directs expression of PG structural genes, we are using site-directed mutagenesis and DNA-protein binding studies.

University of Wisconsin
Madison, WI 53706

195. Biochemical and Molecular Analysis of a Transmembrane Protein Kinase from Arabidopsis thaliana

A.B. Bleecker, Department of Botany *$84,500*

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 initial studies using the kinase promoter with the GUS reporter gene system also indicate that the kinase is present throughout the plant. The kinase is glycosylated, like animal receptor kinases, and has been partially purified from *Arabidopsis* by using lectin columns. The kinase has been expressed in *E. coli*, purified, and found to autophosphorylate on serine and threonine residues, but not on tyrosine residues. As such, it belongs to the small family of receptor-like kinases with serine/threonine specificity. Transgenic plants are 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

196. Enzymology of Biological Nitrogen Fixation

R.H. Burris, Department of Biochemistry *$102,108 (2 years)*

Azospirillum brasilense is a microaerobic nitrogen-fixing bacterium whose fixation can be turned off by ADP-ribosylation of dinitrogenase reductase in response to addition of fixed nitrogen or a shift to anaerobic conditions. DRAT (dinitrogenase reductase ADP-ribosyl transferase, a product of *draT*) catalyzes the ADP-ribosylation, and DRAG (dinitrogenase reductase activating glycohydrolase, a product of *draG*) reactivates dinitrogenase reductase by removing the ADP-ribose from arginine 100 on one of the two subunits. DRAT and DRAG themselves are subject to posttranslational regulation, and we have investigated features of that regulation. The activation of DRAT in response to a shift to anaerobic conditions is transient, but the duration of its activation in response to added ammonium ion depends upon the concentration of the ammonium ion added. In contrast to the response of DRAT, DRAG appears to be active continuously during conditions favoring nitrogen fixation. Thus, the activities of DRAG and DRAT are not always regulated coordinately. Our experiments suggest that immediately following addition of a negative stimulus there is a limited period of "futile cycling" during which DRAT and DRAG are adding and removing ADP-ribose simultaneously from the pool of dinitrogenase reductase.

University of Wisconsin
Madison, WI 53706

197. Molecular Genetics of Ligninase Expression
D. Cullen and T.K. Kirk, Department of Bacteriology

$198,000 (FY 92 Funds/2 years)

Lignin depolymerization is catalyzed by extracellular enzymes of white rot Basidiomycetes such as *Phanerochaete chrysosporium.* In submerged culture, glyoxal oxidase (GLOX) and multiple isozymes of lignin peroxidase (LiP) are secreted at relatively low levels, and production is derepressed under nutrient limitation. Our objectives are to elucidate the genomic organization and regulation of the *P. chrysosporium* genes involved in lignin degradation and to express these genes in the heterologous expression system, *Aspergillus.* Toward these goals, the genes encoding GLOX and six LiP isozymes were cloned and sequenced. Using pulse field electrophoresis, GLOX and LiP genes have been localized to several dimorphic chromosomes. A combination of experimental approaches are being used to construct detailed genetic maps and to establish the nature of chromosome length polymorphisms. LiP and GLOX genes have been shown to be regulated at the transcriptional level, and the expression of LiP genes is dramatically altered in response to culture conditions. Glyoxal oxidase and three peroxidase genes have been expressed in *Aspergillus* under the control of the glucoamylase promoter. Heterologous expression of recombinant enzymes may aid in the development of processes such as biological bleaching of pulps, effluent treatments, and in biopulping.

University of Wisconsin
Madison, WI 53706

198. Organization of the R Chromosome Region in Maize
J. Kermicle, Laboratory of Genetics *$162,000 (2 years)*

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 53706-1569

199. Carbon Monoxide Metabolism by Photosynthetic Bacteria
P.W. Ludden and G.P. Roberts, Departments of Biochemistry and Bacteriology
$90,000

This project focuses on the biochemistry and physiology of the carbon monoxide dehydrogenase system from the photosynthetic bacterium *Rhodospirillum rubrum*. The carbon monoxide dehydrogenase (CODH) from *R. rubrum* carries out the oxidation of CO to CO_2 with a concomitant production of H_2 via an associated, CO-induced hydrogenase activity. CODH is a nickel-, iron-, sulfur-enzyme that can be produced in an apo form (lacking nickel) by starving cells for Ni^{2+}. The purified apo-CODH can be activated by treatment with Ni^{2+}; C^{2+}, Fe^{2+} and Zn^{2+} will also take the place of nickel in the cluster yielding an inactive enzyme. A major effort in the current project period is the purification and characterization of the carbon monoxide-induced hydrogenase activity of the system. Several gene products have been identified as important for this activity and methods for partial purification of the enzyme have been developed. A mutant line of *R. rubrum* with a lesion in a gene downstream of the *cooS* (CODH) gene renders the organism unable to accumulate nickel and process nickel into the active site of CODH. The mechanism of nickel uptake, processing and insertion into CODH and the CO-induced hydrogenase is being investigated. CODH has been crystallized and efforts to obtain higher quality crystals suitable for x-ray crystallography are a major focus of the laboratory.

University of Wisconsin
Madison, WI 53706

200. Starch Synthesis in the Maize Endosperm as Affected by Starch-Synthesizing Mutants
O.E. Nelson, Department of Genetics *$106,522 (FY 92 Funds/2 years)*

The *glucosidase-transferasel (gltl)* locus that is located on the short arm of chromosome 4 close to *sul* has been the subject of continuing attention. The mutant allele, *gltl-1*, is known to condition interesting modifications to starch. This allele, which produces seeds with a modest decrease in seed weight, resulted from a transposable element insertion. A second allele has been isolated following Ethyl Methanesulfonate (EMS) treatment of dry seeds. This new allele, *gltl-9101*, may be a null mutation at the locus. The homozygous mutant seeds are quite shrunken and weigh ca. 42% of nonmutant kernels on segregating ears. For comparison, the *gltl-1* seeds averaged 79% of nonmutant kernels on segregating ears from plants grown in the same rows. The specific activity (μmoles maltotriose/mg protein/minute produced from pullulan) of partially purified preparations of α-1,6-glucosidase extracted from 21 day-old endosperms for *gltl-1* was 10 percent of nonmutant activity, while for *gltl-9101* it was 1 percent. The correlation between the decrease in starch synthesis and the glucosidase activity for the two mutants supports our conclusion that this locus encodes the glucosidase-transferase enzyme. The *gltl* locus is a previously unidentified locus at which nonmutant alleles must be present for maximal starch production.

University of Wisconsin
Madison, WI 53706

201. Feedback Regulation of Photosynthetic Processes
T.D. Sharkey, Department of Botany — *$172,000 (2 years)*

The basic components of feedback from a limited capacity for photosynthetic end product synthesis (primarily starch and sucrose) to electron transport and rubisco have been elucidated. Mechanistic studies of the proposed links of this feedback chain are the focus of the project. The balance between production of triose phosphates and their consumption in end product synthesis is manipulated by environment and by genetics. Tomato plants transformed with a gene from corn for sucrose-phosphate synthase by Calgene shows substantially less feedback behavior than untransformed controls. This is evident in higher carbamylation of rubisco and higher rates of photosynthesis when measured at slightly elevated CO_2. A naturally occurring variant of *Flaveria linearis* shown to lack 90% of its cytosolic fructose bisphosphatase activity, an enzyme essential for sucrose synthesis, exhibits almost no photosynthetic response to elevated CO_2 and also exhibits no growth response. The variant has wildtype levels of FBPase protein as judged by dot blots. Genetic linkage between the FBPase gene and the lack of FBPase activity is now being tested using RFLPs. The results to date indicate that it is worthwhile to transform tomato plants with an FBPase gene in the antisense direction to produce a line of tomatoes with less than normal sucrose synthesis capacity to compare with the high SPS line. The mechanism of reduced rubisco carbamylation will be determined by measuring the amount of rubisco activase protein and mRNA in bean plants grown under feedback or nonfeedback conditions.

University of Wisconsin
Madison, WI 53706

202. Molecular Mechanism of Energy Transduction by Plant Membrane Proteins
M.R. Sussman, Department of Horticulture — *$97,500*

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' 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

203. Analysis of Structural Domains Required for Phytochrome Function by In Vitro Mutagenesis
R.D. Vierstra, Department of Horticulture *$96,000*

Phytochrome is a red/far-red photoreversible photoreceptor that has a central role in light-regulated plant development. In an effort to determine how phytochrome functions at the molecular level, we have exploited a biological assay for active chromoproteins that involves the expression of a chimeric oat phytochrome A gene in transgenic tobacco. Such ectopic overexpression induces a striking "light exaggerated" phenotype which can be used as an *in vivo* assay of receptor function. The goal of the project is to combine this transgenic system with *in vitro* mutagenesis to identify phytochrome domains potentially important to synthesis, dimerization, chromophore attachment, Pr/Pfr phototransformation, Pfr-enhanced degradation, and biological activity. Preliminary mapping has localized several important domains. The minimal domains necessary for chromophore attachment and assembly of the holoprotein into a red/far-red light photoreversible photoreceptor have been mapped to residues 70-399 and 1-672 respectively. A region necessary for dimerization has been identified between amino acids 919-1093. Two domains essential for full biological activity have been found, one near the amino terminus between residues 7-69 and the other at the carboxy terminus between residues 1094 and 1129. The amino-terminal domain is of interest because it undergoes a conformation change during Pr/Pfr transformations and is necessary for many of the physical properties of the chromoprotein. Further refined mapping of these domains is in progress to specifically identify critical residues. 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

204. Novel Biomaterials: Genetically Engineered Pores
H. Bayley *$165,570 (FY 92 funds)**

A selection of nanometer-scale pores is being constructed by genetic manipulation of α-hemolysin (αHL), a protein secreted by the bacterium *Staphylococcus aureus.* The single polypeptide chain of 293 amino acids forms hexameric pores in membranes ~11Å in internal diameter. Our recent focus has been on the mechanism by which the pore assembles. By analyzing the properties of truncation mutants and two-chain complementation mutants and by studying the chemical modification of single-cysteine mutants, a working model for assembly has been devised. Monomeric αHL binds to lipid bilayers and undergoes a conformational change (involving the occlusion of a central glycine-rich loop) that allows the formation of a hexameric prepore complex. The open pore is formed when subunits in this complex undergo a second conformational change after which they span the bilayer. Our studies identified the regions of αHL that are important in each step in assembly and thereby have permitted the design of αHL polypeptides in which pore-forming activity is modulated by biochemical,

chemical or physical triggers and switches. For example, αHL polypeptides with modified central loops can be activated by specific proteases or reversibly inactivated by divalent cations. Now, point mutagenesis and chemical modification are being used to create pores with different internal diameters, with selectivity for the passage of molecules and ions, and which are gated by a variety of inputs. Ultimately, the new pores will be used to confer novel permeability properties upon materials such as thin films, which might be used as components of energy conversion and storage devices, selective electrodes, electronic devices, and ultrafilters.

* Funded collaboratively with the Division of Materials Sciences (DOE)

Yale University
New Haven, CT 06511

205. Molecular Genetics of the R Complex of Maize
S.L. Dellaporta, Department of Biology *$92,000*

R-r controls the production of anthocyanin pigment in plant parts and the aleurone layer of seeds through the production of a family of related transcriptional activating proteins of the helix-loop-helix type. The complex comprises a series of repeated, homologous components arranged in both direct and inverted orientations. These include the P component, a simple R gene, which confers pigmentation of plant parts, and the S subcomplex that consists of the q component, a truncated inactive R gene, and two functional S genes, S1 and S2, that pigment the aleurone. The S genes are arranged in an unusual inverted head-to-head orientation. CHEF-gel analysis has shown that the R-r complex spans more than 250 kb of DNA with the P component separated from the S subcomplex by 190 kb. The unusual, multiply duplicated structure of R-r explains much of the meiotic instability of the complex. Sequence analysis shows that the S subcomplex was derived through the rearrangement of a simple P-like progenitor element. Molecular evidence indicate that these chromosomal rearrangements and gene duplications were mediated by a novel transposable element, doppia.

Yale University
New Haven, CT 06511-7444

206. Spatial Regulation of C4 Genes in C3, C4, and C3/C4 Intermediate Flaveria Species
T. Nelson, Biology Department *$200,000 (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

207. Organization and Control of Genes Encoding Catabolic Enzymes in Rhizobiaceae
D. Parke and L.N. Ornston, Department of Biology *$86,000*

Plant-associated bacteria of the family *Rhizobiaceae* include the plant pathogen *Agrobacterium* 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*. Genes from *Agrobacterium* that catabolize the phenolic substrates *p*-hydroxybenzoate and protocatechuate have been cloned, and their organization in a supraoperonic cluster has been analyzed. A novel regulatory gene, *pcaQ*, has been identified and characterized. In the presence of the inducer ß-carboxy-*cis-cis*-muconate, the *pcaQ* gene product acts as a transcriptional activator of one operon in the genetic cluster. Specific gene probes have been created in order to localize genes for phenolic catabolism to megaplasmids or the chromosome in diverse representatives of this bacterial family. Contrasting with the regulation in *Agrobacterium* and *Rhizobium*, most enzymes of the ß-ketoadipate pathway are expressed constitutively in *Bradyrhizobium*. One enzyme in particular, ß-ketoadipate succinyl CoA transferase, a product of the *pcaE* gene, is expressed at high levels in saprophytic and symbiotic *Bradyrhizobium*. The *pcaE* gene from *Bradyrhizobium japonicum* I-110 will be cloned in order to explore the selective value of its high level of constitutive expression. Mutant strains of *B. japonicum* blocked in the catabolism of phenolics and hydroaromatics have been isolated and used to define pathways of aromatic catabolism in this bacterium. Mutant strains of *Bradyrhizobium* and of the other two genera are being used to investigate the selective value of aromatic catabolism to the bacterial-plant interaction.

Yale University
New Haven, CT 06510

208. Electroenzymology of Plant and Fungal Vacuoles
C.L. Slayman, Department of Cellular and Molecular Physiology
$232,000 (2 years)

This research is an outgrowth of previous work on "Mechanisms and Control of K^+ Transport in Plants and Fungi", in which efforts during the past year have gone along four lines. I) Characterization of a major K^+-specific channel (YPK1) in the plasma membrane of yeast, which has proven to be a strong outward rectifier regulated by membrane voltage and cytoplasmic concentrations of Ca^{++} and H^+; II) Purification of vacuolar membranes from several strains of yeast; III) Completion of work on the mechanisms of uptake of lipid-soluble ions (TPP^+, TPA^+, $TPMP^+$) by *Saccharomyces* and *Neurospora*,

into which the ions enter electrophoretically but never reach equilibrium; and IV) A new collaborative effort to assay cytoplasmic ions (starting with protons) in plants and fungi by means of fluorescent dyes.

Experiments in the coming year should be focussed more specifically on characterization of channels, and channel-regulating cascades, in the vacuolar membranes of *Saccharomyces* and the higher plant, *Arabidopsis thaliana*. Patch- clamping of the tonoplast, chemical purification, and liposome/bilayer reconstitution will be combined toward the following objectives: 1) Identification of the physiologic substrate(s) for the primary vacuolar cation channel of yeast (YVC1); 2) detailed study of that channel's gating kinetics; 3) definition of the physiologic pathway for redox modulation of the channel; and 4) isolation of the channel protein via (calmodulin) affinity chromatography. In addition, 5) a systematic search will be made for other ion-channel types in the yeast tonoplast, as well as for the YVC1-type channel and its congeners in tonoplasts of *Arabidopsis* leaf mesophyl cells. The longer-term goals of these experiments are to elucidate the molecular mechanisms of channel operation in plant/fungal vacuoles; and to understand the roles which channels play in cellular economy and energetic efficiency via this major organelle.

Yale University
New Haven, CT 06511

209. Transfer RNA Involvement in Chlorophyll Biosynthesis
D. Söll, Department of Molecular Biophysics and Biochemistry

$212,000 (2 years)

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 in 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. At present it is unclear whether this is the only pathway of δ-aminolevulinic acid formation in these organisms.

The initial metabolite for the two-step C5-pathway is Glu-tRNA which in the presence of NADPH is converted by the action of an unusual enzyme, Glu-tRNA reductase, to glutamate-1-semialdehyde with the concomitant release of tRNA. Glutamate-1-semialdehyde is the first committed precursor of chlorophyll synthesis. Thus, Glu-tRNA 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 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. In the second step of the pathway an aminotransferase (GSA-amino-1,2-mutase) converts glutamate-1-semialdehyde to δ-aminolevulinic acid.

We cloned and analyzed the genes for these two enzymatic activities from *Arabidopsis thaliana*. Expression of both genes is regulated by light. Promoter analysis, antisense experiments and examination of mutant *Arabidopsis* strains deficient in aminolevulinate synthesis should lead to 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 category. Instead, projects are classified under the overall subject objectives. Likewise, there is no listing of technique areas, 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

As the critical driving mechanism in the conversion of solar energy into chemical energy in living organisms that ultimately results in renewable resources, the coverage includes 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 and many more are being utilized. The intent is to understand the most critical biological energy conversion process upon which most life depends.

Antenna Organization in Green Photosynthetic Bacteria
Abs. 3 Arizona State University *R.E. Blankenship*

The Chlorophyll-Binding Protein CP47 in Photosystem II
Abs. 4 Arizona State University *W.F.J. Vermaas*

Molecular Bases of Photoadaptation in Unicellular, Eucaryotic Algae
Abs. 13 Brookhaven National Laboratory *P.G. Falkowski and J. LaRoche*

Regulatory Enzymes of the Thylakoid: Isolation and Characterization of Protein Kinases and a Protein Phosphatase
Abs. 14 Brookhaven National Laboratory *G. Hind*

Molecular, Genetic and Physiological Analysis of Photoinhibition and Photosynthetic Performance
Abs. 52 **Duke University** *J.E. Boynton, N.W. Gillham and C.B. Osmond*

Nitrogen Control of Chloroplast Development and Differentiation
Abs. 69 **University of Georgia** *G.W. Schmidt*

Photosynthesis in Intact Plants
Abs. 78 **University of Illinois** *A.R. Crofts*

Mechanism of Proton Pumping in Bacteriorhodopsin
Abs. 79 **University of Illinois** *T.G. Ebrey*

Anthropogenic Impacts on Photosynthetic Activity: A Multidisciplinary Context for Research Training
Abs. 85 **University of Illinois** *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*

Transport of Ions Across the Inner Envelope Membrane of Chloroplasts
Abs. 90 **Johns Hopkins University** *R.E. McCarty*

Photoinhibition of PSII Reaction Centers: Photoactivation, Reconstitution and Resynthesis of PSII Reaction Center/Water Oxidizing Complex Components
Abs. 91 **University of Kentucky** *G.M. Cheniae*

Mechanism and Significance of Post-Translational Modifications in the Large Subunit of Ribulose Bisphosphate Carboxylase/Oxygenase
Abs. 92 **University of Kentucky** *R.L. Houtz*

Characterization of Carotenoid and Bacteriochlorophyll Biosynthesis Genes from a Photosynthetic and a Non-Photosynthetic Bacterium
Abs. 94 **Lawrence Berkeley Laboratory** *J. Hearst*

Mechanism of Water Splitting and Oxygen Evolution in Photosynthesis
Abs. 96 **Lawrence Berkeley Laboratory** *M.P. Klein*

Photosynthetic Pigment Proteins and Photosynthetic Light Reactions
Abs. 97 **Lawrence Berkeley Laboratory** *K. Sauer*

Genetic and Biophysical Studies of the Photosynthetic Reaction Center
Abs. 103 **Massachusetts Institute of Technology** *D.C. Youvan*

The Water-Splitting Apparatus of Photosynthesis
Abs. 135 **National Renewable Energy Laboratory** *M. Seibert*

Photosynthetic Electron Transport in Genetically Altered Chloroplasts
Abs. 147 **Ohio State University** *R.T. Sayre*

Regulation of Alternative CO_2 Fixation Pathways in Procaryotic and Eucaryotic Photosynthetic Organisms
Abs. 148 **Ohio State University** *F.R. Tabita*

Membrane-Attached Electron Carriers in Photosynthesis and Respiration
Abs. 161 **University of Pennsylvania** *F. Daldal*

Analysis of the PSII Proteins MSP and CP43'
Abs. 166 **Purdue University** *L.A. Sherman*

The Interaction of Ferredoxin:NADP$^+$ Oxidoreductase (FNR) and Ferredoxin: Thioredoxin Reductase with Substrates
Abs. 181 **Texas Tech University** *D.B. Knaff*

Site-directed Mutagenesis of an Energy Transducing Membrane Protein-Bacteriorhodopsin
Abs. 193 **Wayne State University** *R. Needleman*

Feedback Regulation of Photosynthetic Processes
Abs. 201 **University of Wisconsin** *T.D. Sharkey*

Spatial Regulation of C4 Genes in C3, C4, and C3/C4 Intermediate Flaveria Species
Abs. 206 **Yale University** *T. Nelson*

2. PLANT CELL WALLS

The most dominant biomass products are plant cell walls which consist of polysaccharides, lignins, proteins and other compounds. The category includes 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 and numerous molecular biological procedures, among others. The objective is to be able to obtain more comprehension of the resource that would result in greater productivity and also make it more utilizable.

Molecular Organization in the Native State of Woody Tissue: Studies of Tertiary Structure and its Development 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. 6 **University of Arizona** *M.C. Hawes*

CarbBank - A Structural and Bibliographic Database for Complex Carbohydrates
Abs. 59 **University of Georgia** *P. Albersheim*

The University of Georgia Complex Carbohydrate Research Center (CCRC)
Abs. 60 **University of Georgia** *P. Albersheim and A. Darvill*

The Structures and Functions of Oligosaccharins
Abs. 61 **University of Georgia** *P. Albersheim*

Structural Studies of Complex Carbohydrates of Plant Cell Walls
Abs. 62 **University of Georgia** *A. Darvill*

Mechanisms of Lignin Biosynthesis During Xylogenesis in *Zinnia elegans*
Abs. 64 **University of Georgia** *K-E.L. Eriksson and J.F.D. Dean*

Enzymatic Mechanisms and Regulation of Plant Cell Wall Biosynthesis
Abs. 109 **Michigan State University DOE Plant Research Laboratory** *D. Delmer*

Transcription Factors Regulating Lignin Biosynthesis in Xylem
Abs. 141 **North Carolina State University** *R. Sederoff, D. O'Malley and R. Whetten*

The Molecular Characterization of the Lignin-Forming Peroxidase: Growth, Development, and Response to Stress
Abs. 145 **Ohio State University** *L.M. Lagrimini*

The Structure of Pectins from Cotton Suspension Culture Cell Walls
Abs. 149 **Oklahoma State University** *A. Mort*

Structure and Biosynthesis of the Mixed-linkage ß-D-Glucan of Grasses
Abs. 165 **Purdue University** *N.C. Carpita*

Enzymology and Molecular Biology of Cell Wall Biosynthesis
Abs. 177 **Stanford University** *P.M. Ray*

Characterization of a 1,4,-ß-D-Glucan Synthase from *Dictyostelium discoideum*
Abs. 180 Texas Tech University *R.L. Blanton*

Towards a Detailed Understanding of Structural Variability in Lignins
Abs. 188 Washington State University *N.G. Lewis*

Plant Cell Wall Architecture
Abs. 192 Washington University *J.E. Varner*

3. PLANT RESPIRATION/NUTRITION

Plants as living organisms require energy and inorganic nutrients just as animals do in order to grow and survive. However, in addition to the similarities found in mechanisms and requirements, 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 ion nutrients in sustaining their growth, development and other synthetic 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. 31 University of California - Los Angeles *P.D. Boyer*

Molecular Studies of Functional Aspects of Higher Plant Mitochondria
Abs. 53 Duke University *J.N. Siedow*

Mechanism and Structure of the Plant Plasma Membrane Ca^{2+}-ATPase
Abs. 77 University of Illinois *D.P. Briskin*

Identifying Calcium Channels and Porters in Plant Membranes
Abs. 102 University of Maryland *H. Sze*

Transport, Function and Reaction Mechanism of Vacuolar H^+-Translocating Inorganic Pyrophosphatase
Abs. 163 University of Pennsylvania *P.A. Rea*

The Role of Alternative Respiration in Plants
Abs. 168 Rutgers University *I. Raskin*

Molecular Mechanism of Energy Transduction by Plant Membrane Proteins
Abs. 202 University of Wisconsin *M.R. Sussman*

Electroenzymology of Plant and Fungal Vacuoles
Abs. 208 Yale University *C.L. Slayman*

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 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. In future biotechnological developments where plants are to be used, it is important to know 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 previously were almost intractable.

Control of Sucrose Biosynthesis in Plants by Protein Phosphorylation
Abs. 2 USDA - North Carolina State University *S.C. Huber*

Engineering the Production of Sugar Alcohols in Transgenic Plants: Extending the Limits of Photosynthesis?
Abs. 5 University of Arizona *H.J. Bohnert and R.G. Jensen*

Characterization of Stearoyl-ACP Desaturase
Abs. 15 Brookhaven National Laboratory *J. Shanklin*

δ-Aminolevulinate Biosynthesis in Oxygenic Prokaryotes
Abs. 16 Brown University *S. Beale*

The Magnesium Chelation Step in Chlorophyll Biosynthesis
Abs. 38 Clemson University *J.D. Weinstein*

Gene-enzyme Relationships of Aromatic Amino Acid Biosynthesis in Higher Plants
Abs. 56 University of Florida *R.A. Jensen*

Physiological and Molecular Genetics of *Arabidopsis*
Abs. 116 Michigan State University DOE Plant Research Laboratory *C.R. Somerville*

Control of Triacylglycerol Biosynthesis in Plants
Abs. 122 Michigan State University *J. Ohlrogge*

A National Cooperative for Genetic Engineering of Plant Lipids
Abs. 123 **Michigan State University** *J. Ohlrogge*

Asparagine Synthetase Gene Expression and Plant Nitrogen Metabolism
Abs. 139 **New York University** *G.M. Coruzzi*

Biosynthesis of Hydrocarbons
Abs. 143 **Ohio State University** *P.E. Kolattukudy*

Regulation of Terpene Metabolism
Abs. 186 **Washington State University** *R. Croteau*

Isocitrate Lyase and the Glyoxylate Cycle
Abs. 189 **Washington State University** *B.A. McFadden*

Enhancement of Photoassimilate Utilization by Manipulation of the ADPglucose Pyrophosphorylase Gene
Abs. 190 **Washington State University** *T.W. Okita*

Starch Synthesis in the Maize Endosperm as Affected by Starch-Synthesizing Mutants
Abs. 200 **University of Wisconsin** *O.E. Nelson*

Transfer RNA Involvement in Chlorophyll Biosynthesis
Abs. 209 **Yale University** *D. Söll*

5. PLANT GROWTH AND DEVELOPMENT

It is clear that the productivity of plants for maximal biomass requires that more knowledge be gained about the nature of what controls the way in which plants produce new cells, expand tissues, and differentiate into different organ types. This category includes such types of studies which in the last decade have been completely revitalized by the development of entirely new molecular approaches. What the control mechanisms are over growth is also a critical longstanding question which is included herein.

Role of Zein Proteins in Structure and Assembly of Protein Bodies and Endosperm Texture
Abs. 7 **University of Arizona** *B. Larkins*

Molecular characterization of the Role of a Calcium Channel in Plant Development
Abs. 8 **University of Arizona** *K.S. Schumaker*

Molecular and Genetic Analysis of LEAFY, a Gene Controlling Floral Induction and Flower Development in *Arabidopsis thaliana*
Abs. 18 **California Institute of Technology** *E. Meyerowitz*

Phytochrome from Green Plants: Assay, Purification and Characterization
Abs. 22 **University of California - Berkeley** *P.H. Quail*

Analysis of Genes Essential for Floral Development in *Arabidopsis*
Abs. 24 **University of California - Berkeley** *P. Zambryski*

Protein Translocation and Assembly in Chloroplasts
Abs. 27 **University of California - Davis** *S.M. Theg*

Vacuole Biogenesis in Differentiating Plant Cells
Abs. 28 **University of California - Davis** *T.A. Wilkins*

Structure, Biosynthesis and Role of Complex Protein-bound Glycans
Abs. 30 **University of California- -La Jolla** *M.J. Chrispeels*

The Gibberellin A_{20} 3ß-hydroxylase: Isolation of the Enzyme and Its Molecular Biology
Abs. 33 **University of California - Los Angeles** *B.O. Phinney and J. MacMillan*

Sensory Transduction of the CO_2 Response of Guard Cells
Abs. 34 **University of California - Los Angeles** *E. Zeiger*

Signal Transduction in Plant Development: Chemical and Biochemical Approaches to Receptor Identification
Abs. 37 **University of Chicago** *D.G. Lynn*

Molecular and Physiological Analysis of Cytoplasmic Male Sterility
Abs. 42 **Cornell University** *M.R. Hanson*

Characterization of a Putative Receptor Protein Kinase and its Role in Self-Incompatibility
Abs. 44 **Cornell University** *J.B. Nasrallah and M.E. Nasrallah*

Plant, Cell and Molecular Mechanisms of Abscisic Acid Regulation of Stomatal Apertures
Abs. 54 **Florida State University** *W.H. Outlaw, Jr.*

Molecular Biology of Lea Genes of Higher Plants
Abs. 63 **University of Georgia** *L. Dure*

Regulation of Cell Division in Higher Plants
Abs. 81 **University of Illinois** *T. Jacobs*

Center for the Analysis of Plant Signal Transduction
Abs. 95 Lawrence Berkeley Laboratory W. Gruissem and *S.-H. Kim*

Biogenesis of Plant-specific Cell Organelles
Abs. 111 Michigan State University DOE Plant Research Laboratory *K. Keegstra*

Action and Synthesis of Plant Hormones
Abs. 112 Michigan State University DOE Plant Research Laboratory *H. Kende*

Sensory Transduction in Plants
Abs. 114 Michigan State University DOE Plant Research Laboratory *K.L. Poff*

Molecular Mechanisms of Trafficking in the Plant Cell
Abs. 115 Michigan State University DOE Plant Research Laboratory *N.V. Raikhel*

Environmental Control of Plant Development and Its Relation to Plant Hormones
Abs. 120 Michigan State University DOE Plant Research Laboratory *J.A.D. Zeevaart*

Structure-Function Relationships of ADP-Glucose Pyrophosphorylase: Manipulation of the Plant & Cyanobacterial Genes for Increased Production of Starch in Plants
Abs. 124 Michigan State University *J. Preiss*

Molecular Genetics of Myosin Motors in Plants
Abs. 127 University of Michigan *J. Schiefelbein*

Rapid Regulatory Control of Plant Cell Expansion and Wall Relaxation
Abs. 157 Pennsylvania State University *D.J. Cosgrove*

Role of Ca^{++}/Calmodulin in the Regulation of Microtubules in Higher Plants
Abs. 158 Pennsylvania State University *R. Cyr*

Circadian Rhythms in CAB Gene Expression
Abs. 160 University of Pennsylvania *A.R. Cashmore*

Molecular and Genetic Analysis of *CTR1*: A Negative regulator in the Ethylene Signal Pathway
Abs. 162 University of Pennsylvania *J. Ecker*

Signal Transduction Pathways that Regulate *CAB* Gene Expression.
Abs. 170 Salk Institute for Biological Studies *J. Chory*

Membrane Function in Lipid Mutants of *Arabidopsis*
Abs. 185 Washington State University *J. Browse*

Processing and Targeting of the Thiol Protease, Aleurain
Abs. 191 Washington University *J.C. Rogers*

Biochemical and Molecular Analysis of a Transmembrane Protein Kinase from *Arabidopsis thaliana*
Abs. 195 University of Wisconsin *A.B. Bleecker*

Analysis of Structural Domains Required For Phytochrome Function By *In Vitro* Mutagenesis
Abs. 203 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 expression of desirable genes in plants that will result in 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. 12 Brookhaven National Laboratory *B. Burr and F.A. Burr*

Genetic Analysis of *Adh1* Regulation
Abs. 19 University of California - Berkeley *M. Freeling*

Regulation of Tomato Fruit Growth by MVA and GTP-Binding Proteins
Abs. 20 University of California - Berkeley *W. Gruissem*

The Suppression of Mutations Generated by *Mu* Transposons in Maize
Abs. 39 Cold Spring Harbor Laboratory *R. Martienssen and V. Sundaresan*

Mechanisms and Genetic Control of Interspecific Crossing Barriers in *Lycopersicon*
Abs. 43 Cornell University *M.A. Mutschler*

Regulation of Polyamine Synthesis in Plants
Abs. 67 University of Georgia *R.L. Malmberg*

Soybean Ribulose Bisphosphate Carboxylase Small Subunit: Mechanisms and Determinants of RNA Turnover in Higher Plants
Abs. 68 University of Georgia *R.B. Meagher*

Molecular Characterization of a Maize Regulatory Gene
Abs. 70 University of Georgia *S.R. Wessler*

Development of Innovative Techniques That May be Used as Models to Improve Plant Performance
Abs. 73 University of Georgia *W.W. Hanna and G.W. Burton*

Genetic and Molecular Studies on Cytoplasmic Male Sterility in Maize
Abs. 83 University of Illinois *J.R. Laughnan and S. Gabay-Laughnan*

Organ-Specific Gene Expression in Maize: The *P-wr* Allele
Abs. 87 Iowa State University *T. Peterson*

Post-Transcriptional Regulation of Chloroplast Gene Expression by Nuclear Encoded Gene Products
Abs. 98 Lehigh University *M.R. Kuchka*

Molecular Mechanisms That Regulate the Expression of Genes in Plants
Abs. 110 Michigan State University DOE Plant Research Laboratory *P. Green*

Interaction of Nuclear and Organelle Genomes
Abs. 113 Michigan State University DOE Plant Research Laboratory *L. McIntosh*

Physiological and Molecular Genetics of *Arabidopsis*
Abs. 116 Michigan State University DOE Plant Research Laboratory *C.R. Somerville*

Dosage Analysis of Gene Expression in Maize
Abs. 130 University of Missouri *J. Birchler*

Position Effect as a Determinant of Variegated Pigmentation in Maize
Abs. 131 University of Missouri *K.C. Cone*

Molecular Analyses of Nuclear-Cytoplasmic Interactions Affecting Plant Growth and Yield
Abs. 132 University of Missouri *K.J. Newton*

Transposon-Induced Nuclear Mutations that Alter Chloroplast Gene Expression
Abs. 155 University of Oregon *A. Barkan*

Circadian Rhythms in *CAB* Gene Expression
Abs. 160 University of Pennsylvania *A.R. Cashmore*

Corn Storage Protein: A Molecular Genetic Model
Abs. 169 Rutgers University *J. Messing*

Genetic Engineering with a Gene Encoding a Soybean Storage Protein
Abs. 171 Scripps Research Institute *R.N. Beachy*

Nuclear Genes Regulating Translation of Organelle mRNAs
Abs. 172 Scripps Research Institute *S. Mayfield*

Exploration of New Perspectives and Limitations in *Agrobacterium*-Mediated Gene Transfer Technology
Abs. 173 University of South Carolina *L. Márton*

Regulation of Chloroplast Number and DNA Synthesis in Higher Plants
Abs. 179 Texas A&M University *J.E. Mullet*

The Plant Mitochondrial *mat-r* Gene/*nad1* Gene Complex
Abs. 182 University of Utah *D.R. Wolstenholme*

Organization of the *R* Chromosome Region in Maize
Abs. 198 University of Wisconsin *J. Kermicle*

Starch Synthesis in the Maize Endosperm as Affected by Starch-Synthesizing Mutants
Abs. 200 University of Wisconsin *O.E. Nelson*

Molecular Genetics of the *R* Complex of Maize
Abs. 205 Yale University *S.L. Dellaporta*

7. PLANT STRESS

The ability of plants to grow under sub-optimal environmental conditions is a crucial advantage. 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. 26 University of California - Davis *E. Epstein and J. Dvorak*

Tonoplast Transport and Salt Tolerance in Plants

Abs. 35 University of California - Santa Cruz *L. Taiz*

Effects of Freezing and Cold Acclimation on the Plasma Membrane of Isolated Cereal Protoplasts

Abs. 46 Cornell University *P.L. Steponkus*

Metabolic Mechanisms of Plant Growth at Low Water Potentials

Abs. 51 University of Delaware *J.S. Boyer*

Environmental Stress-Mediated Changes in Transcriptional and Translational Regulation of Protein Synthesis in Crop Plants

Abs. 65 University of Georgia *J.L. Key and R.T. Nagao*

Violaxanthin De-Epoxidase: Biogenesis and Structure

Abs. 74 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 working systems of the host plants. A substantial portion of the activities are now based on using molecular biological approaches combined with the use of mutants.

Phytoalexin Detoxifying Genes and Gene Products: Implications for the Evolution of Host Specific Traits for Pathogenicity

Abs. 9 University of Arizona *H.D. VanEtten*

Determination of Genes and Phenotypes of Bacteria Necessary for Epiphytic Colonization and Survival on Plants

Abs. 21 University of California - Berkeley *S.E. Lindow*

Cloning and Characterization of Genes Determining Disease Resistance in *Arabidopsis-Pseudomonas* Interactions
Abs. 23 University of California - Berkeley *B.J. Staskawicz*

Mechanisms of Inhibition of Viral Replication in Plants
Abs. 45 Cornell University *P. Palukaitis*

Molecular Basis of Disease Resistance in Barley
Abs. 117 Michigan State University DOE Plant Research Laboratory *S.C. Somerville*

Biochemical and Molecular Aspects of Plant Pathogenesis
Abs. 118 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. 154 Oregon State University *W.G. Dougherty*

Regulation of Extracellular Polygalacturonase Production in *Pseudomonas solanacearum*
Abs. 194 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 transferring the 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, reactions and other topics.

Organization and Regulation of the Genes for Nitrogen Fixation in *Rhodobacter capsulatus*
Abs. 36 University of Chicago *R. Haselkorn*

Regulation of Gene Expression in the *Bradyrhizobium japonicum*/soybean symbiosis
Abs. 50 Dartmouth College *M.L. Guerinot*

Cellulose Fermentation by Nitrogen-Fixing Anaerobic Bacteria
Abs. 104 **University of Massachusetts** *E. Canale-Parola*

Molecular Basis of Symbiotic Plant-Microbe Interactions
Abs. 108 **Michigan State University DOE Plant Research Laboratory** *F.J. de Bruijn*

Developmental Biology of Nitrogen-Fixing Cyanobacteria
Abs. 119 **Michigan State University DOE Plant Research Laboratory** *C.P. Wolk*

A Structural Assessment of the Role of Cell Surface Carbohydrates of *Rhizobium* in the Rhizobium/Legume Symbiosis
Abs. 121 **Michigan State University** *R. Hollingsworth*

Catalytic Mechanism of Hydrogenase from Aerobic N_2-Fixing Microorganisms
Abs. 153 **Oregon State University** *D.J. Arp*

Crystallographic Studies of Nitrogenase and Hydrogenase
Abs. 164 **Purdue University** *J.T. Bolin*

Nodulation Genes and Factors in the *Rhizobium*-Legume Symbiosis
Abs. 176 **Stanford University** *S.R. Long*

Plant Recognition of *Bradyrhizobium japonicum* Nod Factors
Abs. 178 **University of Tennessee** *G. Stacey*

Carbon Metabolism in Symbiotic Nitrogen Fixation
Abs. 187 **Washington State University** *M.L. Kahn*

Enzymology of Biological Nitrogen Fixation
Abs. 196 **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 lacking. Thus these studies include genetic, biochemical and physiological approaches in learning how organisms do this so that it may be possible to carry forth these processes in a biotechnological manner.

Cellulose Binding Proteins of *Clostridium cellulovorans* Cellulase
Abs. 25 University of California - Davis *R.H. Doi*

Studies of the Genetic Regulation of the *Thermomonospora* Cellulase Complex
Abs. 48 Cornell University *D.B. Wilson*

Microbiology and Physiology of Anaerobic Fermentations: The Conversion of Complex Organic Materials to Simple Gases
Abs. 66 University of Georgia *H.D. Peck, Jr., L.G. Ljungdahl. L.E. Mortenson and J.K.W. Wiegel*

Hemicellulases from Anaerobic Thermophiles
Abs. 72 University of Georgia *J. Wiegel*

Cellulose Fermentation by Nitrogen-Fixing Anaerobic Bacteria
Abs. 104 University of Massachusetts *E. Canale-Parola*

Physiology and Molecular Biology of Lignolytic Enzymes Systems in Selected Wood-Rotting Fungi
Abs. 125 Michigan State University *C.A. Reddy*

Biochemical Genetics of Lignin Degradation by *Phanerochaete chrysosporium*
Abs. 151 Oregon Graduate Institute of Science and Technology *M.H. Gold*

Oxidative Enzymes Involved in Fungal Cellulose Degradation
Abs. 152 Oregon Graduate Institute of Science & Technology *V. Renganathan*

Characterization of Lignin and Mn Peroxidases from *Phanerochaete chrysosporium*
Abs. 159 Pennsylvania State University *M. Tien*

Molecular Genetics of Ligninase Expression
Abs. 197 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 biochemical pathways including different energy deriving reactions. The projects included in this category focus on attempting to understand the nature of some of these pathways including the unusual pattern of degradation of aromatic compounds as well as polysaccharide breakdown, production of organic solvents and others. Such basic knowledge could afford new ways of converting biomass resources into useful products and also give insights into ways of degrading certain pollutants for possible introduction into bioremediation. This is one of the several areas within the EB program which has been understudied.

Anaerobic Metabolism of Aromatic Compounds by Phototrophic Bacteria: Biochemical Aspects
Abs. 41 **Cornell University** *J. Gibson*

Ethanologenic Enzymes of *Zymomonas mobilis*
Abs. 55 **University of Florida** *L.O. Ingram*

Genetics of Solvent-Producing Clostridia
Abs. 76 **University of Illinois** *H.P. Blaschek*

Molecular Biology of Anaerobic Aromatic Biodegradation
Abs. 88 **University of Iowa** *C.S. Harwood*

One Carbon Metabolism in Anaerobic Bacteria: Regulation of Carbon and Electron Flow During Organic Acid Production
Abs. 126 **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. 128 **University of Minnesota** *P. Rogers*

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

Control of Sugar Transport and Metabolism in *Zymomonas mobilis*
Abs. 136 **University of Nebraska** *T. Conway*

Effect of Community Structure on Anaerobic Aromatic Degradation
Abs. 150 **University of Oklahoma** *M.J. McInerney*

Genetic and Biochemical Analysis of Solvent Formation in *Clostridium acetobutylicum*
Abs. 167 **Rice University** *G.N. Bennett*

Regulation of Alcohol Fermentation by *Escherichia coli*
Abs. 174 **Southern Illinois University** *D.P. Clark*

Enzymology of Acetone-Butanol-Isopropanol Formation
Abs. 183 **Virginia Polytechnic Institute and State University** *J.-S. Chen*

Organization and Control of Genes Encoding Catabolic Enzymes in Rhizobiaceae
Abs. 207 **Yale University** *D. Parke and L.N. Ornston*

12. ONE AND TWO CARBON MICROBIAL METABOLISM

Microorganisms, particularly anaerobic ones, are greatly attuned to the conversion of carbon compounds such as carbon monoxide, acetic acid, methanol and others. For example, the production of methane in the very large number of varied sites e.g., swamps, rumens, rice paddies largely involves using one or two carbon precursor molecules on the part of the methanogens involved. It is another area of much needed attention to be able to understand the types of conversions that the large numbers of organisms are capable of. With such information in hand and with the capabilities of genetic manipulation now possible entirely new bioconversion resources may be feasible. Thus the studies covered include methanogenesis, methylotrophy and other systems.

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

Genetics in Methylotrophic Bacteria
Abs. 17 **California Institute of Technology** *M.E. Lidstrom*

Molecular Biology and Genetics of the Acetate-Utilizing Methanogenic Bacteria
Abs. 32 **University of California - Los Angeles** *R.P. Gunsalus*

Conversion of Acetic Acid to Methane by Thermophiles
Abs. 49 **Cornell University** *S.H. Zinder*

Microbiology and Physiology of Anaerobic Fermentations of Cellulose
Abs. 66 **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. 71 **University of Georgia** *W.B. Whitman*

Genetics of the Methanogenic Bacterium, *Methanococcus voltae* With Attention to Genetic Expression Mechanisms
Abs. 82 **University of Illinois** *J. Konisky*

Exploratory Studies on the Bacterial Formation of Methane
Abs. 84 **University of Illinois** *R.S. Wolfe*

Carbon Metabolism in Methylotrophic Bacteria
Abs. 99 **Los Alamos National Laboratory** *C.J. Unkefer*

Mechanisms of Transcriptional Gene Regulation in the Methanogenic Archaea
Abs. 101 **University of Maryland, Baltimore** *K. Sowers*

Genetics of Bacteria that Utilize One-Carbon Compounds
Abs. 129 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. 144 Ohio State University *J.A. Krzycki*

Structure and Regulation of Methanogen Genes
Abs. 146 Ohio State University *J.N. Reeve*

Enzymological Studies of One-Carbon Reactions in the Pathway of Acetate Utilization by Methanogenic Bacteria
Abs. 184 Virginia Polytechnic Institute and State University *J.G. Ferry*

Carbon Monoxide Metabolism by Photosynthetic Bacteria
Abs. 199 University of Wisconsin *P.W. Ludden and G.P. Roberts*

13. EXTREMOPHILIC MICROBES

How microorganisms cope with exceedingly high temperatures, alkalinity, salinity and other factors is an intriguing serious 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 enzymes that are capable of activity under the same conditions as part of new biotechnological developments.

Membrane Bioenergetics of Salt Tolerant Organisms
Abs. 29 University of California Irvine *J.K. Lanyi*

Sugar Transport and Metabolism in *Thermotoga*
Abs. 40 University of Connecticut *A.H. Romano and K.M. Noll*

Effect of Growth Temperature on Enzyme Folding
Abs. 57 Georgia State University *A.T. Abdelal*

The Metabolism of Hydrogen by Extremely Thermophilic Bacteria
Abs. 58 University of Georgia *M.W.W. Adams*

Hemicellulases from Anaerobic Thermophiles
Abs. 72 **University of Georgia** *J. Wiegel*

Phylogenetic Analysis of Hyperthermophilic Natural Populations Using Ribosomal RNA Sequences
Abs. 86 **Indiana University** *N.R. Pace*

Analysis of Thermally Stable Electron Transport components in the Hyperthermophilic Bacterium, *Pyrodictium brockii*
Abs. 89 **Johns Hopkins University** *R.J. Maier*

Structure and Regulation of L-Glutamate Dehydrogenase in Hyperthermophilic *Archaea* (Archaebacteria)
Abs. 100 **University of Maryland** *F.T. Robb*

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

Bioenergetic and Physiological Studies of Hyperthermophilic Archaea
Abs. 140 **North Carolina State University** *R.M. Kelly*

Structure and Regulation of an Archaebacterial Promoter: An In Vivo Study
Abs. 142 **Ohio State University** *C.J. Daniels*

The Characterization of Psychrophilic Microorganisms and Their Potentially Useful Cold-Active Glycosidases
Abs. 156 **Pennsylvania State University** *J.E. Brenchley*

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 metabolism is one question being pursued with the consideration of bioenergetics as part of the studies. 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. 47 **Cornell University** *V.J. Stewart*

Heavy Metal-lux Sensor Fusions and Gene Regulation
Abs. 75 **University of Illinois - Chicago** *S. Silver*

Studies on the *bo*-type Ubiquinol Oxidase From *Escherichia coli*
Abs. 80 University of Illinois *R.B. Gennis*

Genomic Plasticity and Catabolic Potential of *Pseudomonas cepacia*
Abs. 105 University of Massachusetts *T.G. Lessie*

Molecular Characterization of Bacterial Respiration on Minerals
Abs. 106 Meharry Medical College *R. Blake II*

Biochemistry of Dissimilatory Sulfur Oxidation
Abs. 107 Meharry Medical College *R. Blake II*

15. BIOMATERIALS

The prospects of developing entirely new materials using either organisms or enzymes in the synthesis 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 and Biomolecular Materials
Abs. 93 Lawrence Berkeley Laboratory *M.D. Alper, M. Bednarski, M. Callstrom, J.F. Kirsch, B. D.E. Koshland, B. Novak, P.G. Schultz and C.-H. Wong*

The Effect of Oligosaccharides on Glycoprotein Stability
Abs. 175 Stanford University *C.F. Goochee*

Novel Biomaterials: Genetically Engineered Pores
Abs. 204 Worcester Foundation for Experimental Biology *H. Bayley*

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