DOE/ER-0621P

ANNUAL REPORT AND SUMMARIES OF FY 1994 ACTIVITIES

DIVISION OF ENERGY BIOSCIENCES

U.S. DEPARTMENT OF ENERGY
OFFICE OF BASIC ENERGY SCIENCES
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Cover Picture: Photo taken along Chesapeake and Ohio Canal in Maryland. Shows diversity of plant species. Photo by R. Rabson.

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DIVISION OF ENERGY BIOSCIENCES

SEPTEMBER 1994

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

Program Overview of the Division of Energy Biosciences

The Energy Biosciences program, earlier termed the Biological Energy Research program, was initiated in 1979 for the purpose of fulfilling the need of basic information about plants and microorganisms relating to varied energy matters. The program generates basic information that contributes significantly to future technologies involving alternate fuel generation, petroleum replacements, sustained industrial activities along with means of improving environmental conditions.

From its inception, the program has covered basic research in the areas of plant and non-medical microbial science including physiology, biochemistry, genetics and other disciplinary approaches in order to gain a better understanding of how organisms function and how they are structured. Topics such as photosynthesis, fermentation, adaptation to natural stress conditions, ion uptake, nitrogen fixation, plant-microbe interactions, genetic regulation, metabolic pathways, transport activities and a myriad of other areas have been included in the investigations sponsored. Some of the plants and microorganisms being studied, unlike certain other species, have not received a great amount of research attention, despite the critical role that such organisms have in Nature. In order to achieve the establishment of new biotechnologies, the addition of information emerging from such studies is essential.

As the understanding of biological processes increases, it is becoming clearer that further comprehension will require broader approaches, including multidisciplinary investigations. The fact that the Energy Biosciences program is situated in the Office of Basic Energy Sciences with disciplines including chemistry, materials science, engineering, geosciences and others, facilitates interactions with these other disciplines. Such interactions between programs and scientific disciplines is of growing importance. The Energy Biosciences program has been active in encouraging such interactions.

With respect to interactions with other Federal agencies, Energy Biosciences has been an active participant in the Department of Energy/National Science Foundation/Department of Agriculture Joint Program on Collaborative Research in Plant Biology for almost a decade. (This past year's awards supported by Energy Biosciences are described below.) Energy Biosciences also interacts with the National Science Foundation on training in the research field of microbial physiology upon which much biotechnology development is dependent. The

initiation of a carbohydrate structural database termed "CarbBank" was begun several years ago with the support of Energy Biosciences, and was subsequently joined by the National Institute of General Medical Sciences, National Library of Medicine, National Science Foundation and the Department of Agriculture in support of the project. In addition, foreign institutions have provided assistance in the form of information contributions. This reflects the broad interest in carbohydrate data with respect to the diverse missions of the agencies. Making such information readily available is of high priority. In addition to interactions with other agencies, the Energy Biosciences program has a number of cooperative activities with other sections within the Department. These include projects supported within Basic Energy Sciences as well as interactive collaboration with the Office of Health and Environmental Research and the Office of Environmental Management. One of the effective mechanisms for stimulating interaction within the agency is the BioEnergy Coordinating Committee (BECC), a group that meets to exchange ideas among different sections within the Department, and sometimes with representatives from other agencies as well.

The last few years have witnessed an increased emphasis on the role of basic research in influencing technology development. History shows that without exploratory research, the major advances in industrial development, medical treatment, agricultural productivity and other aspects of today's society would not have occurred. It is hard to predict the potential application of some of the results with respect to at least a fraction of the basic research that is conducted. Nonetheless, there are numerous examples of what might be termed "serendipitous" results that have dramatically impacted civilization. It would have been quite a different world today if Gregor Mendel, the first geneticist of over a century ago, and his numerous successors had never probed into how characteristics are inherited and how the genetic systems operate. Today's young biotechnology industry, with an enormous potential for benefitting mankind, is an outgrowth of this accumulated basic research.

With respect to the Energy Biosciences program, there is a constant transfer of results of many of the supported projects into industrial involvement. Several examples have been cited in prior years' reports. A current example is the work of Dr. William Finnerty in the 1980's on how microorganisms convert the sulfur found in petroleum. This basic information generated earlier is now the foundation of developing biologically based industrial procedures for ridding petroleum of sulfur pollutants prior to combustion. Other examples can be mentioned without citing investigators' names which relate to altering the protein content of plants and microorganisms to achieve commercial objectives by manipulating the organisms, as well as the development of devices for use in artificial lighting for

photosynthesis, and others such as a digital imaging spectrometer useful for detecting pigmented microbial mutants. All in all, there is a highly diverse amount of interaction between Energy Biosciences funded investigators and commercial firms that will undoubtedly result in new products and processes of benefit to mankind. The Office of Basic Energy Sciences will be issuing a document that provides examples of such technology transfer in more detail within the next few months.

One of the features of the Energy Biosciences program is the breadth of the scope of research topics covered. This can be seen in the broad categorization presented at the end of the report (see pages 143-165). The program covers a number of areas that are important and well populated by investigators. In addition, serious efforts are made to generate interest in critical research areas that are underpopulated and needful of attention in order to progress in understanding functions and advance the development of new technologies. One way to stimulate interest and acquaint people with the importance of certain areas is to organize technical workshops in which the status of an area is discussed by groups of experts representing a breadth of scientific disciplines and research interests. The participants in the workshop are asked to outline the research and training needs and determine the technology compensation from the generation of new and innovative knowledge about the topic areas. Representatives from industry are usually in attendance to contribute their perspective on research needs in specific areas. Generally, the outcome of these workshops is summarized in printed form and made available to interested parties. By these activities, the program takes on the broad viewpoint that the research needs are to be addressed not by a single agency, nor by a single country's research activities, but by the broad scientific community. Continuing interactions among investigators from different locations and disciplines are encouraged for the future in order to stimulate progress. In the past couple of years workshops have been held on the topics of plant biochemistry, lignin synthesis and degradation, photosynthetic research related to ecological situations, and phytoremediation (see below).

As our biological science knowledge increases, our appreciation of the complexity of the life process also increases. One of the areas that is needful of more attention relates to organism/organism interactions. For example, the prospect of enhancing the use of both plants and microorganisms for renewing the environmental conditions of certain sites is dependent on having a better understanding of how the various biological systems present in the soil interact. In a recent workshop that was co-sponsored by the Division of Energy Biosciences and the Office of Environmental Management of the Department of Energy on both the basic and applied research needs underlying phytoremediation technology

development, it became very clear that plants are capable of accumulating large amounts of heavy metals, as well as converting soil-borne organic molecules. However, the mechanisms of uptake, accumulation, transport and other components of the processes are virtually unknown. It also becomes very evident that interactions between plants and microorganisms represent a very important aspect of such functions. In addition, the geological nature of the soil or fluid in which the organisms are operative is an important factor as well. These revelations indicate that considerably more investigations are necessary about the mechanisms in which organisms adjust to surroundings and are influenced by them in order to make future bioremediation applications more effective.

During this year, the DOE/NSF/USDA Joint Program on Collaborative Research in Plant Biology again invited applications for projects on Interdisciplinary Training in Plant Research and Multi-institutional Research Coordination in Plant Science. Once the peer review process is completed, the agency representatives decide which agency will fund the projects that received the highest level ratings in the peer review process. It is important to recognize that regardless of which agency funds a project, each of the projects is categorized as a unit of the DOE/NSF/USDA Collaborative Program in Plant Biology. The Energy Biosciences program is to fund two new projects in the multidisciplinary training category. The first of the new projects being supported by the Energy Biosciences program is to Washington State University entitled "Washington State University Interdisciplinary Plant Biochemistry Research and Training Center" with the Director, Dr. Norman G. Lewis. The second new training award was to the University of Pennsylvania entitled, "Structural Basis of Signal and Energy Transduction in Plants" with the Director, Dr. Anthony Cashmore. In both cases, one of the most important emphases will be to train students to be familiar with multiple disciplines for use in approaching research problems. The other awards are being announced by the other two agencies.

The Energy Biosciences program during Fiscal Year 1994 received 139 new research applications following the screening of numerous preapplications. Of the applications received, 21 projects were funded. Funds for the support of most of the new projects were derived from the turnover of some previously supported research. The fact that funds available for the support of plant science and certain aspects of microbiological science are somewhat restricted makes the competitiveness fairly strong. This also applies to other Federal agency programs.

The breakdown of how the resources available to EB were distributed in FY 1994 is indicated in the following table.

	Number of Projects	FY 94 funding (in thousands)	Percent of total funds
University and Non-Profit Institutions	197	$ 18,334	73%
Michigan State University Plant Research Laboratory	11	2,488	10%
Three-Agency Plant Science Collaboration Activities--Universities	5	913	3%
National Laboratories Brookhaven National Lab Lawrence Berkeley Lab Los Alamos National Lab National Renewable Energy Lab.	10	2,320	9%
Small Business Innovation Research (SBIR) contribution, and Miscellaneous		701	3%
Conferences, Educational Activities	7	432	2%
Databases (joint funding)	1	75	>1%
	231	$ 25,263	

An explanation is appropriate about the funding figures indicated with abstracts. Some 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.

One of the very disappointing events of the year was the death of the grantee, Dr. John Laughnan of the University of Illinois. Dr. Laughnan has been a longstanding excellent contributor to maize genetics knowledge including in recent years better understanding of cytoplasmic male sterility.

During Fiscal Year 1994, the Energy Biosciences program provided support for the following conferences, workshops or training activities (two were supported with Fiscal Year 1993 funds):

1. Workshop on Vegetative Development, Marshall, CA, January 7-9, 1994

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

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

4. DOE Lignin Workshop: Research Needs in Lignin Biosynthesis and Biodegradation, Pacific Grove, CA, May 24-27, 1994

5. American Society of Plant Physiologists Course on Plant Biochemistry - 1994, East Lansing, MI, June 27-July 17, 1994

6. Workshop on Phytoremediation Research Needs, Santa Rosa, CA, July 24-26, 1994.

7. Summer Investigations into the Metabolic Diversity of the Microbial World, Woods Hole, MA, Summer 1994

As part of its continuing participation in the Life Sciences Research Foundation's post-doctoral fellowship program, the Energy Biosciences program has initiated three-year fellowship support for the following individuals, whose applications were rated highly by the review committee:

Dr. Hank Bass (Dept. Mol. & Cell Biol., Univ. Calif., Berkeley)
Dr. Kris Niyoki (Dept. Plant Biol., Carnegie Inst. Washington)
Partial funding:
Dr. Jian-Kang Zhu (Lab of Plant Mol. Biol., Rockefeller Univ.)
Dr. David Weiss (Dept. Micro. & Mole. Genetics, Harvard Medical School)

Each of the individuals is working in an area relating to the Energy Biosciences scope of coverage.

One of the most important components of any scientific award system is the use of review procedures that employ highly knowledgeable and thoughtful technical persons. This process has the title of "peer review". The Energy Biosciences program, like most other federal programs that fund research, uses the peer review procedures regularly. As a program we are indebted to the hundreds of scientists who have assisted us in evaluating applications and on-going projects by mail reviews, by panel meetings and by site visit reviews. Without such assistance the program, most likely, would certainly not have the same quality. **Thus, we wish to thank the myriad of reviewers, both in this country and abroad, who have contributed their time and effort to the peer review processing of the program.**

During the year, the program staff was complemented when Dr. James Tavares joined the program. Dr. Tavares had been associated with the Board on Agriculture of the National Research Council for many years prior to his joining the Energy Biosciences staff. The staff members of the Energy Biosciences program are:

Dr. Robert Rabson, Director
Dr. Gregory L. Dilworth
Dr. James E. Tavares
Ms. Patricia A. Snyder
Ms. Mary Jo. Martin

Division of Energy Biosciences
Office of Basic Energy Sciences
ER-17, GTN
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Washington, DC 20585

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Tavares: james.tavares@mailgw.er.doe.gov

Abstracts of Projects Supported in FY 1994

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, Fluorescence Spectroscopy and Photoconductivity

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

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. This latter effort has led us to explore new paradigms concerning the processes of assembly of the individual constituents of the cell wall and the couplings between these processes. Some of the observations which have guided our explorations include evidence for: (1) organization of lignin relative to cell wall morphology; (2) a strong influence of cell wall matrix polysaccharides 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 as well as a high degree of coherence in the organization of the phenylpropane units. The methods used to characterize native tissues include Raman spectroscopy and the Raman microprobe, solid state ^{13}C NMR, fluorescence spectroscopy, and measurements of photoconductivity. We have complemented these methods, where appropriate with liquid state NMR, UV-visible absorption spectroscopy, theoretical modeling of intermolecular interactions, and traditional chemical analytical procedures.

U.S. Department of Agriculture
Beltsville, MD 20705-2350

2. Metabolic Regulation of the Plant Hormone indole-3-acetic Acid

J.D. Cohen and J.P. Slovin, Beltsville Agricultural Research Center *$82,159*

The phytohormone auxin is important for many aspects of plant growth and development. Our understanding of the biochemistry of how auxin is made in plants, and the mechanisms by which plants regulate auxin levels has changed considerably within the last few years. We are now in a position to make rapid advances in understanding how these biochemical processes are related, and how changes in auxin metabolism influences and in turn are influenced by, plant development. The major native auxin found in plants is indole-3-acetic acid (IAA), and for almost 50 years it has been thought that IAA was made from the amino acid tryptophan. Recently we showed that mutant plants that cannot make tryptophan still make IAA, and in very high amounts. Another set of experiments showed that both the traditional tryptophan pathway and the non-tryptophan pathway occur in plants and sometimes both were used by the same plant. An additional source of IAA in plants is the relatively large pool of conjugated IAA stored within plant cells. We are working on four fundamental problems related to how plants get their IAA: 1) the biochemistry of the non-tryptophan pathway to auxin; 2) how a plant decides to use one or the other pathway, or both, during various developmental stages or in response to environmental stresses and stimuli; 3) the regulation and control of an enzyme that releases free IAA from conjugates. The activity of this enzyme changes significantly during a particular stage in development when there is a concomitant dramatic decrease in the level of total auxin in the cells; and 4) auxin turnover (the rate at which IAA is made and destroyed) which is an important component of auxin metabolism. A unique model system we devised for investigating IAA turnover is being used to determine whether IAA turnover changes in response to developmental and environmental events as well as to determine how changes in rates of turnover affect the growth and development of plants.

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

3. Control of Sucrose Biosynthesis in Plants by Protein Phosphorylation

S.C. Huber, USDA/ARS and Departments of Crop Science and Botany, NCSU *$69,905*

Studies have been continued to characterize the control of sucrose-phosphate synthase (SPS), a key enzyme of sucrose formation, by reversible protein phosphorylation. Previously, we identified the major regulatory phosphorylation site as Ser-158, and have now identified two "non-regulatory" seryl residues that are phosphorylated *in vivo*. Consistent with several indirect lines of evidence, these results provide the first direct evidence in support of multisite phosphorylation of SPS. The substrate specificity of the regulatory SPS-protein kinase (SPS-PK), which phosphorylates Ser-158, has been characterized using synthetic peptide analogs (15 to 20-amino acids) of the native sequence. A 19-amino acid synthetic peptide corresponding to the native sequence was a good substrate for SPS-PK *in vitro*, with an

apparent Km for the peptide of 5 to 10 μM. Based on preliminary experiments, basic residues at -6 and -3, relative to the phosphorylatable Ser at position 0, appear to be essential for recognition by the kinase. Thus, the recognition sequence appears to be Basic-X-X-Basic-X-X-Ser-X. Inhibition of SPS phosphorylation and inactivation by Glc-6-P (other common metabolic intermediates had no effect) may be important for *in vivo* control of SPS phosphorylation status. We have demonstrated that, in addition to native SPS, Glc-6-P also inhibits the phosphorylation of a 26-KDa truncated SPS protein as well as synthetic peptides. Thus, it appears that Glc-6-P may be interacting with the protein kinase directly, rather than the substrate protein (SPS), suggesting that SPS-PK may be a metabolite-regulated protein kinase. NR-PK, which is involved in the phosphorylation and inactivation of NR, is also inhibited by a variety of P-esters and may provide another example of a metabolite-regulated protein kinase.

U.S. Department of Agriculture
Urbana, IL 61801-3838

4. Sites of Rubisco Activase Interaction with Rubisco
W.L. Ogren and A.R. Portis, Jr., USDA/ARS *$80,500*

Rubisco activase is the regulatory enzyme for ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco) which initiates photosynthetic carbon acquisition. The primary objectives of this project are to define the nature of the rubisco - rubisco activase interaction and to determine the impact of this interaction on the regulation of the photosynthesis rate. This interaction will be described physically by identifying residues involved in the binding of these two enzymes to each other, mechanistically by identifying residues that affect other essential steps involving hydrolysis of ATP and the release of sugar phosphates from the active side of rubisco, and physiologically by determining how the steady-state rate of photosynthesis is altered by manipulating the activation state of rubisco *in vivo*. These goals will be addressed through the creation of rubisco activase proteins with altered activities by directed mutagenesis and by chimera formation with cDNA's from different species, and by transforming the *Arabidopsis thaliana* rubisco activase-deficient (*rca*) strain with mutant enzymes possessing distinctive properties, followed by determining the photosynthetic characteristics of the transformed plants. Since the rubisco-rubisco activase system is a major factor in determining plant response to the environment, information gained by this research will enhance our knowledge of the capability of plants to adapt to the changing global environment.

Arizona State University
Tempe, AZ 85287-1604

5. Antenna Organization and Regulation in Green Photosynthetic Bacteria
R.E. Blankenship, Department of Chemistry and Biochemistry

$215,340 (2 years)

All photosynthetic organisms contain chlorophyll pigments that function as an antenna, absorbing light and transferring excitations to a photochemical reaction center where energy storage takes place by a series of chemical reactions. The green photosynthetic bacteria are characterized by large antenna complexes known as chlorosomes. The overall objective of this project is to determine the molecular organization of pigments in chlorosome antennas, a well as the mechanisms of excitation transfer and regulation of this unique antenna system. 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 have indicated that the pigments are very strongly coupled, leading to subpicosecond energy transfer. Green sulfur bacteria also contain a redox-activated control of energy transfer efficiency. At high redox potential, most excitations are quenched within the chlorosome, while at low redox potentials almost all are transferred to the reaction center. The quenching effect may be a control mechanism that protects the cell from damage during conditions where light and oxygen are present simultaneously. Recent work has focused on understanding redox activity in the Fenna-Matthews-Olson bacteriochlorophyll *a* antenna protein, which is an intermediate in the energy transfer pathway from chlorosome to reaction center.

Arizona State University
Tempe, AZ 85287-1601

6. The Chlorophyll-Binding Protein CP47 in Photosystem II
W.F.J. Vermaas, Department of Botany *$93,111*

For photosynthesis, light is absorbed by chlorophyll and other pigments that are associated with specific proteins. Subsequently, absorbed light energy is transferred to a particular chlorophyll where this energy is used to initiate a series of redox reactions, which ultimately result in utilization of water and carbon dioxide to produce carbohydrates and oxygen. Our research is concerned primarily with investigating chlorophyll-protein interactions and how these affect the efficiency of light energy transfer. Towards this aim, mutations have been introduced at possible chlorophyll-binding histidine residues of the chlorophyll-binding protein CP47 that is part of the photosystem II complex. For improved resolution of the impact of the mutations, we have genetically deleted photosystem I, which binds a significant amount of chlorophyll. Upon mutation of such histidines in CP47 to residues that are not likely ligands for chlorophyll, an increase in the amount of pheophytin (which is chlorophyll minus the

central Mg) was observed in photosystem II. This is accompanied by a decrease in energy transfer efficiency. The reasons as to why pheophytin in a chlorophyll antenna matrix can lead to a decrease in energy transfer efficiency are beyond the scope of this abstract. An interesting serendipitous outcome of this work is the tentative assignment of the origin of chlorophyll fluorescence emission at 695 nm that is prevalent at low temperature (77° K). This emission was known to be associated with one or few chlorophylls in the CP47 protein, and appears to originate from chlorophyll associated with residue 114 of CP47: mutation of this residue to Gln (which also can serve as chlorophyll ligand) shifts the fluorescence emission peak at 695 nm by 2-3 nm to the blue, without greatly affecting photosystem II function. Mutations in other His residues did not shift the 695 nm emission. This is the first time the environment of a low-energy chlorophyll associated with the core antenna has been tentatively identified.

University of Arizona
Tucson, AZ 85721

7. Engineering the Production of Sugar Alcohols in Transgenic Plants: Extending the Limits of Photosynthesis?

H.J. Bohnert and R.G. Jensen, Department of Biochemistry *$91,180*

Factors limiting photosynthetic carbon assimilation include intrinsic limitations of the photosystems, dark reactions, and metabolism of carbon products. While strategies that circumvent these identified bottlenecks are being explored in many laboratories, we introduced a different approach. A significant amount of carbon in the biosphere is stored as acyclic and cyclic polyols. Different steric forms of polyols are present in virtually every plant, albeit in higher plants they are usually found in small amounts or are absent. Few studies targeting polyol biosynthesis have been carried out. Using gene transformations, we have diverted some carbon into the production of polyols and are investigating their effects on photosynthesis, biomass production and environmental stress tolerance in transgenic plants. The introduced genes lead to the production of either mannitol, sorbitol, or ononitol (methyl-inositol) in tobacco and *Arabidopsis*. Lines have also been obtained that accumulate several polyols. Transgenic plants are phenotypically normal unless polyol production exceeds sucrose production by orders of magnitude in which case the plants show osmotic stress symptoms, indicating that, for example, in tobacco these polyols are not transported from the site of synthesis. Polyols confer increased salt stress tolerance to tobacco albeit to different degrees depending on the type of polyol. *Arabidopsis* expressing increased levels of mannitol may germinate at NaCl concentrations that eliminate germination of wild type plants. Present experiments include measurements of carbon flux through the engineered pathways, and analysis of polyol localization in different compartments.

University of Arizona
Tucson, AZ 85721

8. 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 *$195,200 (2 years)*

Roots of many species are programmed to produce and separate thousands of living cells, root "border" cells, from their root tips daily. The controlled release of living somatic cells into the environment is unique among higher organisms, and its function is unknown. The objective of this work is to test the hypothesis that cell wall degrading enzymes in the root cap of pea play a role in cell wall degradation leading to border cell separation. Activities of a pectinmethylesterase (PME) and a polygalacturonase (PG) are correlated with deesterification of pectin in the root cap, with changes in cell wall pH, and with border cell separation. A root cap expressed PME cDNA has been cloned, and has been shown to be transcriptionally regulated in correlation with border cell separation. This gene will be used to develop transgenic roots with reduced PME activity which can be used to test predictions of a model in which PME is the driving force in initiation of border cell separation. The same approach will be used to test the impact of a root cap PG and other cell wall degrading enzymes whose activity is associated with border cell separation.

University of Arizona
Tucson, AZ 85721

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

B.A. Larkins, Department of Plant Sciences *$93,120*

Mutations that affect the qualitative and quantitative synthesis of maize seed storage proteins, or zeins, typically result in kernels with soft, starchy endosperms. We are investigating the interactions between zein proteins and the role these proteins play in influencing the texture of the mature kernel. There are four structurally distinct types of zein proteins, which are designated alpha-, beta-, gamma- and delta-zeins. These proteins are synthesized on rough endoplasmic reticulum membranes and aggregate within the lumen of the RER to form insoluble accretions. The beta- and gamma-zeins, which are cysteine-rich proteins cross-linked by disulfide bonds, are first to appear in protein bodies. They are primarily found near the surface, while the alpha- and delta-zeins are found in the central region. To investigate the role each of these proteins plays in protein body assembly, we constructed a set of transgenic tobacco plants expressing a gene encoding one of the different types of zein proteins. We have begun to analyze developing seeds from individual transformants, as well as some of their F1 crosses. Concurrently, we have extensively characterized the phenotypes of two different storage protein mutants, *floury2* and *opaque*15, to determine the nature of these genetic lesions and how they alter endosperm texture.

Our studies with transgenic tobacco plants have provided evidence for a specific interaction between alpha- and gamma-zeins during protein body formation. Using the same endosperm-specific promoter, we directed synthesis alpha- and gamma-zein in developing tobacco seeds. However, only gamma-zein was found to accumulate in the mature seed. The alpha-zein gene showed the same temporal pattern of expression, but the level of RNA and protein accumulation was much reduced in comparison with the gamma-zein. Strikingly, seeds from the F1 hybrid of these plants synthesized both types of proteins, with significant amounts of alpha-zein present in the mature seed. Thus, it appears that gamma-zein may interact with alpha-zein and provide a mechanism for its stable accumulation. Experiments to characterize the ultrastructure of the protein bodies formed in these seeds are in progress. We have completed a detailed biochemical and genetic analysis of the *floury2* mutant, which has provided evidence that this gene is responsible for the synthesis of an abnormal alpha-zein protein. We have cloned a gene specifically linked with *floury2* that encodes a 22-kD alpha-zein protein. Sequence analysis reveals an abnormal alpha-helical repeated peptide in this protein that may result in defective protein folding. This could explain the irregular morphology of *fl2* protein bodies, as well as the enhanced synthesis of the 70-kD chaperonin, which is associated with this mutation. We have also characterized a novel mutant that we have designated *opaque15*. This mutation, which maps on the long arm of chromosome 7, reduces synthesis of the 27-kD gamma-zein protein by approximately 70%. This results in a seed with a starchy endosperm that will not germinate. The genetic lesion affects only the gamma-zein proteins; it has no effect on the synthesis and accumulation of alpha-, beta- or delta-zeins. Although we have not completed an ultrastructural analysis of the protein bodies in this mutant, light microscopy revealed a significant reduction in protein body numbers in the youngest endosperm tissue, which is just beneath the aleurone layer.

University of Arizona
Tucson, AZ 85721

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

K.S. Schumaker, Department of Plant Sciences

$146,000 (FY 93 funds/2 years)

During development, plants convert physical and chemical signals (e.g., light, gravity, and phytohormones) into specific growth responses. The pathway from these environmental or physiological cues to a new developmental program involves biochemical and molecular changes in the cells. In several species of moss, addition of the phytohormone cytokinin to cells of the proper physiological stage causes a rapid, transient increase in cytoplasmic calcium which stimulates a cascade of cellular events leading to the formation of buds. Our research is examining the biochemical and molecular properties of a calcium channel that is involved in early events in moss vegetative bud formation.

Using moss protoplasts, we have established the presence of and have biochemically characterized this calcium channel. The channel has similar transport characteristics (voltage-dependence, antagonist and agonist sensitivity) as voltage-dependent calcium

channels in animal cells. In addition, we have shown that one of the cytokinin effects in the induction of bud formation is calcium channel regulation. We are using cell cultures that define the specific stages of moss development to understand when the channel is active and can respond to cytokinin. We are using radio-, azido-labeled antagonist binding to isolated plasma membranes to identify and purify the channel polypeptide. Using methods that rely on conserved sequences determined from molecular genetic analysis of homologous genes in other organisms, we have isolated a portion of the gene encoding the moss channel.

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

University of Arizona
Tucson, AZ 85721

11. Phytoalexin Detoxification Genes and Gene Products: Implications for the Evolution of Host Specific Traits for Pathogenicity

H.D. VanEtten, Department of Plant Pathology *$86,646*

Production of phytoalexins by plants is believed to function as a mechanism of disease resistance. Many fungal pea pathogens have the ability to detoxify the pea phytoalexin pisatin via demethylation. This detoxification may be a means to circumvent a phytoalexin-based resistance mechanism. The detoxifying enzyme, pisatin demethylase, has been studied most thoroughly in *Nectria haematococca*. We have completed an examination of the induction of pisatin demethylating activity in whole cells and the biochemical properties of pisatin demethylase in microsomal preparations from the pea pathogens *Fusarium oxysporum* f. sp. *pisi, Mycosphaerella pinodes*, and *Ascochyta pisi* and compared these properties to those of the enzyme produced by *N. haematococca*. All of the enzymes are cytochrome P450s, based on cofactor requirements, and their inhibition by carbon monoxide, cytochrome P450 inhibitors and antibodies to NADPH cytochrome P450 reductase. In addition, all of the enzymes were selectively induced by pisatin, had a low Km on pisatin and a high degree of specificity towards pisatin as a substrate suggesting the presence in each pathogen of a specific cytochrome P450 for detoxifying pisatin. However, since the pisatin demethylases differed in their pattern of sensitivity to P450 inhibitors and displayed other minor biochemical differences, these fungi may have independently evolved specific cytochrome P450s to detoxify the phytoalexin produced by a common host.

Boston College
Chestnut Hill, MA 02167

12. Osmoregulation in Methanogens
M.F. Roberts, Department of Chemistry *$170,481 (2 years)*

This project is aimed at an understanding of how methanogens deal with osmotic stress and to use these insights for increasing the salt tolerance of other cells. Studies are focused on three different areas: (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 upon alteration of external NaCl (changes in intra- and extracellular concentrations of amino-containing compounds are also monitored by HPLC), (2) ^{39}K and ^{23}Na relaxation studies of those 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 (a key enzyme for the formation of N^{Σ}-acetyl-ß-lysine), and cDPGase (the enzyme that hydrolyzes cyclic-2,3-diphosphoglycerate, the major intracellular solute, to 2,3-DPG) activation by K^{+}. 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 whose activity is modulated by intracellular K^{+}.

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

13. Differential Regulation of Plastid mRNA Stability
D.B. Stern *$168,000 (FY 93 funds/2 years)*

Gene expression in the chloroplast is controlled by a variety of mechanisms, an important one of which is differential mRNA stability. RNA lifetimes in higher plant chloroplasts differ as much as 100-fold, and these relative stabilities often change during plant development. To address the mechanisms by which mRNA stability is regulated, we have focused on the 3' untranslated region, which in chloroplasts generally contains an inverted repeat sequence that can form into a stem/loop structure. We have shown that this stem/loop increases mRNA stability, directs 3' end formation and serves as a binding site for several protein factors. Both petD and rbcL mRNAs contain an eight nucleotide sequence element just downstream of the stem/loop, which we have termed Box II. Certain mutations within Box II prevent the formation of an RNA-protein complex *in vitro* and also destabilize the RNAs, suggesting that protein binding enhances RNA stability. A protein of 41 kd appears to play a major role in stabilizing these RNAs by binding to the stem/loop; the 41 kd protein has been purified and further biochemical and molecular studies are underway. To explore the function of Box II

in vivo, tobacco chloroplast transformation is being used. 3' UTRs containing wild-type or mutant Box II sequences of tobacco chloroplast petD have been cloned downstream of the *uidA* (GUS) reporter gene, and these chimeric genes have been introduced into tobacco chloroplasts by particle bombardment. We are currently carrying out measurements of *uidA* mRNA stability and accumulation, as well as the production of the GUS protein *in vivo*.

Brookhaven National Laboratory
Upton, NY 11973

14. Plant Molecular Genetics
B. Burr and F.A. Burr, Biology Department *$355,000*

Two recombinant inbred families have been used to construct a high density map of the maize genome. The current map has over 1,400 markers. These populations are used by the maize genetics community for rapid mapping of cloned genes. We are now working on the next generation of molecular markers based on simple sequence repeats (SSRs). These loci are highly polymorphic, easy to assay with the polymerase chain reaction, and obviate the use of the laborious Southern blotting techniques. The SSRs will greatly speed up mapping in new populations and will facilitate the use of molecular markers in plant breeding.

We used the recombinant inbred populations to study the nature of genes that have quantitative rather than qualitative effect. We have used telomere length, anthocyanin pigmentation, and carotenoid biosynthesis as model quantitative traits. Preliminary lessons are that regulatory genes or structural genes that control rate limiting steps are the most likely to have quantitative effects. Current emphasis is on isolation and characterization of genes affecting anthocyanin and carotenoid biosynthesis.

Brookhaven National Laboratory
Upton, NY 11973

15. Molecular Bases of Photoadaptation in Unicellular, Eucaryotic Algae
P.G. Falkowski and J. LaRoche, Department of Applied Science *$72,000*

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 within 24 h, solely in response to changes in growth irradiance. Such large changes optimize light harvesting at low irradiance levels while reducing photodamage at higher irradiance levels. This photoadaptation response is not spectrally dependent and does not appear to be mediated by phytochrome. The goal of this research effort is to elucidate the molecular signal transduction pathways that lead to the irradiance-induced changes in the abundance and composition of photosynthetic proteins. Inhibitor studies with the unicellular chlorophyte alga, *Dunaliella tertiolecta*, have revealed that the redox state of the plastoquinone pool specifically has a marked effect on the expression of the chlorophyll a/b binding protein and its message level. The redox state

of this photosynthetic electron transport element is also known to induce protein phosphorylation. Preliminary sequence data 5' to the reading frame of the cab genes, reveals high homology with light regulated elements in higher plants that are responsive to phosphorylated intermediates. We hypothesize that the irradiance response is keyed to photosynthetic electron transport through a phosphorylation cascade which reversibly represses cab gene expression. The details of the cascade are being investigated with both transient expression systems and pharmacological studies.

Brookhaven National Laboratory
Upton, NY 11973

16. Regulation of Energy Conversion in Photosynthesis
G. Hind, Biology Department *$365,000*

This project seeks to characterize specific proteins that are associated with the surface of the thylakoid and function in regulating photosynthetic efficiency.

The distribution of excitation 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). LHC-II phosphorylation is associated with a 64-kDa protein, tp64, that contains presumptive copper redox centers and becomes reversibly phosphorylated more rapidly than LHC-II. Although lacking protein kinase sequence motifs, this protein may confer redox sensitivity on a catalytic partner, which we are purifying from spinach thylakoids. The polyphenoloxidase activity of thylakoids derives from a protein distinct from, but related to, tp64. The structural interrelationship of these metalloproteins is under investigation by sequencing from the cDNA.

The corresponding dephosphorylation of LHC-II is catalyzed by a constitutively active, fluoride-sensitive phosphatase with pH 8.0 optimum. This enzyme was isolated from pea thylakoids as a catalytically active monomer having 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.

The dissipation of excess excitation energy in photosystem II is promoted by enzymic conversion of violaxanthin to zeaxanthin. The reverse process is catalyzed by an epoxidase for which we have discovered novel classes of inhibitor. With insight and assistance from these reagents, we are attempting to isolate and characterize zeaxanthin epoxidase.

Brookhaven National Laboratory
Upton, NY 11973

17. Characterization of Fatty Acid Desaturases and Related Lipid Modification Enzymes
J. Shanklin, Biology Department *$255,000*

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*.

In a new area of investigation we are attempting to define the active site components of the membrane bound desaturases and related hydroxylases using a site directed mutagenesis approach. These experiments will involve expression, purification and spectroscopic characterization of representative members of this class of enzyme.

Brown University
Providence, RI 02912

18. 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. It has become apparent that the five-carbon pathway from glutamate is more widely distributed than was previously thought, and it may be the usual route of ALA formation in phototrophic organisms in addition to being the universal mode in oxygenic species. The macromolecular components of the five-carbon ALA-forming system have been fractionated into three enzymes and a required tRNA component. Cell-free preparations have been obtained in our laboratory from several phototrophic prokaryotes, including oxygenic cyanobacteria and strict anaerobes, that catalyze ALA formation from glutamate by reactions similar to those occurring in plants and algae. We propose to continue the characterization of the enzymes and RNA reaction components derived from phototrophic prokaryotes and from the unicellular alga, *Chlamydomonas reinhardtii*, to compare them to their counterparts

in plants, and to study the regulation of their activity in response to light and nutritional status. The potential of these organisms for molecular genetic studies will be exploited by cloning and sequencing the genes encoding enzymes that catalyze steps of tetrapyrrole biosynthesis. The cloned genes will be used to generate probes to study 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 homologous genes in other algae and higher plants.

California Institute of Technology
Pasadena, CA 91125

19. Genetics in Methylotrophic Bacteria
M.E. Lidstrom, Environmental Engineering Science

$194,000 (FY 93 funds/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 *pqq* genes (genes required for synthesis of the methanol dehydrogenase prosthetic group, pyrroloquinoline quinone) in the facultative serine pathway methanol-utilizer, *Methylobacterium extorquens* AM1. The transcriptional start site has been mapped, and sequences upstream that are similar to those found upstream of other methylotrophy genes have been identified. The transcripts for the first three *pqq* genes have been mapped, and suggest that transcript processing occurs. Gene fusions have been generated to *pqqD*, and their analysis has shown that some of the transcriptional regulators that are required for transcription of *mox* genes are also required for transcription of *pqq* genes, and others are not required. In addition, a comparison of *pqq* production and transcription has indicated that regulation of *pqq* production by methanol is mediated at post-transcriptional levels. We are now focusing on the regulatory network for methylotrophy in this strain, and have sequenced three regulatory genes, *mxbMDN*. *mxbM* and *mxbD* have similarity to members of a two-component regulatory system, while the sequence of *mxbN* did not reveal similarity to any proteins in the data base.

California Institute of Technology
Pasadena, CA 91125

20. Molecular and Genetic Analysis of LEAFY, a Gene Controlling Floral Indand Flower Development in Arabidopsis thaliana
E. Meyerowitz, Division of Biology *$249,290 (2 years)*

LEAFY is a gene whose activity is required for normal floral development. Mutants without LEAFY activity show partial or complete conversion of flowers to inflorescence shoots. When the conversion is partial, the "flowers" that form lack three characteristics of normal *Arabidopsis* flowers. They have no petals or stamens; their organs are arranged in the spiral pattern normal for shoots, and not the whorled pattern characteristic of flowers; and they are subtended by a bract, which is normally the case for shoots, but not flowers. LEAFY thus

sits at the top of three different sets of developmental processes. We have cloned the gene, and have shown that it is the earliest gene known whose high-level expression indicates the future appearance of *Arabidopsis* flowers. In the past year we have completed genetic and molecular studies that show that LEAFY acts to transcriptionally induce the "B" function floral organ identity genes (which are normally responsible for specification of petals and stamens), and acts redundantly with another gene, APETALA1, in inducing the "C" function gene AGAMOUS, which is required for stamen and carpel formation, and for floral determinacy. We have also made antibodies to the LEAFY protein, and found that it is a nuclear protein. Experiments now in progress are examining the factors that induce LEAFY activity, and therefore act in floral induction.

University of California
Berkeley, CA 94720

21. The Molecular Genetics of Ligule Induction
M. Freeling, Department of Plant Biology *$192,060 (2 years)*

To study the "language" of developmental gene regulation, we are investigating the gene pathway that induces the ligule on maize leaves. The ligule and auricle are positioned between the sheath and the blade of the grass leaf. These structures display an incredible diversity of morphology among monocots. In maize several mutants exist which are deficient in the induction and differentiation of these complex structures. Mutants in two genes remove the ligule. Mutants in one gene generate extra ligule. Other mutants alter parts of the ligule/auricle. So far, no mutants act early enough to remove the preligular region in the very young leaf, but one mutant broadens this region. By analyzing these ligule mutants we have been able to establish a preliminary cascade of gene product function. This cascade represents our conceptual framework of how the ligule and auricle develop. Some of the genes are cloned, others are tagged by transposons. We propose to verify and complete the order and interaction of the members of the cascade as well as to identify yet unknown gene/products which play an important role during ligule/auricle development. In addition, we will approach important problems of ligule homology by analyzing the expression of the above mentioned genes within the entire maize plant, as well as in its fellow family member, rice.

University of California
Berkeley, CA 94720

22. Regulation of Tomato Fruit Growth by MVA and GTP-Binding Proteins
W. Gruissem, Department of Plant Biology *$105,730*

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 approaches this problem from different directions, using tomato fruit growth as a model system. One aspect focuses on the temporal and spatial expression of genes for hydroxymethyl glutaryl CoA reductase (HMGR) and the subcellular location of the enzymes. HMGR is encoded by four genes in tomato that are differentially expressed, indicating stringent control over the cell-autonomous synthesis of mevalonic acid in plants. 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. A second aspect focuses on prenyltransferases which utilize farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP) for the modification and biological activation of regulatory proteins. Activities for FPP transferase and GGPP type I and II transferases have been characterized in tomato, and the genes for these enzymes are being cloned. A third aspect focuses on the targets for prenylation enzymes. Proteins that are substrates for FPP transferase and GGPP type II transferase have been identified, cloned, and are being used to characterize their function and prenylation. Together, the experiments will establish the basis to investigate the cross-talk between MVA and sterol synthesis and signal transduction pathways that control division and growth of cells in sink tissues.

University of California
Berkeley, CA 94720

23. Determinants of Environmental Stress Tolerance by Bacteria on Leaves
S.E. Lindow, Department of Plant Pathology *$79,540*

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. Four transposon mutants showing different patterns of reduced growth and survival during physical stresses on leaves have been biologically characterized; the distinctive behaviors of these mutants suggest that several loci are involved in stress tolerance. One locus encodes proteins of 39 and 22.5 kD whose functions may include methionine biosynthesis and detoxification of environmental constituents; the functions of these proteins are still under investigation. At least two of the loci defined in the remaining mutants likely are regulatory since these mutants are altered in tolerance of water stresses, in extracellular polysaccharide production, and in other traits besides epiphytic fitness. The molecular characterization of these two loci is underway.

University of California
Berkeley, CA 94720

24. Phytochrome from Green Plants: Assay, Purification and Characterization

P.H. Quail, Department of Plant Biology *$97,000*

The discovery that the plant regulatory photoreceptor, phytochrome, is encoded by a family of five divergent genes (*PHYA, B, C, D,* and *E*) has focused attention on the twin questions of whether individual family members have discrete photosensory roles in mediating responses to the ambient light environment, and whether each has a similar or different biochemical mechanism of action. We have obtained evidence using photomorphogenic mutants and transgenic plants overexpressing different family members that phytochromes A and B specialize in perception of continuous red (Rc) and continuous far red (FRc) light respectively. As a result the data lead to the conclusion that phytochrome A is responsible for the "far red high irradiance response (FR-HIR)" of etiolated seedlings and phytochrome B is responsible for the "shade avoidance" response of green plants. To map regions of the phytochrome B molecule involved in regulatory activity, we have introduced a series of deletion derivatives of the molecule into *Arabidopsis*. Initial analysis of the phenotype of these transgenic lines has provided preliminary indications of regions within the polypeptide that are necessary for activity.

University of California
Berkeley, CA 94720

25. Molecular Cloning and Characterization of the Arabidopsis thaliana RPS2 Disease Resistance Locus

B.J. Staskawicz, Department of Plant Biology *$211,460 (2 years)*

The RPS2 disease resistance locus specifically confers resistance to strains of *Pseudomonas syringae* that contain the corresponding avirulence gene, avrRpt2. Fine structure RFLP mapping has allowed us to localize this gene to a single YAC clone that contains DNA from chromosome 4. A cosmid contig was constructed in an Agrobacterium binary vector that spans this particular YAC. Employing a flower infiltration transformation protocol we have now identified a single cosmid clone that when transformed into a disease susceptible mutant (rps2-201C) restores the resistance phenotype. Furthermore, we have demonstrated that this cosmid contains at least four cDNA clones that are expressed in wild type resistant plants. We have introduced genomic DNA into disease susceptible plants that corresponds to one of these cDNAs and have demonstrated complementation. We are now in the process of characterizing this cDNA clone to determine its nucleotide sequence. The molecular cloning of the RPS2 gene will now hopefully allow us to determine the molecular basis of plant disease resistance.

University of California
Berkeley, CA 94720

26. Analysis of Genes Essential for Floral Development in Arabidopsis
P. Zambryski, Department of Plant Biology *$90,210*

We are studying several T-DNA tagged mutant lines of *Arabidopsis* that affect different aspects of floral development. The *tousled* mutation produces flowers where there is a random loss of floral organs, and the development of the existing organs is impaired. Molecular cloning of the *TOUSLED* gene shows that it encodes a novel type of protein kinase. Future studies are aimed at 1) detailed analyses of the expression patterns of *TSL* mRNA and protein by *in situ* detection techniques using thin sections of mutant and wild type plant tissues, 2) revealing possible interactions with other genes important for floral development by performing genetic crosses to other mutant lines, and 3) demonstrating the kinase activity of the TSL protein *in vitro* and determining its functional specificity. The *ettin* mutation dramatically affects gynoecium development. All three major domains of the gynoecium, stigma, style, and ovary, are affected. There are major misspecifications of cell types in apical and basal regions, as well as in the lateral and medial planes. A detailed description of wild-type *Arabidopsis* gynoecium development is underway as a basis for better understanding the complex *ett* phenotype. The *petalless* mutation causes a more straightforward phenotype, where mutant flowers are simply devoid of petals. Because of its simple phenotype, and since *ptl* plants are fertile, the *ptl* locus has been predominantly studied by a genetic approach. Preliminary results suggest that *PTL* may act in the same pathways as several genes required for early flower development.

University of California
Davis, CA 95616

27. Cellulose Binding Proteins of Clostridium cellulovorans Cellulase
R.H. Doi, Section of Molecular and Cellular Biology *$105,730*

The overall goal of this project is to determine the subunit composition of the *Clostridium cellulovorans* cellulase and to elucidate the structure and function of the subunits of this multi-subunit enzyme. There are three major subunits in the enzyme which contains more than 10 different subunits. The gene for the largest major subunit, a non-enzymatic scaffolding protein, CbpA, has been sequenced. The amino acid sequence has revealed the presence of several domains including a cellulose binding domain (CBD), a hydrophilic sequence (HLD) repeated four times, and a hydrophobic sequence (HBD) repeated nine times. We have shown that the CBD peptide is capable of binding to crystalline forms of cellulose with a K_d = 1 mM. By Western blot and enzyme immunoassay techniques, we have shown that the HBD sequences are involved in binding the endoglucanase/xylanase subunits of the enzyme. We are currently determining the minimum size of the CBD and the amino acid residues involved in binding of crystalline cellulose. Analyses are being carried out with the HBD

sequence to determine the amino acid sequence involved in binding of HBD to endoglucanases. We will also determine the amino acid sequences in endoglucanases that bind to the HBD.

University of California
Davis, CA 95616

28. Genetic Characterization of Lophopyrum elongatum Salt Tolerance and Associated Ion Regulation as Expressed in Bread Wheat

J. Dvorak, D.W. Rains, and E. Epstein, Departments of Agronomy and Range Science and Department of Land, Air, and Water Resources *$94,090*

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, which is often saline, reduces dependence on this limited resource. The aim of this project is to elucidate physiological mechanisms which enable the highly salt tolerant *Lophopyrum elongatum* to grow in saline environments. Critical genes for the tolerance of salt stress are being identified by investigating gene expression associated with complete or recombined chromosomes of *L. elongatum* with wheat chromosomes in the wheat genetic background. Individual chromosome arms controlling tolerance of suddenly and gradually imposed salt stress in *L. elongatum* and wheat have been identified. It was shown that when wheat and the amphiploid from a cross between wheat and *L. elongatum* are exposed to these salt stress regimes they preferentially exclude Na^+ and accumulate K^+ and amino acid glicinebetaine in the young leaves and that these characteristics are enhanced in the amphiploid. No differences in the accumulation of these elements and compounds between wheat and the amphiploid were found in the old leaves. A major gene, *Kna1*, was identified in wheat chromosome 4D which controls Na^+ exclusion and K^+ accumulation and was shown that genetic manipulation of this single gene enhances field salt tolerance. The gene was mapped relative to molecular markers in chromosome 4D. This will facilitate deployment of this gene in wheat breeding.

University of California
Davis, CA 95616

29. Plant Physiological Aspects of Silicon

E. Epstein, T.W-M. Fan and M.W.K. Silk, Department of Land, Air and Water Resources, and R.M. Higashi, Bodega Marine Laboratory *$105,984*

Silicon is the second most abundant element in soils, the mineral substrate of most of the world's plant life. The soil solution contains silicon, mainly as silicic acid, H_4SiO_4, at appreciable concentrations, on the order of 0.1-0.6 mM. These concentrations are comparable to those of such major nutrient elements as potassium, calcium, sulfur, and others, and well in excess of those of phosphate. As H_4SiO_4 silicon is readily absorbed by plants, and soil-grown plants contain it in amounts comparable to those of the major nutrients.

Its quantitative prominence in plants notwithstanding, silicon is not considered generally "essential," and hence it is not included in the formulation of any of the common nutrient solutions of the plant physiologist. Ample evidence indicates that the plant physiologist's solution-cultured experimental plants, with their abnormally low, contamination-derived silicon content are to an extent experimental artifacts. The artifactual features referred to include disturbances in inorganic nutrition, low resistance to abiotic and biotic stresses, weakened cell wall structure, and failure to maximize leaf exposure to light, all of these items amounting to reduced energetic efficiency of growth and development. A systematic comparison will be made between silicon-poor and silicon-replete plants and their responses studied in terms of mineral nutrition, growth and development, cell wall composition and rheology, and energetics. The plants are rice, *Oriza sativa*, wheat, *Triticum aestivun*, and tomato, *Lycopersicon esculentum*, chosen for their known differential absorption of and responses to silicon.

University of California
Davis, CA 95616

30. Regulation of Embryonic Development in Higher Plants
J.J. Harada, Section of Plant Biology *$182,069 (2 years)*

Higher plants have evolved unique developmental strategies that enable embryos to enter a period of developmental arrest and to emerge from this period of quiescence as vigorously growing seedlings. This mode of development is a necessary requirement for seed formation that has provided higher plants with significant selective advantages. To understand the regulatory pathways that control this transition from embryogenesis to germination, we are studying a single gene recessive mutation of *Arabidopsis thaliana*, designated hairy, that confers a highly unusual embryonic phenotype. The mutant's most striking characteristic is that cotyledons have been converted to leaves as judged by the presence of trichomes on mutant cotyledons. However, the defect appears to involve more than a homeotic conversion of cotyledons to leaves. The shoot apex is activated precociously and mRNAs normally characteristic of both embryonic and postgerminative development accumulate in mutant embryos. These and other results suggest that HAIRY is a central regulatory gene that is involved in maintaining embryonic development, suppressing postgerminative development, and possibly specifying cotyledon identity. Because the mutant was derived from an *Arabidopsis* population mutagenized insertionally with T-DNA and preliminary data indicate that the gene is tagged, the HAIRY gene can be isolated and its role in embryonic development determined. The proposed studies will provide significant basic information about the cellular mechanisms employed by higher plants to adapt to a period of developmental arrest. We may also derive information that will aid in the design of strategies to manipulate embryonic and postgerminative development for applied purposes.

University of California
Davis, CA 95616

31. Cellular and Molecular Characterization of Vascular Plasmodesmata
W.J. Lucas, Section of Plant Biology *$111,550*

Plasmodesmata are special cytoplasmic bridges that interconnect plant cells. It has recently been shown that plasmodesmata have the capacity to selectively traffic macromolecules from cell to cell. We are presently investigating the mechanism(s) by which macromolecules are trafficked within higher plants, with an emphasis on the plasmodesmata that interconnect the phloem sieve elements (SE) and companion cells (CC). Recent findings have demonstrated the presence of a significant range of proteins present in the phloem sap, some of which are likely to fulfill a role in macromolecular transport through the SE-CC plasmodesmata. Rapid and efficient methods have been developed to recover these proteins from the phloem. Ongoing experiments are aimed at the identification and characterization, at the molecular level, of the phloem proteins that are involved in plasmodesmal transport. Our approach involves purification of phloem proteins, direct functional assay of these proteins for intracellular transport function by microinjection methodologies, and further characterization of proteins having the proven ability to traffic through SE-CC plasmodesmata. Expression of plant genes encoding for putative plasmodesmal transport proteins will be analyzed, with emphasis on determining the site of mRNA synthesis and distribution of the protein within the cells of the vasculature. These studies will provide the framework for future studies on the evolution and function of macromolecular trafficking in higher plants. Information of this nature will likely potentiate a wide range of studies on the involvement of plasmodesmata in the control of plant development and physiology.

University of California
Davis, CA 95616

32. Protein Translocation and Assembly in Chloroplasts
S.M. Theg, Section of Plant Biology *$182,000 (FY 93 funds, 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 at least 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 are designed to elucidate the mechanisms that lead to complex formation and enzymatic activity. We are investigating a newly discovered unusual trans-membrane pathway leading to subunit assembly, and are using site-directed mutagenesis techniques to determine the amino acids and forces responsible for binding the different subunits and inorganic cofactors. Mutants that we generate which are still competent for assembly will be further examined for potential effects on the enzyme's activity. In addition, we are investigating the mechanism through which

energy is utilized during translocation of these subunits across the chloroplast membranes in an effort to estimate the true energy costs to eukaryotic cells of their considerable protein trafficking activities.

University of California
Davis, CA 95616-8515

33. Vacuole Biogenesis in Differentiating Plant Cells
T.A. Wilkins, Department of Agronomy & Range Science *$111,550*

The vacuolar compartment undergoes dramatic biochemical and morphological changes during the transition between initiation and rapid cell expansion in developing cotton seed trichomes. Three morphologically distinct vacuole types coexist in trichoblasts located in the protoderm of the ovule. One class of vacuoles is characteristically filled with an unidentified electron-dense material. With the onset of trichome initiation and elongation, dissipation of this electron-dense material occurs coincident with the coalescing of vacuoles. The formation of a prominent central vacuole in rapidly expanding trichomes occurs via fusion of preexisting vacuoles as well as by incorporation of newly synthesized vacuoles. Ultrastructural studies clearly indicate that a majority of the newly synthesized vacuoles are derived by terminal expansion of smooth endoplasmic reticulum. Organelle fractionation and immunolocalization of peripheral vacuolar H^+-ATPase (V-ATPase) subunits revealed that the V-ATPase is localized to the plasma membrane and tonoplast of only newly synthesized vacuoles. The temporal regulation of V-ATPase enzymatic activity reflects the accumulation of V-ATPase transcripts during rapid elongation, indicating that transcription is required to maintain elevated levels of H^+-ATPase activity. Developmental triggers that result in the down-regulation of V-ATPase gene expression and ATPase activity presage the termination of cell expansion.

University of California
Irvine, CA 92717

34. Membrane Bioenergetics of Salt Tolerant Organisms
J.K. Lanyi, Department of Physiology and Biophysics
$306,000 (FY 93 funds/2 years)

The energy costs of salt tolerance in the extremely halophilic bacteria originate from the generation of electrochemical ion gradients across the cytoplasmic membrane that drive sodium extrusion and chloride accumulation. We study the molecular mechanisms of the proton and chloride transporting bacterial rhodopsins and the proton transporting ATPase in these organisms. Studies of the first two proteins, bacteriorhodopsin and halorhodopsin, respectively, explore the thermodynamics of the transport, the chromophore and protein changes that determine the changing connectivity of the active site to the two membrane surfaces during the transport cycle, and on the mechanisms of ion conduction to and from the Schiff base. Site-specific mutations, time-resolved optical multichannel spectroscopy, and through collaborations, retinal analogues, infrared spectroscopy and x-ray diffraction are

methods developed for this work. Studies of the third protein, the membrane ATPase of *H. saccharovorum*, concentrate on the origins of the complex hydrolytic mechanism, the roles played by heavy metals and extraneous ligands that appear to activate or inhibit activity, and shared features with eubacterial and eukaryotic ATPases.

University of California
La Jolla, CA 92093-0116

35. Structure, Biosynthesis and Role of Complex Protein-Bound Glycans
M.J. Chrispeels, Department of Biology *$182,360 (2 years)*

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 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.

This mutant *Arabidopsis* strain has been transformed with the cDNA that encodes human GlcNAc transferase I. The human Golgi enzyme is targeted to the plant Golgi apparatus and functions there to restore the synthesis of complex glycans. We will make fusions between portions of the human cDNA and bovine lysozyme which is used as a reporter protein to uncover the default destination for membrane proteins in plant cells, as well as the information needed to target a membrane protein to the plant Golgi apparatus.

We are also studying the α-amylase inhibitor αAI/arcelin/phytohemagglutinin family of glycoprotein present in bean seeds. The cDNA for αAI has now been expressed in pea (*Pisum sativum*) with a strong seed-specific promoter. These seeds contain up to 1.2% αAI and are resistant to three types of seed-eating weevils: *Calosobruchus maculatus*, *C. chinensis* and *Bruchus pisorum*. We will transform other legumes and we are hunting for genes that could protect seeds against other seed weevils, especially *Zabrotes subfasciatus* and *Acanthoscelides obtectus*. To this end we are cloning αAI genes from wild accessions of the common bean and from other bean species.

University of California
La Jolla, CA 92093-0116

36. Molecular Structure, Function and Physiology of K^+ Uptake Channels in Plants
J.I. Schroeder, Department of Biology *$175,764 (2 years)*

Potassium uptake by higher plant cells is of central importance to plant growth, stomatal regulation, nutrition, tropisms, osmoregulation, enzyme homeostasis and plant membrane

potential control. Elucidation of the molecular mechanisms by which higher plant cells selectivity take up K^+, while diminishing accumulation of toxic metals such as Na^+, Cs^+ or Al^{3+} is of increasing environmental and agricultural concern. Patch clamp studies on guard cells and root hair cells show that K^+ uptake channels provide a molecular pathway for membrane potential control and for low-affinity proton pump-driven K^+ uptake. These "inward-rectifying" K^+ channels interact with metals which are known to show physiological or toxic effects on cation uptake, growth and membrane potentials. *Arabidopsis* ion channel cDNAs have recently been cloned, and we have functionally characterized one of these as an inward-rectifying K^+ channel gene.

The long-term goal of the proposed research is to attain a quantitative description of structural domains of K^+ uptake channel clones responsible for K^+ channel metal block and ion selectivity. Furthermore we will determine whether, and to which extent, K^+ uptake channel structure can influence physiological and toxic effects of metals on membrane potential and cation transport in specific plant cells. These studies will contribute to a structural dissection of ion selectivity and metal block mechanisms for a cation transporter in higher plants. In addition, these studies may contribute important information for the design of strategies for future engineering of K^+ transport traits in specific cell types and for biological transport of toxic cations.

University of California
Los Angeles, CA 90024

37. Molecular Biology and Genetics of the Acetate-Utilizing Methanogenic Bacteria

R.P. Gunsalus, Department of Microbiology and Molecular Genetics $106,698

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 *cdhAB*, have been cloned from *Methanosarcina thermophila* TM-1 and are being DNA sequenced. Their regulation is being examined at the level of mRNA synthesis and decay in response to substrate availability. We will further document events of post-transcriptional processing of *cdhAB* mRNA upon shift to non-acetate growth conditions. In companion studies, we are examining the molecular basis of osmoregulation by this class of methanogens. Depending on the external salinity or levels of dissolved solutes, the cells synthesize osmoprotectants including N-acetyl-ß-lysine, ß-glutamine and α-glutamate. Alternatively, when presented with glycine betaine in the culture medium, they can actively accumulate this compound in an energy dependent fashion. The uptake of this osmolyte is being characterized to document the mechanisms for osmoregulation operative in the Methanosarcina relative to those employed by the eubacteria and eukaryotes. The aim of these studies is to better understand the potentials for adaptation and growth by these acetate-utilizing methanogens in response to environmental change.

University of California
Los Angeles, CA 90024-1606

38. 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 (FY 93 funds/2 years)

The long term objective of our 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 explore methods for isolating the GA_{20}-3ß-hydroxylase that catalyzes the metabolism of GA_{20} to GA_1. Our approach is to clone genes controlling steps in the GA-biosynthetic pathway using transposon tagging. We have three transposon-induced dwarf mutants of maize, one from Ac stocks, the other two from Robertson's mutator (*Mu*) stocks. The Ac mutant is allelic to *d1* (blocked in the metabolism of GA_{20} to GA_1). The two Mu mutants are blocked early in the GA biosynthetic pathway (bioassay data). We use the appropriate transposon to probe Southern blots of genomic DNA isolated from tall and mutant seedlings. We hope to identify a hybridizing band which is unique to the dwarf phenotype. For the *d*-1* Ac mutant, preliminary studies revealed a 7.3 kb SstI genomic DNA fragment which hybridized to our Ac probe and was unique to the dwarf phenotype, however, as we expanded the number of plants examined, we identified dwarfs which lacked the band. We are currently using the same strategy to examine the two Mu mutants, *d*E* and *d*9444*.

University of California
Los Angeles, CA 90024-1606

39. Sensory Transduction of the CO_2 Response of Guard Cells

E. Zeiger, Department of Biology *$188,180 (2 years)*

The stomatal response to CO_2 plays a key role in the regulation of stomatal movements and in the coupling between stomata and the photosynthesizing mesophyll. This research project aims at the characterization of guard cell mechanisms sensing CO_2, and of cellular pathways transducing CO_2 responses. Two metabolic reactions are putative biochemical sensors for the CO_2 signal: carboxylation of phosphoenolpyruvate by PEP carboxylase, and the carboxylation of ribulose bisphosphate by Rubisco. Malate and sucrose, respectively, are the osmotically active end products of these two pathways. Current measurements of osmotica in growth chamber-grown guard cells show that aperture increase during the first half of the opening phase is correlated with increases in potassium and malate. Potassium and malate content then decline, and sucrose becomes the dominant osmoticum in the second part of opening phase. In green house-grown guard cells, potassium increases are accompanied by an increase in chloride during the first phase, without any detectable increases in malate. Potassium and chloride decline in the second half of the opening phase and sucrose increases. Growth chamber and green-house grown guard cells also have contrasting CO_2 sensitivities. These findings indicate that CO_2 sensitivity and counterions for potassium can vary under different environmental conditions, that potassium efflux is not obligatorily coupled

to stomatal closure, and that sucrose plays an important role as a guard cell osmoticum. Current work is characterizing the modulation of these osmoregulatory pathways by CO_2, and the relationship between guard cell sensitivity to CO_2 and osmoregulation.

University of California
Santa Cruz, CA 95064

40. Tonoplast Transport and Salt Tolerance in Plants
L. Taiz, Biology Department *$91,180*

V-ATPases regulate vacuolar solute uptake and play an important role in protecting plant cells from potentially toxic ions such as NaCl. We have been investigating lemon vacuoles as a model system in which to study V-ATPase regulation. Lemon trees have two kinds of vacuoles with greatly differing pHs. In vegetative tissues the vacuolar pH is about 6, whereas in the fruit it can be as low as 2.2. What features of fruit vacuoles allow them to attain such a low pH? One possibility is that the vacuolar proton-ATPase, which is normally kinetically regulated in vegetative tissues, operates close to thermodynamic equilibrium in the fruit. This might be due to the absence of, or insensitivity to, inhibitory regulators in the fruit. Alternatively, a second pump, such as the proton-PPase may augment the activity of the V-ATPase. Finally, the fruit vacuole may be more impermeable to protons. To address these questions we compared the proton-pumping activities and proton permeabilities of vacuolar membranes isolated from epicotyls and fruits. A procedure was worked out for isolating highly active fruit microsomal membranes using 1.5 M MOPS buffer. Although the specific proton pumping activities of the V-ATPases from fruit and epicotyl were similar in presence of 1 mM MgATP, the Km for ATP in the fruit was about half that in the epicotyl. The fruit V-ATPase was much less inhibited by nitrate and ADP than the epicotyl V-ATPase. For example, 50 mM nitrate inhibited the epicotyl V-ATPase by 98%, whereas the fruit V-ATPase was only inhibited by 22% at this concentration. Similarly, 0.2 mM ADP inhibited the epicotyl V-ATPase by 87%, while the fruit V-ATPase was inhibited by 16%. Since the proton-PPase activity of fruit vacuoles was negligible, the low pH of the fruit is due primarily to the activity of the proton-ATPase. We also found that epicotyl vacuolar membranes are significantly more permeable to protons than fruit vacuolar membranes. When the pump is inhibited by the addition of EDTA after having generated a pH gradient, the gradient of epicotyl tonoplast vesicles is completely collapsed after 15 minutes, whereas fruit vesicles hold their proton gradients for up to an hour. We tentatively conclude that the lower pH of fruit vacuoles results from the combined effects of a proton-ATPase with a reduced sensitivity to inhibitors and a membrane that is very impermeable to protons. However, the endogenous inhibitors have not yet been identified.

Carnegie Institution of Washington
Stanford, CA 94305

41. Production of Lipophilic Materials from Plants
C.R. Somerville *$372,039*

Our research is focused on understanding the biochemical and genetic mechanisms that regulate the synthesis and accumulation of lipophilic compounds in plants. One aspect of our work is focused on the isolation of genes for transcriptional factors which regulate storage lipid accumulation. The availability of genes for such factors may permit the production of genetically modified plants that accumulate increased amounts of storage oil. A second aspect of our work is focused on elucidating the mechanisms by which plants secrete certain fatty acids and derivatives such as wax. Knowledge of these mechanisms may permit economic production of new kinds of technically useful fatty acids by large-scale fermentation of genetically modified microorganisms. A third aspect of our work is focused on the identification of genes that encode enzymes required for the synthesis of oxygenated fatty acids such as 12-hydroxy oleic acid which are used as feedstocks for the production of a wide variety of chemicals. The availability of these genes is expected to permit the agricultural production of novel high value fatty acids with applications in the synthesis of polymers and other technical products. The development of new plant varieties that accumulate novel oils is expected to provide expanded markets for the agricultural sector while permitting greater reliance on renewable sources of technical materials.

Carnegie Institution of Washington
Stanford, CA 94305

42. Molecular Basis of Disease Resistance
S.C. Somerville, Department of Plant Biology *$154,188 (2 years)*

We have chosen to work with the powdery mildew disease of *Arabidopsis thaliana*, which is caused by the obligate fungal pathogen *Erysiphe cruciferarum*. Our primary objective is to recover genes encoding disease resistance. The characterization of resistance genes will be an important step in determining key biochemical components of disease resistance. Because chromosome walking is a successful technique for recovering genes from *Arabidopsis*, we will employ this method to isolate clones for powdery mildew resistance genes.

The barley powdery mildew disease is well characterized and provides a frame of reference for studies on resistance in *Arabidopsis*. In parallel the project to clone powdery mildew resistance genes, we will compare resistance mechanisms observed in *Arabidopsis* to those found in barley. This analysis will permit us to identify those *Arabidopsis* powdery mildew resistance genes that display characteristics similar to barley powdery mildew resistance loci. The barley *Ml-a* locus is of particular interest because it is highly polymorphic. An understanding of the genetic mechanism that underlies this high degree of polymorphism will

permit the rational design and utilization of genetically engineered disease resistance genes in crop species.

University of Chicago
Chicago, IL 60637

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

R. Haselkorn, Department of Molecular Genetics and Cell Biology
$184,008 (FY 93 funds/2 years)

The fine-structure physical map of the *Rhodobacter capsulatus* chromosome, obtained by mapping 200 overlapping cosmids with EcoRV, was published last year. Since then the map of SB1003 was extended to two more enzymes and completed by resolving 40 regions of ambiguity using Southern blots to chromosomal digests. Three other strains, selected on the basis of their long range maps determined by pulse field gel electrophoresis, had cosmid libraries prepared and several large contigs were assembled. Then their fine-structure maps were compared to that of the type strain SB1003. The comparisons reveal numerous genome-changing events: long range inversions (more than 500 kb), transpositions, deletions and inversions within inversions. Many of the breakpoints have been identified within cosmids and are being subcloned for sequencing.

The cosmid encyclopedia that defines the chromosome of SB1003 has been cut with EcoRV and fractionated by electrophoresis to provide blots of gene-sized pieces that represent the physical array of the chromosome. These blots have then been hybridized first with total labeled DNA, to provide a normalization standard, and then with differentially labeled RNA corresponding to some condition, such as heat shock, nitrogen starvation, anaerobiosis, etc. The cleanest result to date is for heat shock, which showed 12 chromosomal regions (several genes in each location) responding. For nitrogen stress the results are less clear, requiring further work to define better the physiological conditions needed for RNA preparation.

The University of Chicago
Chicago, IL 60637

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

D.G. Lynn, Department of Chemistry *$187,624 (2 years)*

Striga asiatica is a small chlorophyll-containing angiosperm that has developed the remarkable ability to establish a vascular connection with another plant. The development of the attachment organ, the haustorium, is one of the most rapid organogenesis events known. Since this parasitic ability is wide-spread 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

45. The Magnesium Chelation Step in Chlorophyll Biosynthesis
J.D. Weinstein, Department of Biological Sciences *$86,330*

In plants, photosynthetic membrane biogenesis requires the coordinate synthesis of prosthetic groups, proteins, and various lipids. Chlorophyll and heme, two of the major prosthetic groups, share a common biosynthetic pathway that diverges at the point of metal insertion into protoporphyrin IX (Proto). Iron insertion results in the formation of hemes, while magnesium insertion is the first step unique to chlorophyll formation. This project has had two main thrusts this year. Firstly, we have now completely solubilized Mg-chelatase with low ionic strength buffers and purification in progress; this will further the identification of the enzyme(s) responsible for magnesium insertion. Secondly, we have examined the regulation of precursor flux through the branch point enzymes. Our results suggest: 1) Precursor formation and utilization is tightly coupled to metal ion insertion. 2) The capacity for Mg-Proto formation is ten to thirty times greater than that for heme synthesis. 3) Both ATP and ferrous ion have stimulatory effects on formation and utilization of porphyrin precursors which cannot be explained simply on the basis of their requirement for the two chelation reactions. We have also addressed the question of the localization of Mg-chelatase within the chloroplast. In intact plastids, the enzyme is more sensitive to membrane-permeant than to non-permeant inhibitors. When ATP is supplied by photosynthesis, the activity is not affected by an external

ATP trap. Taken together these results indicate that the enzyme is interior to the inner envelope, rather than exterior to this membrane as had been previously reported.

Cold Spring Harbor Laboratory
Cold Spring Harbor, NY 11724

46. The Suppression of Mutations Generated by Mu Transposons in Maize
R.A. Martienssen and V. Sundaresan

$75,000 (FY 93 funds, 17 1/2 months)

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, resulting in wild-type plants. Recessive alleles of this class at four different loci (*hcf106, a1, Kn1, vp1*) have *Mu1* or *Mu8* elements in the promoter region of the 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. The genetics of suppression have been followed using three markers that have *Mu* dependent phenotypes (*hcf106, Kn1-mum2* and *Les28*). We have determined that suppressible mutations are coordinately regulated in maize, and that somatic variegation in multiply marked stocks can be used as a powerful means for mosaic analysis. The variegation correlates with non-CG methylation of the regulatory *MuDR* transposon. We have developed an RT-PCR technique to rapidly identify *Mu* containing transcripts from lines carrying tagged suppressible mutations, and a genomic PCR method to obtain derivative alleles of *Mu* induced mutations. Two new insertions and a deletion have been recovered at the *hcf106* locus using this method. The intact *MuDR* element and a number of responder constructs have been introduced into tobacco to study the *cis* and *trans* acting components required for transposition and the regulation of *Mu*-suppressible gene expression.

University of Connecticut
Storrs, CT 06269

47. Sugar Transport and Metabolism in Thermotoga
A.H. Romano and K.M. Noll, Department of Molecular and Cell Biology

$168,000 (FY 93 funds/2 years)

Members of the genus *Thermotoga* are extremely thermophilic anaerobes which are among the most slowly-evolving members of the phylogenetic domain *Bacteria*. They ferment sugars to acetate, CO_2, H_2, and lactate as primary products. Enzymes of the Embden-Myerhof-Parnas glycolytic pathway have been identified by others in some *Thermotoga* species strains. However, nothing is known of the mechanisms by which sugars are transported into their cells. The goal of this project is to elucidate mechanisms of sugar transport and

carbohydrate catabolism in *Thermotoga* species. Our early results indicate that *T. neapolitana* can regulate its sugar metabolism *via* catabolite repression. Thus, ß-galactosidase activity is induced by growth with lactose or galactose 10 to 20-fold over that found with other sugars. It is induced 3 to 6-fold by cellobiose. The presence of most sugars (except sucrose) prevents or reduces its induction by lactose. Cellobiose induces ß-glucosidase activity up to ten-fold. Cyclic-AMP is not involved in these regulatory mechanisms, but transport processes may be involved. Non-metabolizable analogs of glucose were found to affect growth of the cells. 2-deoxyglucose was effective in the micromolar range. Mutants resistant to 2-deoxyglucose are being sought to investigate the mechanisms of glucose transport and the regulation of carbohydrate catabolism. The transport of radiolabelled glucose and non-metabolizable analogs of glucose are being measured to optimize the experimental system. The characterization of sugar transport and metabolism in this phylogenetically important bacterium, taken together with studies in other laboratories of sugar metabolism in anaerobic hyperthermophiles of the domain *Archaea* 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

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

J. Gibson, Section of Biochemistry, Molecular and Cell Biology *$82,450*

Compounds containing aromatic rings are produced in very large quantities both biologically and by human activities. Lignin, which is largely composed of aromatic rings, is the second most abundant biopolymer, and therefore an important renewable resource. Many of the man-made compounds are potentially toxic, so that their environmental fate is a matter of serious concern. Because of the slow rates at which many of these substances can be degraded, many are carried into anoxic environments, where they may persist for very long periods, and also contaminate ground water supplies. Degradation of many simple compounds containing aromatic rings occurs in the complete absence of oxygen, by reactions in which the aromatic nucleus is reductively saturated before being opened and led into common biochemical pathways. However, in contrast to the aerobic pathways, many details of the anaerobic pathway have not been resolved, and little is known about the enzymes and electron carriers involved. In this project, the versatile photoheterotrophic bacterium *Rhodopseudomonas palustris* is being used for biochemical analysis of the reductive phase of metabolism. Formation of coenzyme A thioesters, an essential preliminary to reduction, is carried out by a number of ligases with fairly narrow, but overlapping substrate specificities, and three have been purified and characterized; in collaboration with Dr. C.S. Harwood at the University of Iowa, two have been cloned and sequenced. A thioesterase, which appears active only with aromatic or alicyclic CoA thioesters, has also been purified and characterized. This enzyme, like the CoA ligases, is proving a useful tool in analyzing the CoA thioesters formed within intact cells and in extracts. Close collaboration with Dr. C.S. Harwood at the University of Iowa is continuing, so that both molecular genetic and biochemical approaches

to defining the enzymatic and regulatory characteristics of the reductive steps can be integrated. These studies will provide insights that should facilitate overriding the rate-limiting steps in the degradation of aromatic acids.

Cornell University
Ithaca, NY 14853-2703

49. Molecular and Physiological Analysis of Cytoplasmic Male Sterility
M.R. Hanson, Division of Biological Sciences *$93,120*

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

50. 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 *$75,000*

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 F1, and in similar aberrations and skewed segregation ratios in the interspecific F2 and later generations. Since the interspecific barriers impede the transfer of desirable traits from unadapted and wild germplasm to crop species, it is imperative to understand the nature and genetic control of interspecific barriers. We are studying the interspecific barriers using a unique combination of plant materials, including interspecific layer chimeras and isocytoplasmic lines, and a combination of cytological, genetic and molecular techniques. We have determined: 1) the functional basis of unilateral incongruity (UI) including the time and developmental step(s) interrupted and the tissue and genomes involved and 2) the functional basis of hybrid breakdown (HB), the chromosomal regions

associated with HB, and the effects of cytoplasm on non-fecundity. Our continuing goals are to determine 1) the nature of set seed failure in interspecific backcrosses and whether this is related to UI, HB, or a further barrier, Se elimination; 2) the genetic control of UI, and the genomic regions associated with UI; 3) the relationship between SI and UI; and 4) the mechanism of Se elimination, and its relationship with SI. 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

51. Characterization of a Putative Receptor Protein Kinase and its Role in Self-Incompatibility

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

$195,940 (2 years)

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* haplotypes in pollen and stigma, and results in the inhibition of self-pollen at the surface of stigmatic papillar cells. Our work has focused on the characterization of the S-locus Receptor Protein Kinase (SRK), one of two proteins encoded by the *S* locus. SRK is a receptor-like kinase that exhibits serine/threonine kinase activity. The extracellular domain of SRK shares a high degree of sequence similarity with the S-Locus Glycoprotein (SLG), a secreted glycoprotein that is also encoded by the *S* locus. We have shown that both genes are coordinately regulated and expressed exclusively in specialized cells of the pistil and anther. In addition, we have demonstrated a requirement for both SRK and SLG in self-recognition by analyzing self-compatible mutants of *Brassica*. Future work will aim at testing the hypothesis that contact with self-pollen activates the SRK protein which, by phosphorylating intracellular substrates, couples the initial molecular recognition events at the papillar cell-pollen interface to the signal transduction chain that leads to pollen rejection. The study of the *SLG/SRK* gene system has relevance to deciphering the mechanism of receptor-mediated signalling in plants, not only in pollen-stigma interactions, but more generally in other instances of cell-cell communication such as plant-pathogen interactions.

Cornell University
Ithaca, NY 14853

52. Mechanism of Inhibition of Viral Replication in Plants

P. Palukaitis, Department of Plant Pathology *$84,390*

Viruses are a major class of plant pathogens that are responsible for crop losses and reductions in plant biomass. Most natural resistant genes in plants function by blocking virus movement. Studies with cucumber mosaic virus (CMV), which has a host range of over 1000 species, have shown that restrictions on virus movement can involve either the 3a movement protein (MP) or the 3b coat protein (CP). Some forms of resistance involve host-specific

interactions between gene products involved in replication and those involved in movement. Since purified MP can bind and traffic RNA in the absence of replicase, the replicase complex may have an inhibitory effect on virus movement. Such an inhibitory effect is observed in transgenic plants expressing a defective 2a gene, which encodes one of the replication-gene products. While the CP is not required for RNA trafficking, it is required for normal cell-to-cell movement, and in *Cucurbita pepo*, which is resistant to systemic infection by the M-strain of CMV, poor cell-to-cell movement is also observed and maps to the CP gene. In maize, infection by M-CMV is also restricted due to the CP, while infection by LS-CMV is restricted due to the MP. Thus, the CP and MP both have roles in cell-to-cell movement, and host-specific effects on viral movement can be mapped to each of the four gene-products of CMV.

Cornell University
Ithaca, NY 14853

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

P.L. Steponkus, Department of Soil, Crop and Atmospheric Sciences $100,880

A current objective of this project is to determine the mechanistic significance of genotypic differences in the lipid composition of the plasma membrane of rye and oak, which represent the extremes in freezing tolerance of winter cereals. Although the freeze-induced lesions in rye and oak are similar, they occur at significantly higher temperatures in oat. The increased sensitivity of oak to freeze-induced dehydration is associated with greater amounts of cerebrosides and acylated sterylglucosides in the plasma membrane - both before and after cold acclimation. These differences in lipid composition are of mechanistic significance because of their influence on (a) the hydration characteristics of the plasma membrane, (b) the propensity for dehydration-induced lipid-lipid demixing, and (c) the intrinsic curvature of the lipid monolayers. We also initiated studies to determine the effects of COR proteins, which are synthesized by *Arabidopsis thaliana* during cold acclimation, on the cryostability of membranes. Both COR6.6 and COR15am minimize the incidence of freeze-induced fusion of phospholipid liposomes. Although freeze-induced fusion largely occurs over the temperature range at which the liquid crystalline-to-gel phase transition occurs, the proteins do not affect the lyotropically-induced increase in the T_m of either DPPC or DOPC; nor do they alter the propensity for the lamellar-to-hexagonal II phase transition in mixtures of DOPC:DOPE. Therefore the decreased incidence of fusion may be an indirect effect resulting from amelioration of the freezing stresses rather than direct protein-lipid interactions. Although the proteins do not have thermal hysteresis activity, they influence the size and morphology of ice crystals, and solutions of COR proteins contain more unfrozen water between -15 and 0°C than solutions of bovine serum albumin.

Cornell University
Ithaca, NY 14853

54. Genetic Control of Nitrate Assimilation in Klebsiella pneumoniae

V.J. Stewart, Section of Microbiology, Division of Biological Sciences

$181,972 (2 years)

Klebsiella pneumoniae is an enteric bacterium closely related to *Escherichia coli*. Nitrate and nitrite are important nitrogen sources not only for *K. pneumoniae*, but also for many other microorganisms and 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. To date we have concentrated on identifying and characterizing the structural genes involved in nitrate assimilation. DNA sequence analysis coupled with genetic and physiological studies have identified the *nasFEDCBA* operon. The *nasFED* genes apparently encode a nitrate uptake system that shares structural homology with the nitrate uptake system of the cyanobacterium, *Synechococcus* sp. Growth tests with mutants indicate that the *nasFED* genes are required for nitrate but not for nitrite assimilation, suggesting that a separate uptake system for nitrite also exists. The *nasC* and *nasA* genes encode electron-transfer and catalytic subunits, respectively, of assimilatory nitrate reductase. The *nasB* gene encodes assimilatory nitrite reductase. The *nasF* operon is immediately adjacent to the *nar* gene cluster that encodes regulatory and structural components involved in anaerobic nitrate respiration. This gene organization is remarkable because the *nas* and *nar* systems are involved in physiologically-unrelated processes that are under distinct genetic control. Finally, we have isolated insertion mutations that specifically abolish nitrate and nitrite induction of *nasF* operon expression. These insertions define a positive regulatory gene, *nasR*, that is located immediately upstream of the *nasF* operon.

Cornell University
Ithaca, NY 14853

55. Studies of the Genetic Regulation of the Thermomonospora fusca Cellulase Complex

D.B. Wilson, Section of Biochemistry, Molecular and Cell Biology

$172,000 (FY 93 funds/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 are currently refining our 3-dimensional x-ray structure of the E2 catalytic domain to 1.0Å which may allow us to determine the state of ionization of the active site carboxyl residues. We have mutated Asp_{265} to Phe and to Val and find that both mutants lack activity. This supports the idea that Asp_{265} is an essential catalytic residue. We are trying to crystallize several E2cd mutants to see how they change its structure. A number of additional site directed mutants are being prepared based on the 3-d structure to obtain more information about the mechanism of cellulose

hydrolysis by E2. Studies using model substrates suggest that the synergism in the hydrolysis of crystalline cellulose by two different exocellulases such as CBHI and CBHII is due to the fact that one (CBHI) attacks the reducing ends of the cellulose chains and the other (CBHII) attacks the non reducing ends. By this method E3 appears to attack the non reducing ends, E4 the reducing ends of cellulose chains and a third exocellulase E6 also appears to attack cellulose reducing ends. The *T. fusca* protein which binds to the universal actinomycete cellulase regulatory sequence, TGGGACGCGTCCCA has been purified by heparin Sephadex chromatography, gel filtration and affinity chromatography is being characterized. In addition we have shown that this regulatory protein still binds when the terminal A in the sequence is changed to a G. We have also shown that *Streptomyces lividans* cell contain a protein that binds to the regulatory sequence when they are grown on cellobiose.

Cornell University
Ithaca, NY 14853-8101

56. Conversion of Acetic Acid to Methane by Thermophiles
S.H. Zinder, Department of Microbiology *$99,910*

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 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; 2) the demonstration that, in contrast to *Methanosarcina*, *Methanothrix* strain CALS-1 did not accumulate H_2 during methanogenesis from acetate, but instead accumulated CO; 3) the purification and characterization of a thermostable acetyl-CoA synthetase from cell-free extracts of *Methanothrix* strain CALS-1; 4) the demonstration of methanogenesis from acetate and ATP in cell-free extracts of strain CALS-1. Methanogenesis occurred at a high rate (100-300 nmol min^{-1} [mg protein]$^{-1}$), 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; 6) the demonstration of a role for the cell membrane in methanogenesis from acetate in extracts of gently-broken cells. Current research is centered on biochemical factors which allow thermophilic *Methanothrix* to compete with *Methanosarcina*.

University of Delaware
Lewes, DE 19958

57. Metabolic Mechanisms of Plant Growth at Low Water Potentials
J.S. Boyer, College of Marine Studies *$93,120*

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, in stems, gradients in water potential decreased between the vascular tissue and the enlarging cells and growth was inhibited. A few hours later, the extensibility of the cell walls decreased and an extractable 28kD protein accumulated in the wall fraction. To determine whether the protein was present in the cell walls of the intact plants, roots were pressurized sufficiently to force exudate onto the surface of the stems. The exudate contained the protein, which had acid phosphatase activity. There was acid phosphatase activity in the cytoplasm as well. Because the acid phosphatase accumulated in the walls of the stem growing region of plants subjected to water deficiency around the roots, the stem growth was inhibited, experiments are underway to determine whether the phosphatase plays a role in the growth response.

Duke University
Durham, NC 27706

58. 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 *$111,550*

A major goal of this project is to combine molecular genetics, biochemistry and physiology to understand the relationship between photosynthetic performance *in vivo* and the structure of the multifunctional D1 reaction center protein of Photosystem II (PSII). Exposure of plants and algae to sunlight level irradiance often causes photoinhibitor damage to the D1 protein encoded by the chloroplast *psbA* gene, resulting in reduced photosynthetic efficiency. We are using the unicellular green alga *Chlamydomonas reinhardtii* as a model system for our studies on D1 because of its excellent genetics and ease of chloroplast transformation. Replacement of Ala_{251} with Val in the quinone/herbicide binding loop of D1 of cyanobacteria and *Chlamydomonas* had been reported to result in resistance to certain PSII herbicides and to increase sensitivity to photoinhibition. To study this question further, we generated site-directed mutants with 12 of the 20 possible amino acids at D1 position 251. These mutants are of three different phenotypic classes: similar to wildtype, impaired in photosynthetic O_2 evolution or non photosynthetic. Mutants of Ala_{251} to Ser, Gly, Cys or Pro have no effect on phototrophic growth and effect only low level resistance to some PSII herbicides. Mutations of Ala_{251} to Ile, Val and especially Leu result in a reduction in autotrophic growth rate,

increased resistance to several classes of PSII herbicides and reduced photosynthetic efficiency when grown at high irradiance (600μE/m^2/s). The Ala_{251} to Arg, Gln, Glu, His and Asp mutations drastically affect PSII function leading to a non photosynthetic phenotype. We are currently characterizing the Ile, Val and Leu mutants in more detail in order to determine if their photosynthetic defect results from a perturbation in electron transfer on the donor or acceptor sides of PSII. A combination of *in vitro* and *in vivo* biochemical assays are being used to establish whether the phenotype of the non photosynthetic mutants results from blockage of PSII electron transfer, interference with access of protons to the Q_B binding niche or extreme photosensitivity of the D1 protein such that functional PSII centers do not accumulate. We hope to gain a better understanding of the structure/function relationship of the quinone/herbicide binding region of the D1 protein by relating the specific phenotypes of the 12 mutants to changes in amino acid charge and side chain size/shape at D1 position 251. In addition we have isolated mutants which suppress the inability of wildtype *Chlamydomonas* to grow at sunlight level irradiance (188μE/m^2/s), as well as nuclear suppressors of the photosynthetically impaired, high light sensitive Ala_{251} to Ile and Leu mutants of D1. Thus we are now beginning to identify genes whose products are involved in mediating photosynthetic performance under photoinhibitory conditions. These results may guide future investigations in higher plants.

Duke University
Durham, NC 27708

59. Molecular Studies of Functional Aspects of Higher Plant Mitochondria
J.N. Siedow, Department of Botany *$77,600*

Mitochondria isolated from *cms*-T lines of maize are sensitive to toxins (T-toxins) derived from the fungus *Bipolaris maydis* (and related fungi). T-toxin sensitivity is associated with a mitochondrially-encoded, 13 kDa receptor protein, URF13, that interacts with T-toxins to produce pores in the inner mitochondrial membrane. The expression of URF13 in *Escherichia coli* confers T-toxin sensitivity on the bacterial cells and binding studies using radiolabeled T-toxins have established that T-toxin binds to URF13 in a specific and cooperative manner. A structural model has been developed whereby URF13 is postulated to be localized in the membrane as an oligomeric complex, with each URF13 monomer containing three membrane-spanning α-helices, two of which are amphipathic and are postulated to line the aqueous pore. Topological studies have confirmed the validity of the three helical model and chemical cross-linking has been used to establish the oligomeric nature of URF13. Chemical cross-linking in conjunction with site-directed mutagenesis has been used to characterize helix:helix interactions between adjacent URF13 monomers in the oligomeric complex and has led to a refinement of the model in which the URF13 tetramer contains a central four-helical core. Continued application of the combined cross-linking/directed mutagenesis approach will be used to further define both intra- and intermolecular helical interactions within the URF13 oligomeric structure. In addition, attempts to develop constructs for overexpressing URF13 in *E. coli* are underway, with the goal of using purified URF13 in liposome reconstitution studies as a well-defined system for studying URF13:T-toxin interactions.

Florida State University
Tallahassee, FL 32306

60. Plant, Cell and Molecular Mechanisms of Abscisic Acid Regulation of Stomatal Apertures
W.H. Outlaw, Jr., Department of Biological Sciences *$77,600*

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 (GC) pair, a result of accumulation of solutes from the apoplast and synthesis of low MW substances. The specialized metabolism that GCs have evolved to fulfill their crucial role in the plant's physiology extends to more subtle aspects. One focus of our DOE-sponsored research is on the synthesis of stress proteins. One collaborative project identified dual pathways for the elicitation in monocots of *Lea* gene regulation, one ABA-dependent, and a second, ABA-independent. In another project, we find that the accumulation of individual GC proteins is responsive to Ca^{2+} and ABA. Second, we are investigating GC phospho*enol*pyruvate carboxylase (PEPC). One recently finished project determined PEP-Mg^{2+}constants over a range of ionic strengths and temperature. In addition, we find that the kinetic properties of GC PEPC are altered during stomatal opening, a topic we will pursue. Third, we are studying the redistribution of ABA in *Vicia faba*. Our system is a plant with a split-root system (solution culture). We are investigating whether water stress imposed on one part of the root system in a water-sufficient plant will cause leaf stomatal closure by redistribution of root-source ABA.

University of Florida
Gainesville, FL 32611

61. Ethanologenic Enzymes of Zymomonas mobilis
L.O. Ingram, Department of Microbiology and Cell Science *$101,175*

Zymomonas mobilis has been recognized as a potentially important industrial microorganism for the commercial production of fuel ethanol. This organism is capable of extremely rapid glucose metabolism (1 μmoles glucose min^{-1} mg cell $protein^{-1}$). To accomplish this rapid glycolysis, the 13 glycolytic and fermentative enzymes comprise half of cytoplasmic protein. Each activity is expressed from a single gene creating an interesting regulatory dilemma to balance production rates for the hundreds of other gene products needed for biosynthesis during prototrophic growth. Our investigations have identified mRNA stability (10 to 20 min half-life), canonical ribosomal binding regions, low protein turnover, and preferential codon usage as key features which ensure high level expression of glycolytic enzymes. This unusual message stability appears to represent the primary distinction between these fermentative and biosynthetic genes. These enzymes have been mapped on two-dimensional gels and monospecific (based on Western analysis of *Z. mobilis* proteins) antibodies produced. The potential arrangement of glycolytic enzymes into complexes has been investigated using these antibodies. Immunogold ultrastructural studies have co-localized selected activities. Additional studies using monospecific antibodies have been shown co-precipitate complex-like

elements using immunobeads. Using the antibody to glucose 6-phosphate dehydrogenase, most other glycolytic enzymes were also precipitated in contrast to enolase antibodies which appeared quite selective. FPLC gel filtration studies failed to resolve complexed but retained most activities. Each glycolytic gene has been selectively overexpressed in *Z. mobilis* to evaluate flux control. While most were saturating, significant contributions to flux control have been identified for three enzymes thus far: glucose permease (*glf*), glucokinase (*glk*), and glucose 6-phosphate dehydrogenase (*zwf*). It is interesting to note that these represent the first three steps in glucose catabolism. A 15% increase in *Z. mobilis* glycolytic flux (already among the highest known) was achieved by overexpression glucose 6-phosphate dehydrogenase by 45% both in the early stages of fermentation (less than 2 g ethanol/liter) and in the middle stage of fermentation (56 g ethanol/liter) making this enzyme an excellent candidate for improvements in industrial strains. During the course of these studies, *glf* and *glk* were used to construct a recombinant glucose uptake system which can fully replace the PEP-dependent phosphotransferase system in *E. coli*. This new system may be useful for the production of chemicals for biotechnology applications. Other applications resulting from this work include the development of a portable ethanol-production operon (*pdc* + *adhB*) which has now been used to redirect carbon metabolism from organic acids into ethanol in a wide variety of bacteria.

University of Florida
Gainesville, FL 32611

62. Gene-enzyme Relationships of Aromatic Amino Acid Biosynthesis in Higher Plants

R.A. Jensen, Department of Microbiology and Cell Science

$217,000 (FY 93 funds/2 years)

The biosynthesis of aromatic amino acids in higher plants is of great significance, not only because of the role of aromatic amino acids in protein synthesis, but because they are precursors of a vast array of compounds of biotechnological and medical interest. Enzyme levels in the pathway have been shown to be regulated in response to physiological stage of growth and to stress induced by mechanical wounding. We have elucidated the enzyme steps of biosynthesis and allosteric patterns of control for the pathway located in the chloroplast compartment. A separate enzyme network in the cytosol has been partially identified and comprehensive enzymological characterizations will be carried out. The 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.

University of Georgia
Athens, GA 30602-7229

63. The Metabolism of Hydrogen by Extremely Thermophilic Bacteria
M.W.W. Adams, Department of Biochemistry and Center for Metalloenzyme Studies *$87,300*

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 vents. We grow some of the 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 aldehyde ferredoxin oxidoreductase. 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. From the most thermophilic bacterium currently known, *Thermotoga maritima* (T_{max}90°C), we have purified an iron-containing hydrogenase, ferredoxin and a POR that lacks copper. Crystals of ferredoxin, hydrogenase, AOR and POR suitable for structural determinations have been obtained, and the genes for ferredoxin, AOR and POR have been cloned. Currently we are determining the complete amino acid sequences of these proteins and are attempting heterologous expression of their genes in mesophilic bacteria. In addition, 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 hyperthermophilic hydrogenases in industrial energy conversions.

University of Georgia
Athens, GA 30602-4712

64. CarbBank - A Structural and Bibliographic Database for Complex Carbohydrates

P. Albersheim and S. Doubet, Complex Carbohydrate Research Center

*$75,000**

The IBM PC-compatible, computerized database (the Complex Carbohydrate Structure Database, CCSD) and database management system (CarbBank) were created by scientists at the Complex Carbohydrate Research Center (CCRC), with the collaboration of scientists in Europe, to meet the needs of persons interested in carbohydrate science. Building the database and making the software available to researchers worldwide were the initial goals of the project. CCSD release 8 was made available in early 1994 on CD-ROM and by Internet access from the National Center for Biotechnology Information (NCBI) and on CD-ROM from the Protein Information Resource (PIR). Based on estimates by NCBI and PIR for the CCSD8 release, software has been sent to over 600 users worldwide. CCSD8 contains over 27,500 records of complex carbohydrate sequences and corresponding bibliographic information. The next release of the CCSD, number 9, is scheduled for June 1994. CCSD9 will contain over 29,700 records. Over 90% of the records in CCSD9 have been validated by comparison with original citation sources. The CarbBank staff at the CCRC and partners in Europe continue to build the CCSD by finding and abstracting currently published articles that contain complex carbohydrate structures. The CCSD is expected to grow by over 3,500 records in the next year.

The CarbBank staff at the CCRC is also engaged in software development. The DOS version of CarbBank is being enhanced, as necessary, to accommodate the continually growing CCSD. At the same time, CarbBank is being completely re-written so that it will run on a wider range of computer operating systems, including Microsoft Windows, Apple Macintosh, and UNIX. The version for Microsoft Windows is under development, with a December 1994 target release date. Development of versions for the other platforms will proceed as time and resources allow.

The CarbBank project has succeeded in building a database of complex carbohydrate structures and delivering it to users worldwide. The goals now are to continue to build the database and to widen the number of supported computer platforms.

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

University of Georgia
Athens, GA 30602-4712

65. The University of Georgia Complex Carbohydrate Research Center (CCRC)
P. Albersheim and A. Darvill, Complex Carbohydrate Research Center
$1,720,000 (FY 93 funds/2 years)

The University of Georgia Complex Carbohydrate Research Center (CCRC) has a multidisciplinary faculty and staff who serve as a national resource for basic research in complex carbohydrate science. The CCRC has nine tenured or tenure-track faculty and one adjunct faculty member; three members of the staff have non-tenure-track faculty appointments. This grant supports research, analytical services, and training in plant and microbial complex carbohydrates. Four of the CCRC's 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. Educational activities involve the training of undergraduate and graduate students, postdoctoral research associates, and visiting scientists. Eleven undergraduate and 29 graduate students are currently pursuing research projects or Ph.D. degrees in the CCRC; six undergraduate and 12 graduate students are working in plant or microbial carbohydrate science. Four week-long laboratory training courses are held annually for ~40 scientists from institutions and industries located throughout the United States. The faculty and staff of the CCRC are currently involved in more than 112 internal or external collaborative research projects of which 51 are part of the plant and microbial carbohydrate program. The plant and microbial carbohydrate program has provided service to 154 individuals by analyzing 564 samples in the time that the service program 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 198 corporations during the same time period.

University of Georgia
Athens, GA 30602-4712

66. The Structures and Functions of Oligosaccharins
P. Albersheim, Complex Carbohydrate Research Center
$434,664 (FY 93 funds/30 months)

Oligosaccharins are a class of signal molecules involved in regulation of plant growth, development, and defense against pests. The research supported is designed to elucidate how oligosaccharins (oligosaccharides with regulatory functions) function *in planta* by studying proteins that generate and process oligosaccharins as well as protein receptors for oligosaccharins. The research projects that we are investigating include determining: (i) how oligogalacturonides participate in the establishment of rhizobia-legume symbioses, (ii) the role of fungal endopolygalacturonases and plant polygalacturonase inhibitor proteins in pathogenesis, (iii) the role of fungal proteins that inhibit pathogenesis-related ß-1,3-glucanases of host plants, (iv) whether plant tissues contain endogenous "nodulation"

factors, (v) the effect of oligogalacturonides on the metabolism of auxin in tobacco stem explants, (vi) the mechanism by which *endo*glucanases and xyloglucan oligosaccharides control growth of pea stems, and (vii) the role of *endo*xylanases and arabinosidases in the pathogenesis of rice by *Magnaporthe grisea*, and (viii) the mechanism by which *endo*xylanases elicit defense responses in tobacco cells.

University of Georgia
Athens, GA 30602-4712

67. Structural Studies of Complex Carbohydrates of Plant Cell Walls
A. Darvill, Complex Carbohydrate Research Center

$1,016,660 (FY 93 funds/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 the primary cell walls of plants. The structural studies emphasize the detailed analyses of the 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, (ii) using monoclonal antibodies to locate specific polysaccharides in different cells and tissues, (iii) studying the three-dimensional conformations of cell wall polysaccharides to determine how poly- or oligosaccharide conformation affects the structural framework of the cell wall, and (iv) analyzing structures of cell wall polysaccharides extracted from *Arabidopsis* plants that have mutations affecting their wall structures. 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 and its constituents.

University of Georgia
Athens, GA 30602-7229

68. Mechanisms of Lignin Biosynthesis During Xylogenesis in Zinnia elegans
K-E.L. Eriksson and J.F.D. Dean, Department of Biochemistry *$96,030*

Lignin plays important roles in plant structure, function and defense. This project seeks to identify the enzymes regulating lignin deposition in secondary cell walls. We have found that oxygen-dependent phenoloxidase activity is tightly correlated with lignification of vascular tissues in *Zinnia* stems. This activity is also associated with lignin deposition in walls of individual *Zinnia* cells which have been induced to differentiate in suspension culture. High-salt extracts of *Zinnia* stems suggest that a single enzyme may be responsible for this phenoloxidase activity. The size, isoelectric point and substrate specificity of the *Zinnia* enzyme resemble those of laccases isolated from the lignifying xylem of several tree species. The *Zinnia* enzyme has not been purified enough to positively identify it as a laccase; however, laccase activity is dependent upon copper, and the *Zinnia* enzyme shows reduced activity in plants grown under copper-deficient conditions. Our results suggest that the oxygen-dependent phenoloxidase from *Zinnia* catalyzes polymerization of lignin precursors in vascular cell walls. Using Ce^{3+} deposition to localize and quantify H_2O_2 production in *Zinnia* stems, we have found that although a small amount of H_2O_2 is produced in the walls of vascular cells undergoing lignification, substantially more H_2O_2 is produced in the non-lignified walls of collenchyma cells. The observation that oxygen-dependent enzymes may actually play a greater role in lignification of vascular tissues than do H_2O_2-dependent enzymes has had a profound effect on our concept of lignin biosynthesis.

University of Georgia
Athens, GA 30602-7229

69. 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, Department of Biochemistry and Microbiology *$527,300 (FY 93 funds/17 1/2 months)*

Biomass generated by photosynthesis is degraded by consortia of anaerobic microorganisms to organic compounds, which can be used as industrial feedstock chemicals, and to gases or liquids to use as alternate fuels. Included in these consortia are microorganisms that grow and reproduce at extreme temperatures and pH. Enzymes from such microorganisms often have high stabilities to heat or extreme pH, and they have industrial uses. Our work involves the isolation and characterization of microorganisms from different types of consortia and studies of their interactions. A major part of our work deals with characterization of enzymes from the anaerobic microorganisms, and it includes studies of mechanism of action, expression and genetic manipulations. Among our objectives is to develop vectors to perform transformations of *Desulfovibrio* and *Clostridium* species. This is especially important for studies of metal-containing enzymes such as hydrogenases, formate dehydrogenases, and CO dehydrogenases. These enzymes are expressed in *E. coli* but not as active proteins

probably due to their complex structures of metal centers. To study their functions by site-directed mutagenesis we must develop methods to express them in heterologous or homologous cells. Biomass is acted upon by secreted hydrolytic enzymes such as cellulases, xylanases, esterases, and proteinases. We have isolated several such enzymes from alkaliphilic (newly discovered) and cellulolytic clostridia, and anaerobic fungi, and we are now studying their expressions both in heterologous and homologous cells. Of importance is the finding that esterases and xylanases are interacting to efficiently solubilize lignocellulolytic material. A major problem in anaerobic microbiology is the generation of energy during autotrophic growth. It is studied with the acetogens *Clostridium thermoaceticum* and *C. thermoautotrophicum*. These studies involve electron transport, ATP-generation, and H_2-cycling. Similar work is done with hydrogenosomes that are organelles of the anaerobic fungi. They evolve H_2 as a means of disposing electrons and purportedly generate ATP. Little is known about the function of hydrogenosomes.

University of Georgia
Athens, GA 30602

70. Why Do Plants Have Two Pathways of Polyamine Synthesis?
R. L. Malmberg, Botany Department *$199,820 (2 years)*

Polyamines are small positively charged compounds that have been hypothesized to be involved in a wide variety of plant physiological and developmental functions. Polyamine biosynthesis in higher plants differs from that in animals in several respects. One of the notable differences is the presence of two pathways to putrescine synthesis, from arginine via arginine decarboxylase, found in some kingdoms but not others, and from ornithine via ornithine decarboxylase, probably found in all organisms. We are exploring regulatory and functional differences between arginine and ornithine decarboxylases in plants. This includes: characterization of an unusual enzyme activation of arginine decarboxylase, defining both the activation site and the activating enzyme; an analysis of ornithine decarboxylase structure and regulation; the production of transgenic plants to determine the relative functions of ornithine and arginine decarboxylases; and a screen for new mutants in *Arabidopsis thaliana* that lack ornithine or arginine decarboxylase enzyme activities for a variety of possible reasons.

University of Georgia
Athens, GA 30602

71. Soybean Ribulose Bisphosphate Carboxylase Small Subunit: Mechanisms and Determinants of RNA Turnover in Higher Plants

R.B. Meagher, Department of Genetics *$93,120*

The determinants of RNA turnover were explored with two diverse systems: the first dealing with the mechanisms of *rbcS* mRNA (encoding the ribulose bisphosphate carboxylase small subunit)degradation in soybean, petunia and *Arabidopsis*; the second examining the roles of poly(A) binding proteins (PABP) in RNA metabolism. We have shown that *rbcS* mRNA is degraded into a discreet set of 5' and 3' proximal products and developed an *in vitro* system which would generate the same products from endogenous and exogenous *rbcS* mRNA (Tanzer and Meagher, 1994, MCB, in press). The kinetics suggest that cleavage site selection is random and not processive, thus, an endonuclease may be involved. This year we began mapping the precise 5' and 3' ends generated *in vivo* and *in vitro* in order to identify a consensus sequence or structure for these processing sites. A streamlined assay was developed for this ribonuclease in order that the enzyme can be purified.

The *Arabidopsis* PABPs are encoded by a large diverse gene family (Belostotsky and Meagher, 1993, PNAS, 90:6686) and are differentially expressed. The *PAB5* gene is specifically expressed in developing pollen and stipules. When expressed from a yeast promoter, *PAB5* rescues a yeast *PAB1* deletion, restoring viability and almost normal poly(A) tail processing. Using this complementation system we have created a series of temperature sensitive mutations in *PAB5*. Related future experiments focus on identifying *Arabidopsis* cDNAs which when expressed suppress these conditional mutations, presumably because their products interact with PABPs. We are also attempting to identify the functions of *PAB5* at the plant level by examining the phenotype of *Arabidopsis* lines deficient in *PAB5* expression using co-suppression and antisense suppression.

University of Georgia
Athens, GA 30602

72. Nitrogen Control of Chloroplast Development and Differentiation

G.W. Schmidt, Department of Botany *$89,240*

One of the major environmental factors that limits growth and development of plants and algae is the availability of nitrogen. Deficiency of this nutrient is diagnosed as chlorophyll deficiency reflecting specific effects on development of chloroplasts. Our work concerns the molecular, biochemical and physiological consequences of prolonged adaptation to nitrogen-limiting conditions as well as the cellular responses that ensue upon nitrogen provision. The analyses are performed with *Chlamydomonas reinhardtii* grown in N-limited continuous culture systems to enable precise regulation of formation of the photosynthetic apparatus as a function of the rate of nitrogen supply. While chloroplast gene expression is primarily posttranscriptional, many nuclear genes encoding pigment-binding proteins and

several enzymes of photosynthetic carbon metabolism are regulated at the transcriptional level. The loci are being characterized with respect to their nitrogen-responsive regulatory elements and temporal patterns of gene expression during nitrogen repletion. Another effort concerns identification of the components for chlororespiration, a thylakoid-localized oxidative electron transport activity which is especially prevalent in nitrogen-deficient cells. Complexes implicated in chlororespiratory electron flow have been purified and antibodies against the major subunits obtained as means of identifying their genes and mode of regulation by nitrogen availability. Ultimately, we hope to determine whether chlororespiration is an activity that enhances viability of cells whose photosynthetic capacities are compromised by long-term nitrogen-deficiency conditions.

University of Georgia
Athens, GA 30602

73. Post-transcriptional Regulation of the R/B Gene Family in Maize and Rice
S.R. Wessler, Departments of Botany and Genetics *$97,000*

The *R/B* genes encode a family of transcriptional activators that control the distribution of anthocyanin pigments in the maize plant. This sensitive, non-lethal phenotype has facilitated the isolation of over 50 naturally occurring *R* alleles, each displaying a distinctive pigmentation pattern. Previous studies indicated that the phenotypic differences conditioned by different *R/B* alleles reflect differences in their regulation rather than their products. A role for differences in transcriptional regulation of *R* and B alleles has been documented. In addition, we have demonstrated that an upstream open reading frame (uORF) composed of 38 codons and found in most *R* alleles characterized to date, represses *R* expression post-transcriptionally. Experiments indicate that uORF is a general repressor of translation reinitiation that can repress any downstream gene in maize or in the mammalian rabbit reticulocyte translation system. Thus far, we have documented three levels of translational repression, (i) leaky scanning, mediated by a suboptimal uORF AUG, (ii) ribosome stalling, due to the numerous rare codons of uORF and (iii) the uORF peptide, which prevents translation reinitiation, possibly by binding to components of the ribosome. The mechanism whereby the uORF peptide prevents ribosome reinitiation is under investigation.

We have also found that an active *R* homolog, isolated from a red variety of rice, encodes truncated *R* proteins from alternatively spliced transcripts. The fact that these truncated proteins are missing one of the *R* proteins' three nuclear localization sequences has led us to hypothesize that alternative splicing has evolved to repress *R* activity by restricting its nuclear localization. Experiments are underway to test this notion.

Although the *R* gene product is dispensable, we believe that these diverse post-transcriptional mechanisms of gene regulation have evolved to repress *R* expression because its overexpression may interfere with normal plant development.

University of Georgia
Athens, GA 30602

74. Biochemistry and Genetics of Autotrophy in Methanococcus
W.B. Whitman, Department of Microbiology *$162,000 (2 years)*

Even though methanogenic bacteria catalyze the terminal step in the anaerobic oxidation of organic matter, these bacteria have a very narrow substrate specificity. From this perspective, the carbon metabolism of the methanococci has been examined. These bacteria contain the complete Embden-Meyerhof pathway for the oxidation of endogenous glycogen to pyruvate even though sugars are not taken up. Although pyruvate oxidoreductase (POR) is present in sufficient quantity to oxidize the pyruvate formed from sugars, in resting cells this reaction only occurs in the absence of hydrogen gas, which is the physiological electron donor. This property would be expected from the normal role of the enzyme in pyruvate biosynthesis. Purification and characterization of the POR indicates that it is a complex oligomer composed of at least four different types of subunits. However, the kinetic properties of the enzyme closely resemble those of the catabolic PORs characterized in other archaebacteria and eubacteria. To facilitate development of a genetic system in methanogens, a polyethylene glycol transformation system was developed in *Methanococcus maripaludis*. Using an integration vector designed by Sandbeck and Leigh, transformation frequencies as high as 10^5 transformants per microgram of DNA were obtained. Using this transformation system, continuing work will develop additional vectors for cloning in methanococci.

University of Georgia
Athens, GA 30602

75. Hemicellulases from Anaerobic Thermophiles
J. Wiegel, Department of Microbiology and Center for Biological Resource Recovery *$166,000 (FY 93 funds/2 years)*

The long term goal of this research effort is to obtain an anaerobic thermophilic bacterium that efficiently converts various types of hemicellulose-containing biomass to ethanol over a broad pH range. The strategy is to modify the profile 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 *T. ethanolicus* JW200 utilizes xylans, its xylanase, acetyl(xylan) esterase and O-methyl glucuronidase activities are barely measurable and are regarded as the rate limiting steps in xylan utilization by this bacterium. To identify possible enzyme donors and to extend our presently limited knowledge of hemicellulases in anaerobic thermophiles, we characterize the hemicellulolytic enzymes from this and other anaerobic thermophiles. In addition to the xylosidase/arabinosidase from *T. ethanolicus*, an enzyme that exhibits different T-optima for the two activities, we have characterized two xylosidases, two acetyl(xylan) esterases, and an O-methyl glucuronidase from *Thermoanaerobacterium* strain JW/SL-YS485. We will continue with the characterization of xylanases from newly isolated slightly acidophilic,

neutrophilic and slightly alkalophilic thermophiles. We have cloned, subcloned and partially sequenced the 165 kD (2 x 85 kD) xylosidase/arabinosidase from *T. ethanolicus* and started to clone the esterases and xylosidases from *Thermoanaerobacterium* JW/SL-YS485. Further, we will start to develop a shuttle vector and continue to apply electroporation of autoplasts as a method for cloning into *T. ethanolicus*.

University of Georgia
Tifton, GA 31793

76. Development of Innovative Techniques That May be Used as Models to Improve Plant Performance
W.W. Hanna and G.W. Burton, Department of Agronomy *$48,500*

The objectives of this project are to: (1) establish the cytoplasmic diversity of germplasm in the weedy subspecies of the primary gene pool and demonstrate its value, (2) identify mechanism(s) for transfer of germplasm from the secondary gene pool to the cultivated species and evaluate the plant breeding potential of this germplasm, and (3) transfer gene(s) controlling apomixis from the tertiary gene pool to cultivated pearl millet for the purpose of producing true-breeding hybrids. Species within the genus *Pennisetum* are being used as test organisms. The approach uses plants of wild species with different genetic and cytoplasmic backgrounds, ploidy levels, and chromosome numbers crossed and backcrossed with different genotypes of diploid and tetraploid pearl millet. Resistance to a number of the major diseases are being 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. About 85% seed abortion in the apomictic plants has been found to be due to poor endosperm development. Genes in the secondary gene pool are being evaluated for their potential contribution for producing superior grain and forage hybrids. New cytoplasms are being identified and transferred to the cultivated species.

University of Hawaii
Honolulu, HI 96822

77. Violaxanthin De-Epoxidase: Biogenesis and Structure
H.Y. Yamamoto, Department of Plant Molecular Physiology *$92,700*

Violaxanthin de-epoxidase (VDE) is localized in the lumen of thylakoids and, in the presence of ascorbate and low pH, converts violaxanthin in the membrane to zeaxanthin. *In vivo*, zeaxanthin forms under conditions where light is in excess relative to the requirement for CO_2 fixation. VDE is of interest because zeaxanthin increases the rate of non-radiative (heat) dissipation energy, thereby protecting PSII against the potentially damaging effects of excess light. VDE has been purified to a single major component on 2-D SDS PAGE by lipid-affinity precipitation with monogalactosydiacylglycerol. N-terminal and internal amino-acid sequences of the protein have been determined. Nucleotide probes will be generated using primers

based on the VDE sequences by polymerase chain reaction. Polyclonal antibodies will be generated to the complete protein and to the peptide of the N-termines. cDNA expression libraries from *Lactuca sativa*, cv. Romaine will be probed with DNA or antibodies and positive clones will be sequenced by standard methods. The complete cDNA sequence is expected to yield insights about structure. Translation and transport studies will answer biogenesis questions. Cloning of VDE will 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

78. Heavy Metal-lux Sensor Fusions and Gene Regulation
S. Silver, Department of Microbiology and Immunology *$99,910*

We will study the bacterial oxidation of arsenite to arsenate and the reduction of arsenate to arsenite, as these processes are carried out by different microbial species from environmental sources. We will determine how broadly these enzymatic activities are found in nature. We will work on the molecular genetics of arsenate reductase (recently discovered in our laboratory) and determine the properties of these enzymes and their governing genes in both Gram positive and Gram negative bacteria. A mutagenic analysis will define key amino acid residues and the reaction mechanism. Purified enzyme protein will be studied *in vitro*, to determine its reductive coupling, physical properties and cellular location. A similar approach will be undertaken with arsenite oxidase. However, since arsenite oxidase was only recently purified, the first step will be to isolate and characterize its genetic determinant and study its gene(s) and their regulation. Whereas we believe that arsenate reductase occurs widely in many types of arsenate-resistant bacteria, it seems more likely that arsenite-oxidase is more limited, to Pseudomonads that show arsenite-resistance without arsenate resistance. This hypothesis will be tested. Once the gene for arsenite oxidase is in hand, molecular genetic analysis will be undertaken prior to further enzyme work. These studies will provide a base for understanding of conversions of arsenic oxyanions of differing toxicity and their interconversions in a natural microbial arsenic cycle that will affect the availability and toxicity of environmental arsenic to higher organisms including man.

University of Illinois
Urbana, IL 61801

79. Genetics of Solvent-Producing Clostridia
H.P. Blaschek, Department of Food Science *$102,820*

Systems for the genetic manipulation of the acetone-butanol-ethanol (ABE) fermentation microorganism, *Clostridium acetobutylicum* continue to be developed. A macrorestriction map of *C. acetobutylicum* ATCC824 chromosome was generated using a combination of the restriction enzymes MluI, SalI, and SmaI. The primary structure of the chromosomal map was generated using two-dimensional reciprocal digestion with SalI and MluI. Analysis of SmaI/SalI and SmaI/MluI two-dimensional PFGE gels allowed for alignment of internal DNA fragments. Chromosomal DNA embedded in agar blocks was digested with SalI, SmaI, or MluI, and was subsequently separated by TAFE using three different run conditions. This resulted in a 5.7 Mb macrorestriction map consisting of 92 restriction sites. The location of 17 previously cloned *C. acetobutylicum* genes was determined by Southern hybridization. Additional verification of heterologous expression of *C. cellulovorans engB* gene in *C. acetobutylicum* was demonstrated using Western Blot Analysis. Transcriptional analysis of the *C. cellulovorans* endoglucanase gene, *engB*, in both *E. coli* and *C. acetobutylicum* demonstrated multiple transcription initiation sites, none of which corresponded to the site determined in *C. cellulovorans*. We conclude that transcriptional control of the engB gene is less stringent in heterologous backgrounds and postulate that expression of the *engB* gene in *C. cellulovorans* is increased in the presence of cellulose.

University of Illinois
Urbana, IL 61801-4798

80. Mechanism and Structure of the Plant Plasma Membrane Ca^{2+}-ATPase
D.P. Briskin, Department of Agronomy *$80,999 (FY 93 funds/2 years)*

The plasma membrane Ca^{2+}-ATPase couples ATP hydrolysis to Ca^{2+} extrusion at the plasma membrane. In this project, studies have been conducted to examine the structure and mechanism of this enzyme. Based upon differential detergent solubilization, a soluble preparation of the enzyme was produced that was relatively free from the plasma membrane H^+-ATPase. This soluble enzyme preparation when previously washed with EGTA was further enriched by affinity chromatography using a calmodulin sepharose column. Further characterization of this enriched enzyme preparation is currently underway. Using [γ-^{32}P]ITP, the mechanism of the plasma membrane Ca^{2+}-ATPase was examined in native membrane fractions. The use of this radiolabeled, alternative substrate allowed the observation of a rapidly turning over phosphorylated intermediate involved in the mechanism of nucleoside triphosphate hydrolysis. Phosphorylation was found to be Ca^{2+}-dependent, La^{3+}-enhanced but inhibited by erythrosin B. Additional studies will be conducted on the mechanism of this enzyme using the enriched enzyme preparation. Using radiation inactivation analysis, the quarternary structure of the plasma membrane Ca^{2+}-ATPase was examined in the native

membrane. Membrane fractions were rapidly frozen, irradiated with γ-rays using a ^{60}Co source and then residual ATP-dependent $^{45}Ca^{2+}$ transport was examined in the thawed vesicles following irradiation. Residual Ca^{2+}-transport activity was found to decline in an exponential manner with increasing radiation dose and based upon target theory, a molecular size of about 245 kDa was determined. This suggests that the plasma membrane Ca^{2+}-ATPase is present as a dimer in the native membrane.

University of Illinois
Urbana, IL 61801

81. Photosynthesis in Intact Plants
A.R. Crofts, Department of Physiology and Biophysics *$109,610*

The goal of the project is to understand how photosynthesis functions in intact plants. Under natural conditions, plants experience large differences in light intensity over the day and within the canopy, and can adapt to extreme variations in local humidity, but are able to maintain optimal rates of photosynthesis by control mechanisms which modulate input of excitation to match availability of CO^2. The mechanisms of control, and integration with the physiology of the leaf, are poorly understood. An important component of the feed-back mechanism is the proton gradient. Previous work has demonstrated that lumenal pH exerts a control on several reactions of the photosynthetic chain, and over qe-quenching and zeaxanthin formation, which control excitation delivery. Our main effort will be to understand how these different effects of lumenal pH are coordinated. We have developed portable and laboratory based instruments and methods for studying photosynthesis in intact plants, and a substantial program of research using these instruments is underway in the lab and in the field. We are studying the partial reactions of the oxygen evolving complex, the intermediate electron transfer chain, including the cytochrome b_6/f complex, and its interactions with the quinone pool, and the down-regulation of photosynthesis through qe-quenching and zeaxanthin formation. An important component of the project is a laboratory based program of research to establish in simpler systems the parameters through which observation of photosynthetic electron transport and energy coupling in intact plants can be measured. We will continue the development of new instruments, and extend the research programs and collaborations established around the apparatus previously constructed.

University of Illinois
Urbana, IL 61801

82. Mechanism of Proton Pumping in Bacteriorhodopsin
T.G. Ebrey, Department of Physiology and Biophysics *$84,390*

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

83. Studies on the bo_3-type Ubiquinol Oxidase from Escherichia coli
R.B. Gennis, Department of Biochemistry *$115,430*

The bo_3-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 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. 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). Our current model assigns all of the metal ligands and 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. We are focussing our efforts on a region connecting transmembrane helices II and III. Several mutations in this "loop" decouple proton pumping from the redox chemistry.

University of Illinois
Urbana, IL 61801

84. Regulation of Cell Division in Higher Plants
T. Jacobs, Department of Plant Biology *$182,000 (FY 93 funds/2 years)*

We are interested in biochemical mechanisms that mediate the integration of plant developmental signals with the regulatory machinery driving the mitotic cell division cycle in plants. First, we must understand the points of entry of cellular regulatory networks into the cell division engine per se. To this end, our project is aimed at characterizing gene and protein elements that guide plant cells into and out of the G1, S, G2, and M phases of the mitotic pathway. Passages in this progression are controlled all or in part by cyclin-dependent protein kinases (CDKs), an ever-growing and varied family of cellular regulators. In model yeast and animal cell systems, the activity, substrate specificity, and, in some cases, subcellular localization, of these enzymes is dependent upon their phosphorylation states and their associations with regulatory cyclin subunits. We have cloned three CDK and four cyclin cDNAs from pea. We have identified a strong but unspecified CDK enzyme activity peak in S-phase and a lesser one at the G2/M transition. We continue to work toward: 1) verifying the authenticity of our clones by functional tests in heterologous systems; 2) characterizing the localization of expression of each CDK and cyclin with antibody and nucleic acid probes in situ; and 3) determining the makeup and activity kinetics of the various complexes formed by these proteins at critical points in the mitotic cycle.

University of Illinois
Urbana, IL 61801

85. Genetics of the Methanogenic Archaebacterium, Methanococcus voltae with Attention to Genetic Expression Mechanisms
J. Konisky, Department of Microbiology *$91,180*

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. We have recently turned our attention to characterizing the expression of the structural gene that encodes the *M. voltae* S-layer protein. The S-layer is composed of a hexagonal array of approximately 400,000 protein subunits and completely covers the cell surface. Since S-layer protein is the most abundant protein found in this methanogen, characterization of its structural gene promises to reveal interesting features of gene expression.

Using the cloned S-layer gene (*slpA*) as probe in Northern-blot based experiments, we have found that the level of expression of slpA depends on the physiological state of cells. For example, we have found that when *M. voltae* is grown at low pH in a medium containing reduced sodium, *slpA* gene expression is much reduced. In order to study transcription regulation of the slpA gene, we are undertaking studies to define its promoter structure as well as those structural features that relate to differential expression. The goal of these

studies is to define mechanisms whereby methanogens are able to respond to changes in their environment utilizing mechanisms that involve differential gene expression. These studies are relevant to the flux of biological methane formation in the natural environment since marine methanogens, such as *M. voltae*, reside in an environment that undergoes periodic changes in salt concentration and pH.

University of Illinois
Urbana, IL 61801-3707

86. Genetic Studies on Cytoplasmic Male Sterility in Maize
J.R. Laughnan and S. Gabay-Laughnan, Department of Plant Biology *$82,450*

The objective of this project is to determine the basic mechanisms of cytoplasmic male sterility (CMS) in maize and to understand the bases for both nuclear and cytoplasmic reversions to fertility. Genetic studies involve attempts to identify cases of insertion of transposable controlling elements into nuclear *cms-T* and *cms-S* restorer genes to afford their later molecular characterization and use as hybridizing clones. We are also searching for cases in which a transposable *cms-S restorer-of-fertility (Rf)* gene is inserted into already cloned wild-type genes. A number of newly-arisen *Rf* genes have been shown to be transposable, as has the standard *Rf3* gene, and we are continuing efforts to characterize these transposition events. Genetic studies will indicate whether *Rf* elements transpose to a large number of different sites or whether there are preferred sites for insertion. A number of spontaneous *Rf* genes have arisen in inbred nuclear backgrounds and these fall into two classes, functional and nonfunctional. We are studying the unique properties of the nonfunctional restorer genes. The molecular determination of CMS is in the mitochondrial DNA (mtDNA). Cytoplasmic reversion to fertility is accompanied by mtDNA alteration. Through collaborative arrangements we have identified a region of the mtDNA that is rearranged in all cytoplasmic revertants regardless of nuclear background. The expression of this region is affected by cytoplasmic reversion and by the standard nuclear restorer, *Rf3*, while the restorers carried by three other sources do not affect transcription. We are collaborating on studies of the newly arisen *Rf* genes dealing with the transcription and translation of the *cms-S*-associated region.

University of Illinois
Urbana, IL 61801

87. Exploratory Studies on the Bacterial Formation of Methane
R.S. Wolfe, Department of Microbiology *$68,487*

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, such as sediments, the rumen of ruminants, sanitary landfills, and sewage sludge digesters. We are conducting

exploratory studies designed to encourage the role of protozoa in methanogenic biomass digesters. We are exploring ways of simplifying the culture of methanogens in liquid and solid media, so that these organisms may be more readily employed as research tools by the scientific community. These studies include the sensitivity of methanogens to a variety of factors such as reducing agents, ions, ionic strength, and oxidizing agents. For example, under certain conditions of stress, cells of 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, to determine its structure, its role, and its relationship to known coenzymes which may be modified when cells are under stress. Our goal is to define how sensitive, anaerobic methanogenic-cells survive under stress in terms of biochemistry.

University of Illinois
Urbana, IL 61801

88. 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 *$308,693*

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 broad knowledge base and the experimental skills required to make progress with these complex issues. An interdisciplinary outlook and multidisciplinary training are especially important for future research in photosynthesis where many of the most significant issues span the range from physics to agronomy. Detailed information about mechanism, derived largely 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 also encourages the involvement of undergraduate students by providing summer fellowships for research in faculty labs. A workshop on Photosynthesis and Global Climate Change is offered each summer for high school and community college teachers. Our aim is to assist them in sparking interest in science, *per se*, and in communicating the nature of the scientific method, and to provide a basis for critically assessing the information flow on this societally important issue. Further efforts will be 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

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

N.R. Pace, Department of Biology *$50,440**

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, and the sequences provide for subsequent identification of organisms and the design of probes for further studies.

16S rRNA genes in DNA isolated from high-temperature environmental samples are isolated by cloning, directly or following amplification by polymerase chain reaction, for sequence 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. Many 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. 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

90. Organ Specific Expression in Maize: The P-wr allele
T. Peterson, Department of Zoology and Genetics
$188,521 (FY 93 funds/2 years)

In maize, the *P* gene acts as a transcriptional regulator of the flavonoid biosynthesis genes in the pathway leading to the production of a flavonoid pigment termed phlobaphene. This pigmentation, as well as the expression of the *P* gene, is restricted to specific maize floral organs. We have previously investigated the expression of the *P-rr* allele which specifies pigmentation of the kernel pericarp and cob glumes. In contrast, the *P-wr* allele produces pigmentation only of the cob glumes, and thus the kernel pericarps of *P-wr* genotypes are colorless. Thus the aim of this project is to determine the mechanism(s) leading to organ specific expression of the *P-wr* allele. As a first approach to this problem, we have found that both the colorless pericarps and the red cob glumes of *P-wr* genotypes contain the *P-wr* specific transcripts. Using polymerase chain reaction methods, we have amplified *P-wr* specific cDNAs from pericarps and cob glumes. As compared to *P-rr*, the *P-wr* 3' end and thus the -COOH terminus of the protein contain a unique sequence. We are currently using this unique *P-wr* sequence to further investigate the expression properties of the *P-wr* locus using Northern blot hybridizations, functional testing in pericarps and cob glumes, and generation of specific peptides and antibodies. The results of this study should help to elucidate fundamental regulatory mechanisms which produce specific patterns of gene expression during plant development.

Iowa State University
Ames, IA 50011-1020

91. Regulation of Carotenoid Biosynthesis: The immutans Mutant of Arabidopsis
S. Rodermel and D. Voytas, Department of Botany *$80,510*

The *immutans (im)* variegation mutant of *Arabidopsis thaliana* contains green- and white-sectored leaves due to the action of a nuclear recessive gene. The mutation is somatically unstable, and the degree of sectoring is influenced by light and temperature. Whereas the cells in the green sectors contain normal chloroplasts, the cells in the white sectors are heteroplastidic for normal and abnormal, non-pigmented plastids. This indicates that the plastids in *im* white cells are not affected equally by the nuclear mutation and that *im* expression is "plastid autonomous." In contrast to other variegation mutants with heteroplastidic cells, the defect in *im* is not maternally-inherited. The white tissues of *immutans* accumulate phytoene, a non-colored C_{40} carotenoid intermediate. This suggests that *im* controls, either directly or indirectly, the activity of phytoene desaturase (PDS), the enzyme that converts phytoene to zeta-carotene in higher plants. However, *im* is not the structural gene for PDS. As a first step toward characterizing the Immutans protein, the goal of our research is to clone *immutans* by chromosome walking and functional complementation. Wild type and mutant alleles of the gene will be sequenced and the

expression of the gene will be studied in mutant and wild type tissues. The expression of the *Arabidopsis* PDS gene will also be studied in the wild type and mutant tissues to determine how the mutation affects this step of carotenoid biosynthesis. This information should offer great insight into the complex circuitry that regulates nuclear-organelle interactions.

University of Iowa
Iowa City, IA 52242

92. Molecular Biology of Anaerobic Aromatic Biodegradation
C.S. Harwood, Department of Microbiology *$57,230*

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 environmental pollutants, 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 cloned three regions of the *R. palustris* chromosome involved in aromatic acid degradation. One includes the gene for 4-hydroxybenzoate-CoA ligase, the first enzyme of 4-hydroxybenzoate degradation, and another region includes the gene for benzoate-CoA ligase, which initiates benzoate degradation. The expression of both genes is controlled by an unlinked regulatory gene, termed *aadR*, which encodes a transcriptional activator specific to aromatic degradation. These three genes will be used to identify environmental signals that regulate expression of anaerobic aromatic degradation. We have evidence that additional benzoate/4-hydroxybenzoate degradation genes are linked to the ligase genes, and we are working to identify them. Our ultimate aim is to use the genes to 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

93. Bacterial Nickel Metabolism and Storage
R.J. Maier, Department of Biology *$93,382*

Nickel is an essential element for the growth of many microorganisms, as nickel is a component of several microbial enzymes. From studies on bacterial mutants in Ni-containing ureases and hydrogenases, it has recently become clear that the Ni-requiring organisms contain enzymes that deal with intracellular nickel metabolism and subsequent insertion into the Ni-enzyme. To determine the routes of nickel metabolism and incorporation into a bacterial enzyme (hydrogenase) in the N_2-fixing bacterium *Bradyrhizobium japonicum*, mutant strains of the bacterium have been studied, and some of these contain amplified nickel-

sequestering ability that is coordinately regulated with hydrogenase. *B. japonicum* takes up nickel with a high-affinity binding system, the nickel is stored intracellularly, and putative nickel-storage components have been identified. Genes within several operons have been characterized which encode Ni-metabolism factors, and the role of these in intracellular nickel metabolism and incorporation of the metal into hydrogenase has been studied. The genes encode domains which act as nickel-binding ligands, and at least one of the gene products binds some other divalent metal ions in addition to nickel. The long-term goal is to understand the number, nature, and function of the components involved in bacterial nickel metabolism.

Johns Hopkins University
Baltimore, MD 21218

94. Transport of Ions Across the Inner Envelope Membrane of Chloroplasts
R.E. McCarty, Department of Biology *$96,030*

Little is known about the mechanisms by which ions cross the chloroplast inner envelope membrane, the permeability barrier of the chloroplast. Among the inorganic ions that must cross the envelope are Ca^{2+}, Cl^-, Mg^{2+}, Mn^{n+}, SO_3^-, H^+ and Fe^{n+}. Using inner envelope vesicles loaded with the fluorescent Ca^{2+} indicator, Fura-2, evidence for Ca^{2+} transport from the medium into the vesicle interior was obtained. The transport rate shows saturation kinetics with respect to Ca^{2+} concentration indicating the possible participation of a proteinaceous Ca^{2+} transporter.

Pyrene, a trisulfonated hydroxypyrene derivative, is an excellent fluorescent pH indicator and may be trapped inside inner envelope vesicles. The addition of ATP to these vesicles caused an acidification of the vesicles' interior that is revered by the ionophore, gramicidin. Several inhibitors, including vanadate and N-ethylmaleimide block acidification totally but inhibit ATPase activity only partially. Thus, the chloroplast inner envelope membrane may contain an H^+-ATPase.

University of Kentucky
Lexington, KY 40546-0091

95. 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/3 years)*

Our research continues to focus on reactions affecting disassembly of the Photosystem II Reaction Center/Water Oxidizing Complex, the reassembly of this complex and, particularly, the reassembly of the Mn/Ca-cluster of the Water Oxidizing Complex (WOC). It is generally accepted that this cluster occurs as a tetra-nuclear Mn-cluster with μ-oxo bridges between several, if not all, of the $Mn^{\geq 3+}$ ions in the cluster. The assembly of the $(Mn)_4$-cluster is driven by the PSII Reaction Center via a multiquantum process (photoactivation). On the other

hand, the stoichiometric relationship between the $(Mn)_4$-cluster and the Ca of WOC remains controversial and little is known regarding the assembly of Ca into the $(Mn)_4$ cluster. Highly active O_2-evolving BBY-type PSII membranes (≥800 O_2/Chl·h) from wheat/spinach and 17kD/23kD/LHCP-less PSII particles (≥1800 O_2/Chl·h) with determined abundances of Chl/P680 were used in an analyses of the stoichiometry of the PSII functional Mn/Ca. Using procedures which removed the 17/23kD polypeptides from PSII membranes and selectively dissociated the Ca without dissociation of the $(Mn)_4$-cluster and without causing irreversible loss of O_2-evolution capacity, we observed that oxygen evolution by PSII membranes was linearly proportionate to the abundance of Ca between the limits of 2 to 1 Ca per Reaction Center. This relationship was observed independent of the light regime used in assays of oxygen evolution. Complete dissociation of the $(Mn)_4$-cluster from PSII membranes by NH_2OH-or Tris-treatment diminished the Ca abundance from 2 to 1 per Reaction Center. With the 17kD/23kD/LHCP-less PSII particles, O_2-evolution was proportionate to the abundance of Ca between the limits of 1 to zero Ca per Reaction Center, and NH_2OH-or Tris-treatment of PSII particles caused virtually complete dissociation of both the $(Mn)_4$-cluster and the 1 Ca per Reaction Center necessary for O_2-evolution. Such results confirm conclusions reached by Dr. Sakae Katoh's group - namely, WOC contains only one Ca per $(Mn)_4$-cluster; additionally, the results suggest that the binding of the Ca is dependent on the existence of the $(Mn)_4$-cluster. Accordingly, analyses were made using NH_2OH-extracted PSII membranes of the stoichiometry of Mn and Ca binding during reassembly/photoactivation of functional O_2-evolving complexes. Over the course of photoactivation of NH_2OH-PSII, the abundance of functionally bound Mn increased maximally from ~zero to 3.8 Mn per Reaction Center and the functionally bound Ca increased maximally from ~1 to 1.95 Ca per Reaction Center (Δ~0.95 Ca/3.8 Mn). This ratio of 1 Ca per 4 Mn ligated functionally was observed throughout the entire time-course of photoactivation when done at optimum conditions. It was also observed when photoactivation was carried out over wide extremes of sub-optimal conditions. Apparently, the synthesis of the $(Mn)_4$-cluster also results in the synthesis of the binding site for the 1 Ca per Reaction Center which is required in oxygen-evolution.

University of Kentucky
Lexington, KY 40536-0084

96. Acetyl-CoA Cleavage and Synthesis in Methanogens: Mechanistic, Enzymological, and Metabolic Studies

E. DeMoll, Department of Microbiology & Immunology and D.A Grahame, Uniformed Services University of the Health Sciences, Bethesda, MD

$90,000

This project will give us a better understanding of the biochemical mechanisms involved in acetyl-CoA synthesis and cleavage in methanogens -- a reaction catalyzed by the acetyl-CoA decarbonylase/synthase (ACDS) enzyme complex. Also, we will characterize the role of this multienzyme complex in the regulation of growth of methanogens. We have shown that *Methanosarcina barkeri* synthesizes acetyl-CoA from tetrahydrosarcinapterin, coenzyme A, and CO_2 plus reduced ferredoxin (formed with H_2 by a specific ferredoxin-reducing hydrogenase). Experiments will study the kinetic mechanisms of this reaction. We will

examine the enzymatic mechanism of acetyl-CoA cleavage and synthesis. In addition, we will test our hypothesis that when the methanogens are growing with C_1 units as sole carbon source, carbon flow into anabolic pathways is regulated at the level of the ACDS complex by the available reducing equivalents. In methanogens growing with acetate as sole carbon and energy source we will study whether regulation of carbon flow into anabolic versus catabolic pathways is or is not controlled via the ACDS complex.

University of Kentucky
Lexington, KY 40546

97. 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 *$87,300*

The species and site-specific post-translational methylation of Lys-14 in the large subunit (LS) of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is catalyzed by S-adenosylmethionine (AdoMet):Rubisco LS (lysine) $^{\epsilon}$N-methyltransferase (Rubisco LSMT). This enzyme belongs to a class of protein methyltransferases (Protein Methylase III's) for which there is no known protein or DNA sequence. Pea Rubisco LSMT was purified to homogeneity utilizing a novel affinity purification technique developed in our laboratory, and internal protein sequence obtained for five peptic polypeptides. Degenerate oligonucleotide probes constructed against two of the peptide sequences successfully directed the amplification of a single 317-bp PCR product using first-strand pea cDNA as a template. The PCR product contained internal sequence identical to one of the peptic polypeptides from purified Rubisco LSMT. Additionally the PCR product hybridized in Northern blot analysis to a pea mRNA of the expected size (~1.8 kb) and abundance. A full-length cDNA of Rubisco LSMT was subsequently obtained by screening a λgt10 pea cDNA library, and utilizing 5' PCR-RACE. The 1823 bp cDNA for Rubisco LSMT encoded for a 489 amino acid polypeptide with a molecular mass of 55 kd, and an amino-terminal region with characteristics of chloroplast transit peptides. All five of the peptic polypeptide sequences obtained from purified Rubisco LSMT were found in the deduced amino acid sequence of the Rubisco LSMT full-length cDNA clone. Northern blot analysis of pea tissues showed that the Rubisco LSMT gene was expressed in an organ-specific manner and was modulated by light.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

98. Enzymatic Synthesis and Biomolecular Materials
M.D. Alper, J.F. Kirsch, D.E. Koshland, P.G. Schultz and C.-H. Wong, Center of Advanced Materials *$167,000**

The goal of this research is the use of natural biological processes and molecules or variants of them 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.

Work is also progressing on the synthesis of organic thin films which mimic the biological membrane to alter interfacial and surface properties, and to fabricate sensor devices. Thin film sensor have been developed to detect influenza virus, botulism and cholera toxins.

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

Lawrence Berkeley Laboratory
Berkeley, CA 94720

99. Center for the Analysis of Plant Signal Transduction
W. Gruissem and S.-H. Kim, Structural Biology Division *$46,000*

In mammalian as well as in yeast cells, several proteins involved in signal transduction and cell trafficking, e.g., trimeric G-proteins and the Ras, Rap/Rho and Rab/Ypt family proteins, are modified by farnesyl (FPP) and geranylgeranyl (GGPP) intermediates from the sterol biosynthesis pathway. It appears that prenylation of these proteins increases their affinity to the membrane, but it may also serve as a mechanism to modulate signaling and growth as a function of the metabolic state of the cell. In an attempt to understand the role of the sterol biosynthesis pathway in plant signal transduction and growth control, we are studying the role of protein prenylation in tomato during cell division, growth, and specific signaling processes. Genetic and enzymatic approaches are in progress to identify and clone plant prenyl transferases. Using the genetic approach, a protein with homology to the mammalian farnesyl transferase beta-subunit has been cloned. Currently, we are in the process of cloning the protein farnesyl transferase alpha-subunit. Using the enzymatic approach in combination with

specific substrates, assays for protein farnesyl transferases have been designed. We have established enzymatic evidence in tomato for the presence of the three known prenyl transferases, FPP transferase, GGPP type I and type II transferases. Work is in progress to purify the enzymes and clone the genes for the protein subunits.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

100. DNA Topology and Photosynthetic Apparatus Assembly in the Bacterium *Rhodobacter capsulatus*

J. Hearst, Structural Biology Division *$263,000*

The purple non-sulfur photosynthetic bacterium *Rhodobacter capsulatus* develops an extensive intracytoplasmic membrane system that contains the photosynthetic apparatus. This normally occurs during anaerobic growth. But this bacterium is also capable of respiratory growth in the presence of oxygen, allowing photosynthetic mutants to be maintained and propagated. Thus, the effects of genetic lesions in pigment biosynthesis and in the structural proteins involved in energy transfer and electron transport can be studied.

A model for general control of gene expression for aerobic and anaerobic metabolism has postulated a role for DNA topology in regulating the transition between metabolic modes in *Salmonella typhimurium*. This model, also extended to other facultative anaerobes, proposes that DNA gyrase is essential for anaerobic gene expression, while topoisomerase I is required for expression of genes for aerobic metabolism.

In *R. capsulatus* the enzyme DNA gyrase has been involved in the expression of genes for anaerobic metabolic processes such as nitrogen fixation and photosynthesis. Since DNA supercoiling is homeostatically regulated by balancing the antagonistic activities of gyrase and topoisomerase I, and in order to assess the connection between supercoiling and anaerobic gene expression, we are directing our efforts toward obtaining *R. capsulatus top A* mutants. We will explore the possibility of relating this activity with photosynthetic membrane assembly as well as with pigment biosynthesis.

The first step in this project is to determine the nucleic acid sequence for the gene encoding topoisomerase I (*top A*) from *R. capsulatus*. Three experiments are underway to locate the *top A* gene:

i) Isolation of the *top A* gene from a pJRD 215 cosmid library of wild-type *R. capsulatus*. For this purpose, the *E. coli* strain AS17, which exhibits a temperature-sensitive-lethal phenotype that is rescued by *top A+* plasmids, is being transformed by electroporation. The complementation of the AS17 temperature-sensitive growth phenotype by a cosmid expressing the *R. capsulatus top A* gene is being determined by assaying cell viability at 30°C and 37°C. AS17 transformants are being grown in Luria broth containing kanamycin or tetracycline. Serial dilutions of these cultures are plated and incubated at permissive (30°C)

and nonpermissive (37°C) temperatures, and the number of cells forming colonies are counted.

ii) Southern hybridization is being carried out using probes selected from sequence similarity between *top A* genes from several microorganisms (i.e., *Bacillus firmus, E. coli, Saccharomyces cerevisiae* and *Vaccinia virus*). A specific probe is hybridized to DNA fragments from the digestion of *R. capsulatus* genomic DNA by either Pst I or Ava II are hybridized. The selected DNA fragments are being analyzed in order to isolate the *top A* gene from the pJRD 215 cosmid library of wild-type *R. capsulatus* by colony hybridization.

Polymerase chain reaction (PCR) techniques could be used as a way of amplifying the sequences from the above mentioned microorganisms to use them as probes in further experiments.

iii) *R. capsulatus* topoisomerase I isolation is being carried out using a procedure that takes advantages of the binding properties of this enzyme to heparin and to single stranded DNA.

The ultimate goal of these experiments is to clone the gene encoding topoisomerase I from *R. capsulatus* in order to study its relationship with photosynthetic membrane assembly and with pigment biosynthesis. These studies could lead us to a further understanding of the photosynthetic system from purple bacteria toward higher plants.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

101. Mechanism of Water Splitting and Oxygen Evolution in Photosynthesis
M.P. Klein and V.K. Yachandra, Structural Biology Division *$234,000*

Photosynthetic oxygen evolution is catalyzed by a cluster of four Manganese atoms which are the site of water splitting and accumulation of four charges induced by four successive photons. The oxidation states of the Mn in four (of the five) of these intermediate or S-states have been determined from Mn K-edge X-ray absorption spectra, for samples prepared by microsecond flashes at room temperature and characterized for S-state composition by EPR. Oxidation of Mn accompanies the first flash from the dark stable S_1 to the S_2 state, little or no change in oxidation is induced by the second flash that induces the S_3 state and sizable reduction follows the third flash leading to the S_4-S_0 states. The fourth and fifth flashes restore the S_1 and S_2 states. The structure of this cluster in the S_1 and S_2 states, as determined by EXAFS, is consistent with a pair of di-m-oxo bridged Mn dimers with Mn - Mn distances of 2.72 Å and 2.85 Å linked by a mono-m-oxo bridge with Mn - Mn distance of 3.3 Å. Replacement of Ca with Sr establishes that Sr(Ca) is within 3.5 Å of the Mn cluster. Small reproducible changes in some of the Mn - Mn distances are induced upon binding of NH_3, a water analog. The directions of the shorter Mn - Mn vectors are at about 60° relative to the membrane normal while that of the 3.3 Å vector lies at about 30° with respect to the membrane normal.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

102. Photosynthetic Pigment Proteins and Photosynthetic Light Reactions
K. Sauer, Structural Biology Division *$297,000*

The mechanism by which solar energy is utilized in plant photosynthesis involves many steps of excitation transfer in the process of distributing it to the reaction centers of photosynthetic membranes, all within a nanosecond following the absorption of visible light photons. We have investigated the role of the organization of the antenna chlorophyll proteins associated with Photosystem I of higher plants as well as the association of phycobilisomes with thylakoid membranes of cyanobacteria using fast time-resolved fluorescence measurements. This latter study was greatly aided by the use of pigment-deletion mutants designed to explore the function of long-wavelength emitting pigments of the phycobilisome cores in characterizing the coupling between the phycobilisome and the chlorophyll-containing thylakoids. In this investigation, as well as that of the Photosystem I complex, spectroscopic measurements made at low temperature were especially informative. Independent absorption and fluorescence studies of Photosystem II have demonstrated that excitation is essentially equilibrated among the antenna pigments associated with the reaction centers within about 15 picoseconds. Understanding the basic physics underlying this rapid and extensive excitation transfer is a challenge that we have explored using the pigment-protein C-phycocyanin (C-PC). X-ray crystallographic structural information for C-PC has enabled us to carry out detailed excitation transfer calculations using Förster inductive-resonance transfer theory. We have obtained excellent agreement between the theoretical predictions and our time-resolved fluorescence relaxation measurements for C-PC monomers and trimers, showing that the Förster mechanism is sufficient to explain the observed behavior.

Lehigh University
Bethlehem, PA 18015

103. Post-Transcriptional Regulation of Chloroplast Gene Expression by Nuclear Encoded Gene Products
M.R. Kuchka, Department of Molecular Biology $178,000 (FY 93 funds/2 years)

We are interested in the process by which nuclear gene products participate in the expression of specific chloroplast genes. As a model for nuclear involvement in chloroplast gene expression, we have focused on the synthesis of the chloroplast encoded D2 protein of photosystem II, a process which requires the product of at least one nuclear gene for the stabilization of the D2-encoding transcript (psbD), and the products of at least three nuclear loci for the translation of the psbD transcript into the D2 protein. Mutant strains of *Chlamydomonas reinhardtii* defective in either psbD message stabilization or translation have been the focus of our experiments. The 5' untranslated (UTR) region of psbD mRNA is known to be an important *cis*-acting element for both transcript stabilization and translation. We are currently testing for the binding of proteins from wild type and mutant cell extracts to the 5' UTR of psbD RNA in order to understand their potential roles in different stages of

psbD gene expression. We have identified four proteins from wild type cell extracts which specifically bind to this region of psbD mRNA by *in vitro* uv-crosslinking analyses. In strains affected in psbD transcript stability, all four proteins bind the 5' UTR of psbD in ways indistinguishable from wild type. Mutants affected in psbD translation sometimes show modified forms of one or more of these proteins. The relevance of these proteins and the relation of their psbD sequence binding to D2 protein synthesis are being pursued. We are currently seeking to identify the nuclear genes encoding factors essential for psbD message stabilization and translation. Several different approaches are being used, including RFLP mapping and chromosome walking, and cloning by complementation with cosmid libraries of *Chlamydomonas* DNA. Additionally, new nuclear mutant strains defective in chloroplast gene expression have been generated by insertional mutagenesis. Strains defective in the synthesis of D2 will be the focus of future experiments.

Los Alamos National Laboratory
Los Alamos, NM 87545

104. Carbon Metabolism in Methylotrophic Bacteria
C.J. Unkefer, Isotope and Nuclear Chemistry Division *$131,000*

The purpose of this project is to understand the unique aspects of the metabolism and biochemistry of methylotrophic bacteria which grow on reduced one carbon compounds such as methane, methanol, or methylamine. Methylotrophs possess a unique system for oxidizing alcohols which involves the PQQ-dependent methanol dehydrogenase (MDH). The MDH oxidation of alcohols is coupled directly to the electron transport chain, and is used to drive the net synthesis of ATP. When growing on methane or methanol, methylotrophs derive all of their energy from this process. The MDH cofactor PQQ (pyrroloquinoline quinone) is one of the three o-quinone enzyme cofactors that have been identified. PQQ is unique among this group in that it must be biosynthesized as distinct molecule. Using ^{13}C labeling and NMR spectroscopy have shown that PQQ is biosynthesized from the amino acids glutamate and tyrosine. We are continuing to probe the biosynthesis of PQQ. Molecular genetic studies in other laboratories have identified PQQ biosynthesis genes. All three organisms studied contain an open reading frame that encodes for a 23-29 amino acid peptide. We are testing the hypothesis that this peptide is the precursor for the biosynthesis PQQ. We are currently synthesizing the *Methylobacterium extorquens* AM1 peptide labeled with L-[3'-5'-$^{13}C_2$]tyrosine and will examine extracts for their ability to oxidize this peptide. During our PQQ biosynthesis studies we generated ^{13}C-labeled samples of PQQ which were used to probe the mechanism of MDH. Using NMR spectroscopy we demonstrated that upon formation of the enzyme-substrate complex the alcohol binds to the C-5 carbonyl of PQQ to form a hemi-ketal structure. In addition, when cyanide, a competitive inhibitor of MDH binds reversibly to PQQ as a cyanohydrine at the C-5 carbonyl. The ability of methylotrophs to grow on C_1 compounds gives them considerable industrial potential because they could be used to produce a variety of useful compounds from inexpensive and renewable precursors such as methanol or methane. Their ability to oxidize a wide variety of chemicals make them potentially important for treatment of waste streams. For the potential of these organisms to be realized, we must understand fundamental aspects of the their physiology.

University of Maryland
Baltimore, MD 21201

105. Structure and Regulation of L-glutamate Dehydrogenase from Hyperthermophilic Archaea (Archaebacteria)

F.T. Robb, Center of Marine Biotechnology *$86,388*

Pyrococcus furiosus, a marine hyperthermophile, has the most thermostable glutamate dehydrogenase (GDH) currently known along with the GDH from the deep sea isolate, ES4. GDH represents up to 2% of the total soluble protein of these cells, indicating a major role in metabolism. ES4 GDH has the highest denaturation temperature (113.5°C) recorded so far for a protein of this size. The *gdh*A genes from *P. furiosus*, ES4 and *Thermococcus litoralis* have been cloned and sequenced. The deduced amino acid sequence (420 residues) of GDHs from ES4 and *T. litoralis* corresponded to the N-terminal amino acid sequence obtained from the pure proteins, with a relatively high hydrophobicity and fewer sulfur-containing residues than mesophilic GDHs. A definitive structure is now required. This will be achieved by crystallography, associated with mutagenesis studies. In *P. furiosus* and *T. litoralis* GDH crystallization occurs readily, and diffraction data from *T. litoralis* GDH are at 3.4 A resolution. GDH from *P. furiosus* was overexpressed in *E. coli*. Heat treatment of *E.coli* extracts triggered increased GDH activity due to the assembly of the inactive monomers into active hexamers. The specific activity, and half life of 7.5 hours at 100°C, of the recombinant GDH assembled *in vitro*, was equivalent to that of *P. furiosus* GDH. The controlled assembly of active hexameric GDH has potential for general studies on multimeric proteins.

A family of genes whose expression is regulated by maltose was identified from a *P. furiosus* subtraction library by a procedure that minimized retrieval of ribosomal RNA sequences. This approach is applicable to studying gene regulation in organisms that are not amenable to classical genetic techniques.

University of Maryland
Baltimore, MD 21202

106. Mechanisms of Transcriptional Gene Regulation in the Methanogenic Archaea

K.R. Sowers, Center of Marine Biotechnology *$82,456*

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. An *in vitro* transcription assay has been developed using a template that contains the promoter and 300 base pairs of the 5' end of *cdh*A, and the *lac* terminator sequence. Transcription initiation factors are currently being isolated by assaying cell fractions with the *in vitro* assay. 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. Concurrently, an expression vector is being constructed to study transcriptional regulation of *cdh in vivo*. Results of this study will determine whether gene regulation in the Archaea functions by mechanisms that are analogous to the other two linkages 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

107. Identifying Calcium Channels and Porters in Plant Membranes
H. Sze, Department of Botany *$89,439 (FY 93 funds/2 years)*

Calcium transport proteins are dominant regulators of the signaling function of Ca^{2+}, and of Ca^{2+} required for secretion and for protein folding in the ER. 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, regulation and subcellular location. Unlike animals, an endomembrane-associated Ca^{2+}-pumping ATPase from carrot cells has characteristics like a plasma membrane Ca^{2+} pump. The pump is stimulated by calmodulin, and insensitive to inhibitors of SER Ca^{2+}-ATPases. The Ca^{2+} pump, detected as a Ca^{2+}-dependent phosphoenzyme of 120 kD, was partially purified using a calmodulin-affinity column. Interestingly, several isoforms of Ca^{2+}-ATPases may exist as multiple [^{125}I] calmodulin-binding proteins of ~110-125 kD were detected in endomembranes. The results suggest that plants may possess several types of Ca pumps in which the expression and activity may be differentially regulated depending on the developmental stage, cell type or subcellular membrane. To distinguish among the different Ca pumps, we are taking a molecular approach. Studying the structure and regulation of Ca^{2+} pumps is an important step towards understanding how plants grow and develop.

University of Massachusetts
Amherst, MA 01003

108. Cellulose Fermentation by Nitrogen-Fixing Anaerobic Bacteria
S. Leschine, Department of Microbiology *$184,300 (2 years)*

In anaerobic natural environments cellulose and other components of plant biomass are degraded by the combined activities of many diverse microorganisms which exist as microbial

communities and engage in a wide range of metabolic interactions. We are simulating processes occurring in natural environments by establishing biologically-defined heterogeneous bacterial communities that we use as *in vitro* systems for quantitative studies of cellulose degradation, particularly under conditions of combined nitrogen deprivation. A second aspect of our research is the investigation of the mechanisms by which components of the multicomplex cellulase-xylanase system of mesophilic, N_2-fixing bacteria catalyze the depolymerization of crystalline cellulose and other plant cell wall polysaccharides. This enzyme system consists of at least seven distinct extracellular, high-molecular-weight multiprotein complexes, each with different enzymatic and structural properties. We are exploring the possibility that a 125,000-M_r glycoprotein which is found in all complexes, and apparently lacks enzyme activity, functions as a scaffolding and/or cellulose-binding protein in the multicomplex cellulase-xylanase system. In addition, we are examining this enzyme system for activities associated with the degradation of the hemicellulosic portion of biomass. 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 carbon and nitrogen cycling in anaerobic environments. Furthermore, the information obtained will be valuable for the development of practical applications, such as the bioconversion of plant biomass (e.g., agricultural, forestry, and municipal waste materials) to fuels such as ethanol.

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

109. Molecular Basis of Symbiotic Plant-Microbe Interactions
F.J. de Bruijn *$250,505*

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 rhizospheres". 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

110. Enzymatic Mechanisms and Regulation of Plant Cell Wall Biosynthesis
D. Delmer *$240,505*

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

111. Molecular Mechanisms That Regulate the Expression of Genes in Plants
P. Green *$245,505*

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

112. Biogenesis of Plant-specific Cell Organelles
K. Keegstra *$260,505*

We are continuing studies on the biogenesis of plastids and plant cell walls. The aspect of plastid biogenesis currently under investigation is the transport of cytoplasmically synthesized precursors into chloroplasts. In particular, we are focusing on identifying and characterizing the components of the transport apparatus responsible for mediating this translocation process. Cross-linking studies have identified 75-kD and 86-kD proteins of the chloroplastic envelope membranes as putative transport components. We will isolate cDNA clones encoding both of these proteins and prepare antibodies against both proteins. We will employ these tools to investigate the function of these two proteins during transport. Additional cross-linking efforts are continuing with the goal of identifying other components of the transport apparatus.

Our studies of cell wall biogenesis are focused on the glycosyltransferases involved in synthesizing the complex carbohydrates present in cell walls. We are currently purifying the fucosyltransferase and galactosyltransferase involved in the synthesis of xyloglucan, a major hemicellulosic polysaccharide present in the primary walls of dicots. The fucosyltransferase has been solubilized and partially purified. Purification efforts are continuing with the goal of preparing antibodies against each protein and isolation of cDNA clones. These tools will be used to study the cell and developmental biology of wall biosynthesis.

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

113. Action and Synthesis of Plant Hormones
H. Kende *$260,505*

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 *in situ* hybridization. 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. In addition, we are identifying genes in the intercalary meristem whose expression is regulated by gibberellin, the plant hormone that is ultimately responsible for rapid growth of rice stems. This is being done using differential display of mRNA. 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

114. Interaction of Nuclear and Organelle Genomes

L. McIntosh *$255,505*

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 "spitting" 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

115. Sensory Transduction in Plants

K.L. Poff *$230,505*

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 phototropism in

flowering plants. This response is under the control of the blue light photoreceptor pigment system(s) which control(s) numerous light responses. 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 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 genetic, physiological and biophysical characterization of mutants and ecotypes that differ in their phototropic response. 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

116. Molecular Mechanisms of Trafficking in the Plant Cell
N.V. Raikhel *$250,505*

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: vacuoles, 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 the 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

117. Biochemical and Molecular Aspects of Plant Pathogenesis
J.D. Walton *$245,505*

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 genes 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

118. Developmental Biology of Nitrogen-Fixing Cyanobacteria
C.P. Wolk *$250,505*

Anabaena and related filamentous cyanobacteria utilize solar energy to fix nitrogen gas within spaced cells called heterocysts. We are characterizing the genes, and their roles, that allow *Anabaena* to fix nitrogen in the presence of oxygen. Our emphasis is on genes, denoted *het*, that are required for the patterned formation of heterocysts. Genetic studies indicate that the contiguous genes *hetM, hetN* and *hetI* may be required for the synthesis of a substance that affects pattern formation. Proteins HetM and HetN show similarity to polyketide synthases, while HetI is similar to proteins involved in the synthesis of acylated cyclic peptides, and can functionally replace one of them. Studies of similar enzymatic systems suggest that intermediates in a synthesis catalyzed by HetM and HetN might be covalently bound to HetM via a phosphopantetheine cofactor. We are seeking, by specifically labeling the cofactor, to identify and characterize bound intermediates. Gene *hetC* is located ca. 1 kb 5' from *hetP*.

Over a 2-kb region of DNA, the product of translation of *hetC* is similar to ATP-binding-cassette proteins that export products from bacterial cells. Nearer to its N-terminus, HetC has a domain similar to kinases. We are attempting to determine what product HetC may export, and whether HetC interacts with HetP. This work will facilitate understanding of cellular differentiation, pattern formation, and biological conversion of solar energy. Other studies have illustrated the potential of nitrogen-fixing cyanobacteria as agents of bioremediation.

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

119. Environmental Control of Plant Development and Its Relation to Plant Hormones

J.A.D. Zeevaart *$240,505*

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 identify 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. In long-day rosette plants, such as spinach, stem growth in long days (LD) is mediated by gibberellins (GAs). We have found that there are no qualitative differences in the GA composition between plants growing as rosettes in short days (SD) and bolting plants in LD. However, the GA levels are greatly increased during LD treatment, and it is this rise in GAs, specifically in the shoot tip, that causes stem growth. The objective is to determine at the biochemical (enzyme activity) and molecular level (mRNA) how LDs stimulate GA biosynthesis. Specific steps under investigation include *ent*-kaurene synthesis, the first committed step in GA biosynthesis, and the three-step conversion of C_{20}- to C_{19}- GAs that is presumably catalyzed by a multifunctional enzyme.

Abscisic acid (ABA) is a plant hormone whose synthesis is greatly stimulated when plants experience a water deficit. Conversely, ABA is rapidly inactivated to phaseic acid (PA) when plants are rehydrated. The objective of our studies is to find out how the metabolism of ABA is regulated by the water status of the tissue. In order to characterize the mechanisms regulating the pathway, we are screening for mutants that are impaired in either ABA biosynthesis, or in the formation of PA.

Michigan State University
East Lansing, MI 48824-1101

120. Xylan-Degrading Enzymes of Cytophaga xylanolytica
J.A. Breznak, Department of Microbiology *$94,627*

Xylan is a heteropolysaccharide consisting of a ß-1,4-linked xylopyranose backbone, to which are attached side groups of arabinofuranose, O-methyl glucuronic acid, and acetate. It is the most abundant of the so-called "hemicelluloses" and, next to cellulose, is the second most abundant polysaccharide in nature, where it occurs mainly in plant cell walls. We are studying xylan degradation by a newly-discovered, but seemingly ubiquitous species of *Cytophaga, C. xylanolytica*, a gliding bacterium that retains almost all of its xylanolytic activity as a cell-associated complex. Initial efforts at dissecting the enzymatic constituents of the "xylanase" complex have resulted in the purification of the α-L-arabinofuranosidase component, which is a 170 kDa trimer consisting of 55 kDa subunits and which is bifunctional, possessing a small amount of endo-ß-xylanase activity as well. We now seek to purify other major enzymes of the xylanase repertoire, which in this bacterium include: endo-ß-xylanase; ß-xylosidase; and α- and ß-glucosidase. We also seek to determine the precise cellular location of these enzymes; the extent to which they act synergistically with each other in xylan hydrolysis; and the organization and regulation of the genes encoding them. The latter effort has been facilitated by our recent discovery that *C. xylanolytica* genes encoding endo-ß-xylanase, ß-xylosidase, and α-L-arabinofuranosidase can be cloned and expressed in *Escherichia coli*. Results of this study should increase our limited understanding of xylan degradation by procaryotes and refine our current concept of the biochemical ecology of cytophagas -- a group of bacteria widely acclaimed for, but poorly understood with respect to, their biopolymer-degrading ability.

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 *$74,690*

In our laboratory, we are interested in determining what aspects of the bacterial cell surface chemistry are important in delineating the specificity of the interaction between *Rhizobium* and legume plants. One of the areas we have addressed is the effect(s) of environmental conditions on the bacterial surface chemistry. Results so far indicate that factors such as oxygen, pH, and carbon source are important in determining the kinds of carbohydrate molecules which exist on the cell surface. There is also a definite link between the bacterial cell surface lipids and the normal lipid chemistry of plant cells. We have determined that *Rhizobium* is capable of synthesizing lipid molecules such as sulfoquinivosyl diacylglycerol and digatactosyl diacylglycerol in addition to other lipids normally found exclusively in plants and photosynthetic organisms. In the case of *Bradyrhizobium japonicum* USDA110, there is

a dramatic shift towards the synthesis of such lipids exclusively in the bacteroid form. One of the most important results we have obtained so far is that the N-acetylglucosamine-containing lipooligosaccharides which have been implicated in host specificity normally occur on the surface of bacteria. The methods we use include NMR spectroscopy, mass spectrometry, infrared spectroscopy, light and electron microscopy and immunochemistry.

Michigan State University
East Lansing, MI 48824-1312

122. Control of Triacylglycerol Biosynthesis in Plants
J. Ohlrogge, Department of Botany and Plant Pathology *$86,330*

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 (FY 93 funds/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 and Cyanobacterial Genes for Increased Production of Starch in Plants

J. Preiss, Department of Biochemistry *$162,000 (FY 93 funds/2 years)*

Structure-function relationships of the ADP-glucose 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 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 ADP-glucose pyrophosphorylase has been isolated and expressed in *Escherichia coli*. Likewise, the cDNA clones of the large and small subunits of the potato ADP-glucose pyrophosphorylase have been expressed in *E. coli*. The expression of these genes and prior chemical modification studies on the purified spinach leaf ADP-glucose 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. "Mutant", active plant enzymes that are less sensitive to allosteric inhibition by phosphate or that may not require the allosteric activator, 3-phosphoglycerate, for activity will be constructed. These could be used to produce transgenic plants having increased amounts of starch. Recent studies have shown that the potato tuber subunit has catalytic activity in absence of the large subunit but requires about 20 more times of activator for activity. Thus, the small subunit is inactive in the presence of physiological concentrations of activator. Attempts will be made to see if the affinity of the small subunit for activator can be increased.

Michigan State University
East Lansing, MI 48824-1101

125. Physiology and Molecular Biology of Ligninolytic Enzyme System in Selected Wood-rotting Fungi

C.A. Reddy, Department of Microbiology

$166,000 (FY 93 funds/2 years)

The white rot basidiomycete, *Phanerochaete chrysosporium*, produces during secondary metabolism two families of extracellular heme proteins designated lignin peroxidases (LIPs) and manganese-dependent peroxidases (MNPs) as key components of its lignin degrading enzyme system. There has been some progress on our understanding of the physiology and molecular biology of the above enzymes in the recent past. However, very little is known about the regulation of *LIP* and *MNP* gene expression in wood-grown cultures of *P. chrysosporium* in response to key environmental signals such as C, N, and Mn(II). This is one of the major objectives of this project. Several experiments have been completed with poplar wood as the substrate. Further studies will focus on determining the pattern of *LIP* and *MNP* gene expression in *P. chrysosporium* cultures grown on different wood species such as pine, spruce, maple, and oak. We will continue our studies on the analysis of the LIP-MNP enzyme systems of selected white-rot fungi and in a brown-rot fungus, *Gleophyllum trabeum* and compare these to the ligninolytic enzyme system of *P. chrysosporium*. We will continue ongoing work on the analyses of genomic DNA sequences in selected wood-rot fungi that are homologous to the LIP genes of *P. chrysosporium*.

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 (FY 93 funds/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* and *Anaerobiospirillum succinoproducens* 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. succinoproducens*, CO_2 services as a catabolic electron acceptor, and its addition converts glucose metabolism from a lactate fermentative process into a succinate respiratory process. The *A. succinoproducens* PEP carboxykinase has been previously purified in our laboratory, the *pck*A gene has been cloned in *E. coli*, and its sequencing is almost completed. Expression of the gene in *E. coli* is underway to gain more understanding of this key enzyme.

In degradation of acetic acid to the 1-C end products CO2 and CH4 by *Methanosarcina barkeri*, we have completed the studies of 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 syntrophic 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 *$79,540*

Although plants are immobile, their cells exhibit a variety of intracellular transport processes. These include cytoplasmic streaming, directed vesicle transport, nuclear rotation, and organelle movements. Many of these processes are thought to be important for the normal growth and development of plants. To examine the molecular basis of these processes, and their role in plant development, we are characterizing genes that encode the actin-based motor protein, myosin, in the higher plant *Arabidopsis thaliana*. Several myosin-like gene fragments were isolated by using the polymerase chain reaction with primers derived from animal and fungal myosins. Using these gene fragments, we have identified at least nine different myosin-like genes in *Arabidopsis*. We are currently focusing our attention on one of these genes, *MYA1*, whose product displays structural similarity to a class of myosins found in yeast, chicken, and mouse. Myosins in this class appear to play a role in the intracellular trafficking of organelles. Experiments in progress are designed to examine the location of the *MYA1* product within the *Arabidopsis* plant and to determine the effect of inhibiting or overexpressing the *MYA1* gene. These studies are focused on the role of *MYA1* in the developing root, because of the numerous advantages that the *Arabidopsis* root possesses for studies of cell and organ development. We are also analyzing some of the other myosin-like genes from *Arabidopsis*, by using molecular genetic approaches to characterize their structure, expression, and function. These studies are designed to lead to a better understanding of molecular motors in plants and their role in plant development.

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 *$188,180 (2 years)*

The overall objective of this project is to elucidate the detailed mechanism by which solvent-forming bacteria such as *Clostridium acetobutylicum* regulate the well-known shift in fermentation pathway between alcohol-acetone and organic acid production. We eventually

want to isolate and describe: (1) the regulatory genes and protein elements that determine induction of synthesis of the solvent-pathway enzymes; and (2) how this regulation system interacts with the sporulation induction and development program and with related pathways such as granulose and exopolysaccharide formation in clostridia. The recent physiologic data on factors that promote the switch to solvent production by *C. acetobutylicum* suggest a two-signal system; and molecular experiments predict that transcriptional control of specific enzyme induction occurs previous to the switch. Thus, we are searching for regulatory elements that may involve two types of environmental sensors and also DNA binding proteins that act as enhancers or positive control proteins.

The research is designed to identify and characterize the regulatory elements that mediate induction of solvent-pathway enzymes in *C. acetobutylicum* as well as the other attendant metabolic and developmental events. We will use the probes and clones we have produced from 44 pleiotrophic Tn916 transposon-induced mutants to continue to analyze for complementation groups of presumptive regulatory mutants. With the *E. coli-C. acetobutylicum* hybrid plasmid, pKR13, we have constructed, together with a reasonably efficient electrotransformation technique, we will form a *C. acetobutylicum* genomic library in *E.coli* and attempt complimentation of both chemically induced and Tn916 insert induced solvent-negative, granulose-negative, and Spo$^-$ mutants. We will attempt to identify the function and protein products of presumptive regulatory mutants in order to add to knowledge of the nature of the regulatory mechanisms in these organisms.

University of Minnesota
Navarre, MN 55392

129. Genetics of Bacteria that Utilize One-Carbon Compounds

R.S. Hanson, Gray Freshwater Biological Institute — *$81,480*

Methylotrophic bacteria are those bacteria that grow on one carbon compounds including methane, methanol, methylamines and some halomethanes. The bacteria that utilize methanol or methane synthesize up to 20% of their soluble protein as the quinoprotein, 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 MDH activity. We have cloned the regulatory sequences, promoter and a Shine-Delgarno sequence located upstream of the MDH large subunit gene (*mox* F) from *Methylobacterium organophilum* XX, a facultative methylotroph, adjacent to *xyl*E as a reporter gene in a new vector (pHX200). This 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. These genes are designated *mox* Q, *mox* E, *mox* B, *mox* M, *mox* N, *mox* D. The *mox* genes, Q, E and B are specifically required for expression of *mox* F while the *mox* genes M, N, and D are required for the expression of several other methanol inducible genes as well as *mox* F. These six regulatory genes are being sequenced and their roles in transcription of *mox* F are being investigated.

Genes whose expression is induced by methanol contain conserved septamers upstream from their promoters which may serve as binding sites for positively acting regulatory elements.

University of Missouri
Columbia, MO 65211

130. Cellulose Synthesis and Morphogenesis
T.I. Baskin, Division of Biological Sciences *$160,050 (2 years)*

The goal of this research is to understand plant morphogenesis. The project will quantify the relationship between morphogenesis and alignment of cellulose microfibrils. Experiments will use growing roots of *Arabidopsis thaliana*, because we have several cellulose-deficient mutants with unusual morphology, and single cells elongating in culture. The spatial distribution of expansion, in length and radius, will be measured for wildtype, mutants, and plants exposed to microtubule- or microfibril-disrupting herbicides. Measured roots will be fixed and sectioned; and alignment of cellulose microfibrils will be quantified in two ways: with polarized light microscopy, and with electron microscopy of metal replicas of the inner-most layer of the wall. Similar experiments will be done with tissue culture cells, only the walls of these cells will be studied in rapidly-frozen, deep-etched material. Comparison of microfibril alignments among cells of different shapes will reveal the relationship between cell shape and cellulose alignment. Comparison of data from polarized light and electron microscopy will show the relative importance of microfibrils in the inner-most layer. Finally, the quantity and degree of organization of cellulose in different tissues of the mutants will be related to their aberrant morphology. The work will deepen understanding of how microfibrils shape the cells and tissues of higher plants. This research will uncover basic mechanisms of plant morphogenesis and can thus be expected to have a positive impact on applied efforts to optimize plant growth for human benefit.

University of Missouri
Columbia, MO 65211

131. Dosage Analysis of Gene Expression in Maize
J. Birchler, Division of Biology *$93,120*

The goal of this project is to understand further the basis of dosage sensitive regulatory effects on gene expression in maize. These effects act directly or inversely on the quantitative level of gene products when the dosage of specific chromosomal segments is varied. There are six specific questions addressed in this project. First, the structural and regulatory genes for the anthocyanin pigment pathway, which have been previously cloned, will be analyzed via Northern blots in a comprehensive set of segmental dosage series to examine the relationship of the direct and inverse effects on the pathway with the effects of other regulatory genes already defined. These studies should help clarify how dosage regulators interact in regulatory hierarchies. Secondly, the dosage regulators will be tested

as to whether they exhibit evidence of parental imprinting in the endosperm, as has been suggested for other regulatory genes. This will be tested by comparing dosage series produced maternally versus paternally and then examining the degree of effects on the collection of proteins expressed in the endosperm. Thirdly, combinations of multiple inverse and direct acting chromosomal segments, that are effective on the mRNA level expression of the *Globulin-1* gene, will be combined to learn the interaction properties as to whether they are cumulative, nonadditive or synergistic. Fourthly, larger aneuploids will be examined for threshold effects on gene expression as opposed to modulations found in smaller segmental aneuploids. Fifth, a collection of RFLP loci selected for expressed genes in leaves will be tested in the respective monosomics and segmental dosage series to determine the generality of structural gene dosage effects, dosage compensation, direct effects and inverse effects. Lastly, the patterns of gene expression will be examined in an extensive ploidy series from 1-7x as a comparison to the aneuploidy studies. The dosage sensitive nature of the studied effects bears not only on the problem of gene regulation but also on the molecular bases of aneuploid syndromes and dosage compensation.

University of Missouri
Columbia, MO 65211

132. Position Effect as a Determinant of Variegated Pigmentation in Maize
K.C. Cone, Division of Biological Sciences *$122,000 (FY 93 funds/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. Plants carrying *Pl-Bh* exhibit an unusual variegated pattern of pigmentation in nearly all organs of the plant, including the kernel. The nucleotide sequence of *Pl-Bh* DNA is virtually identical to that of wild-type *Pl* DNA; however, *Pl-Bh* DNA is more heavily methylated. Our hypothesis is that the variegated pattern of *Pl-Bh* expression and its altered tissue-specificity are due to a position effect. To address this idea, we are looking for derivatives in which *Pl-Bh* expression is altered, perhaps as a result of a change in a *cis*-linked factor. Thus far, we have recovered two genes that modify expression of *Pl-Bh*. One is a trans-dominant enhancer of anthocyanin expression in anther tissue. The second is a trans-dominant suppressor of blotchy pigmentation in the kernels. Current efforts are aimed at mapping these modifiers and determining whether they act by altering methylation of *Pl-Bh*. Additional experiments are underway to continue the search for *cis*-acting modifiers of Pl-Bh.

University of Missouri
Columbia, MO 65211

133. Molecular Analyses of Nuclear-Cytoplasmic Interactions Affecting Plant Growth and Yield

K.J. Newton, Division of Biological Sciences *$92,150*

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 phenotypes. Plants that have certain maize nuclear genotypes in combination with cytoplasmic genomes from the most 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. We are investigating the effects of maize alleles of two nuclear genes, *Rcm1* and *Mct*, on teosinte mitochondrial function and gene expression. When the recessive *rcm* allele is present with the teosinte mitochondrial genotypes, the plants are short and slow-growing and kernels are reduced in size. Attempts to clone this locus using transposon tagging are in progress. The product of the dominant *Mct* allele specifically alters the cytochrome oxidase subunit 2 transcript pattern in the mitochondria from the perennial teosintes by activating transcription from a novel mitochondrial promoter. Further studies of this interaction--genetically, biochemically and by *in vitro* analysis, may help us better understand promoter selection and the initiation of transcription in higher plant mitochondria.

University of Missouri
Columbia, MO 65211

134. Genetics of the Sulfate-Reducing Bacteria

J.D. Wall and B.J. Rapp-Giles, Biochemistry Department *$84,390*

The genetics of the sulfate-reducing bacteria (SRB) of the *Desulfovibrio* genus are being developed. As interest rises in the application of these microbes to bioremediation and biodegradation, tools for the manipulation of the sulfate reducers are in more demand. We are continuing to develop vectors such as those for probing for SRB promoters and for mobilizable cosmid library construction. To complement the use of cosmids and to make the SRB more genetically accessible, we are attempting to make the SRB sensitive to bacteriophage lambda. The demonstration of successful transposition by Tn*5* derivatives at low frequencies and much higher frequencies by Tn*7* has spurred construction of "improved" Tn*7* derivatives for random mutagenesis. Tn*10* derivatives are still to be tested. The physical map of *D. desulfuricans* G200 is in progress with the construction of linker libraries for the rare cutting restriction endonucleases. Reiterated sequences that cause ambiguities within the mapping are being identified.

Mount Sinai School of Medicine
New York, NY 10029

135. The Respiratory Chain of Alkaliphilic Bacteria
T.A. Krulwich, Department of Biochemistry *$102,820*

Extremely alkaliphilic *Bacillus* species successfully confront the challenge of special energy demands, growing at higher molar growth yields on non-fermentative carbon sources at pH 10.5 than at near neutral pH. The high concentration and array of respiratory chain complexes are an important part of this capacity. A dissection of the respiratory pathway, its components, and their role in alkaliphily is in progress. During the coming year experiments will focus on: a panel of pH 10.5-minus mutants, many of which have alterations in respiratory chain components; completion of the cloning and characterization of genes encoding alkaliphile cytochrome *d*; and functional studies of a newly identified operon that encodes one or more alkaliphile hemoglobin genes whose products complement *Escherichia coli* strains that are deficient in terminal oxidases.

National Renewable Energy Laboratory
Golden, CO 80401

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

Structural and functional studies of the O_2-evolving core complex of photosystem II (PSII) have emphasized orientational aspects of the integral proteins that make up the complex and electron transport properties on the donor side of the reaction center (RC). Surface-enhanced Raman scattering, a distance-sensitive (on a 5-10 Å scale) probe, was used to determine the general location of the pigments found in the core complex. Of particular interest was the discovery that the heme group of cytochrome b_{559} is located between the D1-D2 RC complex and CP47, one of the proximal antenna proteins of PSII. The functional PSII core complex also binds a tetrameric Mn cluster required for catalyzing water-splitting activity. When the Mn cluster is removed, either diphenylcarbazide (DPC) or Mn^{+2} can act as artificial donors to PSII. When both are present in spinach, a different phenomenon can occur; Mn at μM levels inhibits light-driven DPC (≥ 50 μM) donation non-competitively. To further understand the mechanism of DPC and Mn interaction, we investigated the effects of substituting Zn^{+2} and Co^{+2} (which are not PSII donors but inhibit DPC donation) for Mn and determined that Mn^{+3} (formed in a single turnover of PSII at a site different from the one that binds Zn and Co) was the inhibiting species. These results support the use of Mn inhibition of DPC donation as a means to identify PSII amino acid residues that bind Mn. Similar studies with *Synechocystis* are examining DPC/Mn interactions in site-directed mutants. However, the K_ms for DPC for but not Mn^{+2} donation in wild type are quite different from those of spinach requiring additional evaluation of Mn inhibition in the cyanobacterium.

University of Nebraska
Lincoln, NE 68588-0118

137. Control of Sugar Transport and Metabolism in Zymomonas mobilis
T. Conway, School of Biological Sciences *$192,000 (FY 93 funds/2 years)*

Glucose transport in *Zymomonas mobilis* 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. Current work is designed to elucidate facilitator expression, function, and communication with the glycolytic enzymes. Fusions of the *glf* promoter to *lacZ* are being used to discern the architecture of this efficient promoter and have implicated a region that appears to be involved in increasing transcription in the presence of fructose. Kinetic studies of the facilitator prove that the *glf* gene product is indeed a facilitator and that it can function properly in recombinant *E. coli*. The facilitator exhibits low affinity, high velocity glucose transport properties and does not appear to carry fructose. An additional carrier for fructose is implicated and catabolite choice at the level of solute exclusion is indicated. The recombinant *E. coli* model is proving to be very useful for studying the *Z. mobilis* transporter.

University of Nebraska
Lincoln, NE 68588-0118

138. Tomato Bushy Stunt Virus and DI RNAs as a Model for Studying Mechanisms of RNA Virus Replication, Pathogenicity and Recombination
T.J. Morris, School of Biological Sciences and A.O. Jackson, University of California, Berkeley *$246,376 (2 years)*

Our research emphasizes analysis of tomato bushy stunt virus (TBSV) determinants that are important in replication, pathogenicity and recombination. TBSV is a small RNA virus encoding five genes. It has a broad dicot host range and causes serious diseases of plants. Different TBSV strains vary in virulence by generating linear deletion mutants called defective interfering RNAs (DI RNAs) which compete with parental virus and reduce the severity of disease symptoms. We have sequenced TBSV and a number of distinct DIs and have analyzed biologically active transcripts generated in vitro from clones of both the parental virus and several of the DIs. We have used protoplasts and several host plants to evaluate the biological properties of the viruses, chimeric viral genomes and DI RNAs derived from the viruses. The availability of these cloned derivatives and results obtained in previous grant periods will permit implementation of the following objectives for the renewal period: 1) To complete characterization of structures important in replication of the DI RNAs by isolating important *cis* acting elements; 2) To continue to investigate *de novo* generation and evolution of DI RNAs by refining our existing RNA recombination based model; 3) To evaluate genes and elements important in viral RNA replication and movement through mutagenesis; and 4) To apply yeast as a genetic tool for analysis of TBSV replication which should permit us to

utilize selective genetic methodology developed in yeast to study TBSV replication and to identify host genes involved in virus replication.

University of Nebraska
Lincoln, NE 68583

139. Mechanistic Enzymology of CO Dehydrogenase from Clostridium thermoaceticum
S.W. Ragsdale, Department of Biochemistry *$94,090*

Clostridium thermoaceticum and other anaerobic microbes perform CO_2 and CO fixation by a recently discovered pathway that is distinct from most other metabolic pathways. Unique aspects include a preponderance of enzyme-bound intermediates, the involvement of metal-carbon bonds at key steps, and a strategy of carbon-carbon bond formation that is similar to some well studied organometallic reactions in solution. A key enzyme in the acetyl-CoA pathway is the metalloenzyme, carbon monoxide dehydrogenase (CODH). It interconverts CO and CO_2 and performs the final steps in the synthesis of acetyl-CoA. The site of assembly of acetyl-CoA is a nickel/iron-sulfur cluster in which CO binds to iron. The site of CO oxidation is another iron containing center. Our goals for the upcoming year are: (1) to determine the structure of the site of CO oxidation and define the elementary steps of this reaction, (2) to identify whether nickel or iron forms the bond with the methyl group in the methyl-CODH adduct, (3) to define the elementary reaction steps in acetyl-CoA formation by CODH, and (4) to compare the CODH from *C. thermoaceticum* with the CODH from acetoclastic methanogens and photosynthetic bacteria.

New York University
New York, New York 10003

140. Asparagine Synthetase Gene Regulation and Plant Nitrogen Metabolism
G. Coruzzi, Department of Biology *$98,940*

We have been using a molecular-genetic approach to study the role of asparagine synthetase in plant nitrogen metabolism. Recently, we have begun to study AS gene regulation in *Arabidopsis* with the intention of using genetic approaches to understand the role of AS in regulating plant growth and development. We have shown that there is a single gene for glutamine-dependent asparagine synthetase (Asn1) in *Arabidopsis*, which is expressed only in shoots of dark-grown plants. We have shown that the negative effects of light on Asn1 gene expression are mediated in part via the photoreceptor phytochrome, and in part via light-induced changes in metabolites. We have shown that high carbon (e.g., sucrose) represses the expression of Asn1 in *Arabidopsis*. The sucrose-repression of Asn1 expression is alleviated when a nitrogen source is exogenously applied. Taken together, our working model for metabolic control of the Asn1 gene is that asparagine acts as a shunt to store nitrogen and that this pathway is activated in response to a high N:C ratio in a plant. We have devised several strategies to select for or create mutants which affect asparagine synthetase in *Arabidopsis* that should enable us to further test the hypothesis that AS is a key enzyme

that regulates the flow of nitrogen in plants. These mutant analyses should also enable us to get at the factors which mediate light and metabolite control of AS1 gene expression. We are also identifying cis-acting DNA elements involved in negative regulation of AS1 by light in transgenic plants. In a genetic screen, we are using a positive selection strategy to select for mutants in a putative repressor that affects AS1 expression in the light.

North Carolina State University
Raleigh, NC 27695-7905

141. Bioenergetic and Physiological Studies of Hyperthermophilic Archaea
R.M. Kelly, Department of Chemical Engineering

$182,456 (FY 93 funds/2 years)

Efforts to better understand the mechanisms underlying the metabolic patterns in the hyperthermophilic archaea are being investigated. Focus has been on the role of sulfur reduction in the overall bioenergetics of heterotrophic hyperthermophiles and on intracellular proteolysis. Continuous culture studies focusing on sulfur reduction and oligosaccharide utilization in three hyperthermophiles (*Pyrococcus furiosus, Thermococcus litoralis* and ES4) are underway. Emphasis has been placed on the determination of maximal growth yields, induction and repression of key enzymatic activities associated with putative glycolytic pathways, and differential gene expression under varying growth conditions. A novel and, perhaps, primitive intracellular proteolytic complex (previously designated as protease S66) in *P. furiosus* has been isolated and the gene encoding the subunit of the complex has been cloned and sequenced. Among other issues, the role of this complex in protein turnover and stress response is being examined in the context of this organism in addition to comparing it to other complexes in eubacterial and eukaryotic cells. The overall objective of this study is to elucidate key physiological characteristics of these organisms at the cellular, biochemical and genetic levels.

North Carolina State University
Raleigh, NC 27695-8008

142. Transcription Factors Regulating Lignin Biosynthesis in Xylem
R. Sederoff, R. Whetten, M. Campbell, and D. O'Malley, Department of Forestry

$101,679

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

biosynthetic pathway. Our target genes include those encoding enzymes phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumarate CoA ligase (4CL), caffeate 3-O-methyl transferase (OMT), cinnamyl alcohol dehydrogenase (CAD), and laccase (LAC). In this project we will identify coding sequences and begin characterization of allelic variation leading to genetic mapping of the genes for these enzymes. We will identify the genes specifically active in the formation of lignin in xylem of loblolly pine, and begin the isolation of promoters for these genes. A long term objective is the identification of transacting factors involved in regulation of transcription in lignin biosynthesis. We have begun to characterize genes encoding transcription factor homologs from differentiating xylem. Our experiments should lead to information on the nature of the signal transduction pathways regulating these enzymes in development and in response to environmental stimuli. Understanding these mechanisms is important for strategies of genetic engineering of wood properties in forest trees.

Ohio State University
Columbus, OH 43210

143. An In Vivo Analysis of Archael Transcription Signal and the Regulation of Heat Shock Promoters

C.J. Daniels, Department of Microbiology *$187,935 (2 years)*

Using a plasmid based genetic exchange system for the halophilic archaeon *Haloferax volcanii*, we have developed methods for the analysis of transcriptional control signals in this organism. With a yeast tRNA gene serving as a reporter gene, we are investigating the requirements for efficient and accurate initiation and termination of transcription. A detailed mutational analysis of the *H. volcanii* tRNALys BoxA promoter region has revealed that the archaeal promoter most closely resembles the eukaryal TATA-like promoters and that all Archaea share a common promoter structure. Analysis of sequences located upstream of the *H. volcanii* tRNALys promoter TATA element have also revealed a positive acting element for this promoter. In related studies, we have used this reporter system to investigate the requirements for transcription termination in this organism. Preliminary data shows that oligo-T sequences are efficient and specific signals for termination *in vivo*. We are extending this analysis to examine native termination elements and the effects of DNA bending on transcription termination. As a paradigm for regulated gene expression in *H. volcanii*, we have cloned and characterized the HSP-60-like protein encoding gene from this organism. This gene exhibits a strong transcriptional induction (20 fold) in response to heat shock. Functional analysis of the promoter region of this gene, using the yeast tRNA reporter, indicates that an "enhancer" element upstream of the TATA sequence is responsible for heat shock induction of transcription. A detailed analysis of this element is underway.

Ohio State University
Columbus, OH 43210

144. Mechanisms of Microbial Applications
C.J. Daniels and W.R. Strohl, Department of Microbiology *$62,465*

Graduate programs specializing in Microbial Biology have declined in recent years, greatly reducing the opportunities for advanced training in this important research area. As one mechanism to rebuild interest in Microbial Physiology and to train new scientists, we propose a three week summer course focused on the theme of mechanisms of microbial adaptation. The course will contain both lecture and laboratory components targeted to senior level graduate students and industrial scientists not working primarily in microbial physiology research areas. Current topics in the mechanisms of microbial adaptation will be surveyed in lectures by the faculty participants and these will be supplemented with presentations by experts in the field. Lectures will address: adaptive response of microorganisms in fermentations, global response mechanisms, regulation of microbial autotrophism, carbon metabolism in methanogens, and biodegradation and bioconversion by microorganisms. The accompanying laboratory exercises will illustrate current practical problems in the topic areas and expose the students to modern experimental techniques and conceptual approaches. These experiments will provide the students with experience in fermentation technology, characterization and quantitation of proteins and metabolic intermediates, enzyme activity measurements and basic kinetic analyses, genetic approaches for the identification of regulated genes, and the anaerobic techniques needed to purify oxygen-sensitive proteins. Laboratory sessions will prepare the students to challenge and expand their current research and to undertake new approaches in their experimental systems.

A second inherent goal of the course is to establish a significant interactive community of research scientists in the field of Microbial Physiology. Introducing students to leading scientists in an informal but intense science setting should stimulate a sense of excitement and community, and set the foundation for a network of younger and experienced researchers.

Ohio State University
Columbus, OH 43210

145. Biosynthesis of Hydrocarbons
P.E. Kolattukudy, Biotechnology Center *$186,000 (FY 93 funds/2 years)*

Alkanes are found in all types of organisms from bacteria to higher animals and plants. 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 enzyme which catalyzes decarbonylation of fatty aldehyde to alkane and CO has been purified from a green algae *Botrycoccus braunii*. Studies on this enzyme include determination of subunit composition, substrate specificity, retention of aldehyde H, and

reversibility of the reaction. Antibody has been prepared against this enzyme, and a cDNA library was constructed. Two types of fatty acyl-CoA reductases have been found. A fatty acyl-CoA reductase purified from *B. braunii* generates fatty aldehyde whereas another microsomal reductase solubilized and purified to homogeneity yields fatty alcohol with hardly detectable levels of aldehyde intermediate. The N-terminal amino acid sequence of the former showed significant homology to the fatty acid reductase of *Vibrio harveyi*. The aldehyde generating reductase must participate in hydrocarbon production. The fatty alcohol generating reductase must be participating in wax ester production.

Ohio State University
Columbus, OH 43210

146. Transmethylation Reactions During Methanogenesis from Acetate or Methylamines in Methanosarcina barkeri

J.A. Krzycki, Department of Microbiology *$187,000 (2 years)*

Methanosarcina barkeri is capable of growth on acetate, methanol, and various methylamines with the concomitant production of methane. We are involved in the elucidation of the biochemical pathways responsible for utilization of these substrates. In previous studies, we had identified several proteins methylated following inhibition of *in vitro* methanogenesis from acetate. Maximal methylation was observed using an inhibitor of methylreductase, the final enzyme of methanogenesis from all substrates, indicating possible roles of these previously unidentified proteins in methane formation. Two methylated corrinoid proteins with molecular masses of 480 and 29 kDa have been isolated, and we are now investigating their physiological roles.

The smaller corrinoid protein is a monomethylamine methyltransferase present constitutively in cells grown on acetate. It is a monomer with a single corrin ring. Although up to 0.3% of soluble protein in cells grown on acetate, it is highest in cells grown on trimethylamine. The 29 kDa corrinoid protein co-purified with another protein when assayed for monomethylamine dependent methylation of CoM. The second protein was identified as a methyl-B12:coenzyme M methyltransferase (MTII) isozyme most abundant in trimethylamine grown cells. We are currently pursuing other proteins required for the conversion of monomethylamine to methane, as well as the analogous enzymes required for trimethylamine and dimethylamine dependent methanogenesis.

A physiological role is yet to be established for the larger corrinoid protein, however, it is 3-5% of total protein in cells grown on acetate and much lower in cells grown on methanol or trimethylamine. The operon encoding the 2 subunits of 40 and 30 kDa has been sequenced, and the latter subunit found to have high homology to the B12 binding fragment of methionine synthase from *E. coli*. The purified 480 kDa methylated corrinoid protein is rapidly demethylated by coenzyme M, but not dithiothreitol or homocysteine, resulting in the Co (I) state. The purified protein is capable of methylating coenzyme M at a high rate with saturation kinetics, using iodomethane as an artificial methyl group donor. This data strongly

indicates the protein is involved in one of the methanogenic pathways, and we are now examining possible physiological methylating substrates.

Ohio State University
Columbus, OH 43210-1096

147. The Molecular Characterization of the Lignin-Forming Peroxidase: Growth, Development, and Response to Stress

L.M. Lagrimini, Department of Horticulture *$133,753*

The tobacco anionic peroxidase has been implicated in the biosynthesis of lignin in vascular plants, however, we have little indication of how this enzyme may function in other metabolic processes. We have cloned and sequenced the tobacco anionic peroxidase gene, and joined its promoter and upstream sequences to the GUS reporter gene. This chimeric gene was transformed into tobacco to aid in the characterization of peroxidase gene expression. In young seedlings GUS activity is localized to the epidermis and trichomes of the shoot. In maturing leaves and stems, GUS activity is also found in the metaxylem and endodermis. Older shoots have reduced GUS activity in the epidermis and trichomes, and now shows strong activity in the developing secondary xylem. GUS activity was low in the roots, and restricted to the cortex of old coarse roots. The response of the peroxidase promoter to chemical stimuli was assessed in tobacco mesophyll protoplasts. The promoter was not responsive to cytokinins, ethylene, gibberellic acid, abscisic acid, salicylic acid, jasmonic acid, or heat shock. However, the promoter was suppressed with the addition of auxin (IAA). The suppression by auxin is most interesting in light of several observations showing the tobacco anionic peroxidase's involvement in IAA catabolism. Transgenic plants with an antisense RNA construct for the anionic peroxidase fused to the CaMV 35S promoter were obtained with less than 5% of the peroxidase activity of control plants. These plants were similar to controls, however, they grew at a significantly slower rate. Surprisingly, lignin levels in leaf and stem tissue were identical to controls. It is possible that >95% suppression of the peroxidase gene is required to see an observable difference in lignin content. Transgenic plants with xylem-specific, root-specific, and leaf-specific promoters joined to peroxidase sense and antisense sequences are under analysis for altered lignin content.

Ohio State University
Columbus, OH 43210

148. Structure and Regulation of Methanogen Genes

J.N. Reeve, Department of Microbiology *$109,125*

The goal of this project is to determine the structure, organization and mechanisms of regulation of expression of genes responsible for methanogenesis, and to relate these parameters to the functions of their encoded gene products. We have shown that in growing *Methanococcus vannielii* cells there are ~180 transcripts of the *mcr* operon that encode methyl coenzyme M reductase (MR), the enzyme that synthesizes methane. These transcripts have

a half-life of ~15 min at 37°C and transcription of this operon could not be stimulated by partially inhibiting methanogenesis. *Methanobacterium thermoautotrophicum* (*M.t.*) strains contain two isoeznymes, MRI and MRII, and the *mcr* and *mrt* operons that encode these enzymes, respectively, have been cloned and sequenced and their growth phase dependent transcription demonstrated. The *mrt* operon has been located immediately downstream from the *mvhDGAB* gene cluster that encodes the methyl-viologen reducing hydrogenase and polyferredoxin in *M.t.*ΔH. Purified preparations of native and recombinant polyferredoxin have been analyzed by several spectroscopic techniques demonstrating the presence of [4Fe:4S] clusters. The role of the polyferredoxin in electron transfer during methanogenesis is under investigation. Antibiotic resistance-conferring genes have been introduced into plasmids isolated from strains of *M. thermoformicicum* that are closely related to *M.t.*ΔH, and these plasmids used as donor DNAs to screen electroporation protocols for transformation of *M.t.*ΔH.

Ohio State University
Columbus, OH 43210

149. Photosynthetic Electron Transport in Genetically Altered Chloroplasts
R.T. Sayre, Departments of Biochemistry and Plant Biology *$100,880*

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

150. Regulation of Alternative CO_2 Fixation Pathways in Prokaryotic and Eukaryotic Photosynthetic Organisms

F.R. Tabita, Department of Microbiology *$194,000 (2 years)*

Carbon dioxide is a ubiquitous, but highly oxidized form of carbon found throughout the biosphere. We are investigating how anaerobic photosynthetic bacteria and aerobic eukaryotic marine diatoms control the process by which they reduce and metabolize CO_2 through routes that differ from the Calvin reductive pentose phosphate pathway. In particular, we are interested in the molecular signals that influence switches in CO_2 metabolic paths in both groups of organisms, with initial studies focused on the bacteria. Using mutant strains of nonsulfur purple photosynthetic bacteria (*Rhodospirillum rubrum* and *Rhodobacter sphaeroides*) containing deletions in the RubisCO genes, it was recently established that CO_2 could be assimilated under both photoheterotrophic and photolithoautotrophic growth conditions. Both biochemical and molecular biological approaches have been used to probe the mechanisms that control the alternative CO_2 fixation pathway(s) in the two organisms. Using transposon mutant strains of *R. sphaeroides* that were incapable of photoheterotrophic growth with CO_2 as electron acceptor, a plasmid was isolated that could complement the mutant. A small DNA fragment, containing the site of the original transposition, was subsequently isolated and shown to be important for growth under conditions where CO_2 fixation is required. Current work is focused on these fragment and flanking sequences. We have also noted that RubisCO negative strains of both *R. rubrum* and *R. sphaeroides* synthesize specific and unique proteins under growth conditions which favor active CO_2 fixation. These proteins are being isolated to determine their role and importance in the alternative CO_2 fixation pathway. A rapid growing, genetically tractable green sulfur bacterium (*Chlorobium tepidum*), which fixes CO_2 through the reductive tricarboxylic cycle, is also being used to study the molecular control of this well described alternative CO_2 fixation pathway. *Chlorobium* mutants that are deficient in CO_2 fixation capacity have been isolated and are currently being characterized.

Oklahoma State University
Stillwater, OK 74048

151. The Structure of Pectins from Cotton Suspension Culture Cell Walls

A. Mort, Department of Biochemistry *$91,769*

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 imidazole 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. We have determined the pattern of esterification of the 50% methyl esterified homogalacturonan. The pattern appears to be an alternation of esterified and non-esterified residues. If we extract this 50% esterified homogalacturonan with enzymes, it co-solubilizes along with RGI. Our hypothesis is that in cotton, as in apple and various other plants [Schols and Voragen, *Carbohydr. Res.* 256, 83-95 (1994)], the RGI and this HG are interspersed within one polymeric unit. We are attempting to determine the range of sizes of the poly-GalA sections. Preliminary results indicate a broad range centered around ~20 residues. The low ester homogalacturonan accounts for about 20% of the weight of the walls, and the length of the polygalacturonic acid stretches is much longer (up to at least 70 residues).

We will attempt to determine if other 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.

University of Oklahoma
Norman, OK 73019-0245

152. Effect of Community Structure on Anaerobic Aromatic Degradation
M.J. McInerney, Department of Botany and Microbiology *$81,480*

We studied the effect of hydrogen utilization kinetics on the rate and extent of benzoate degradation by an anaerobic syntrophic bacterium, strain SB. The maximum velocity for benzoate degradation decreased when a hydrogenotroph with lower maximum velocity for hydrogen use served as the syntrophic partner. The half-saturation constant (k_m) for benzoate degradation decreased when nitrate rather than sulfate served as the electron acceptor. Nonlinear regression analysis of progress curve data showed that acetate inhibition did not account for the existence of a threshold for benzoate degradation. Cocultures of SB used fatty acids from 4 to 18 carbons in length in addition to benzoate, which distinguishes SB from other known syntrophic bacteria. Enzymatic studies suggested that benzoate was activated by a coenzyme A ligase reaction and degraded by a pathway with the formation of glutaconyl-CoA as an intermediate. We isolated a new species of obligately halophilic, fermentative anaerobe, *Halobacterium salsugo*, and a new species of dissimilatory iron-reducing bacteria. The iron reducer coupled the oxidation of either acetate or hydrogen to iron reduction, and grew by dissimilatory cobalt reduction.

Oregon Graduate Institute of Science and Technology
Portland, OR 97291-1000

153. Biochemical Genetics of Lignin Degradation by Phanerochaete chrysosporium

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

$125,130

The objective of this research is to further our understanding of the lignin degradative system of the basidomycete *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. We are examining the oxidation of a variety of model dimers, aromatic pollutants and polymers with these enzymes, including the oxidation of cytochrome *c* by lignin peroxidase. We are also examining the effect of various chelators on the manganese peroxidase reaction and trying to determine whether this enzyme can oxidize recalcitrant substructures in lignin. 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 has facilitated 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* and have used this system to disrupt a gene encoding MnP isozyme 1. Disruptions of genes encoding other MnP and LiP isozymes are underway. These mutants will be used to elucidate the role of MnP and LiP in the degradation of lignin and aromatic pollutants.

Oregon Graduate Institute of Science and Technology
Portland, OR 97291-1000

154. Oxidative Enzymes Involved in Fungal Cellulose Degradation

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

$83,420

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 cellulose-degrading cultures of *Phanerochaete chrysosporium*, *Sporotrichum thermophile*, and *Coniophora puteana*, a brown-rot fungus. In

the presence of limiting nutrient nitrogen, cellulose-degrading cultures of *P. chrysosporium*, produce both lignin and cellulose-degrading enzymes. In this study, contribution of CDH to lignin degradation and the interaction of CDH with lignin peroxidase will be examined. Mechanism of electron transfer between the heme and flavin moieties of CDH will be determined by spectroelectrochemical methods. Experiments to understand the biochemical mechanisms responsible for the enhancement of cellulose hydrolysis by CDH will be performed.

Oregon State University
Corvallis, OR 97331

155. Catalytic Mechanism of Hydrogenase from Aerobic N_2-Fixing Microorganisms
D.J. Arp, Laboratory for Nitrogen Fixation Research *$162,000 (2 years)*

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

Oregon State University
Corvallis, OR 97331-3804

156. Understanding and Targeting a Novel Proteinase/Substrate Interaction
W.G. Dougherty, Department of Microbiology *$96,030*

Plant viruses continue to negatively impact biomass production worldwide. Potyviruses, a ubiquitous group of RNA viruses efficiently vectored by aphids, comprise one of the more serious virus threats to production agriculture. Using the potyvirus tobacco etch virus (TEV) as a model, we have been developing antiviral strategies that will permit us to design virus resistant germplasm. TEV expresses its genetic information initially as a large molecular

weight polyprotein that is extensively proteolytically processed by a viral-encoded proteinase. This proteinase is referred to as the NIa proteinase and is unique in its requirement to bind to a cleavage site sequence that spans a linear seven amino acids motif. Over the past year we have been examining the different forms of the NIa proteinase that exist in a cell. We have shown that the 27kDa NIa proteinase will cleave off its carboxy-terminal 24 amino acids to generate a truncated 25kDa protein. This is an obligatory mono-molecular event. We have been able to express and purify to homogeneity the two different versions of the TEV NIa proteinase. The 25kDa version of the NIa proteinase displays altered proteolytic activity and specificity. Both versions of the proteinase are being examined in kinetic studies and both are being crystallized for eventual structure studies. These studies will lead to a more complete understanding of the TEV NIa proteinase and provide a framework to design and test inhibitor molecules.

University of Oregon
Eugene, OR 97403

157. Transposon-Induced Nuclear Mutations that Alter Chloroplast Gene Expression

A. Barkan, Institute of Molecular Biology *$180,000 (FY 93 funds/2 years)*

The long term goal of this project is to use mutant phenotypes to identify nuclear genes that control the expression of chloroplast genes. Recent work has focused on two nuclear mutants of maize with unique phenotypes. Analysis of chloroplast transcripts, polysomes, and protein synthesis indicate that the *crp1* mutation causes defects in the processing of *petD* mRNA and in the translation of *petA* mRNA; the expression of most chloroplast genes is unaffected. Mutant chloroplasts lack the monocistronic *petD* mRNA but contain normal levels of its polycistronic precursors. The loss of monocistronic *petD* mRNA is correlated with a loss of *petD* protein synthesis, suggesting that the missing *petD* transcript is essential for high rates of *petD* translation. A mechanism by which processing to a monocistronic form could activate the translation of *petD* sequences was suggested by computer-generated secondary structures, in which the *petD* start codon lies in a hairpin in the polycistronic RNA but remains unpaired in the monocistronic transcript. These results provide strong evidence that the extensive RNA processing typical of chloroplast transcripts can play a role in controlling gene expression. Although the *petA* mRNA is normal in structure and abundance in *crp1*, it is translated poorly. Thus, the CRP1 gene is essential for the processing and translation of a subset of chloroplast mRNAs. We are currently investigating the biochemical bases for these defects.

crp2 defines a nuclear gene that is necessary for transcriptional fidelity in the chloroplast. A global reduction in the translation elongation rate is correlated with transcription of the inappropriate DNA strand of many genes. We hypothesize that the translation defect is a consequence of the production of "antisense" RNAs arising from this aberrant transcription. The CRP2 gene may encode a factor that increases the specificity of transcription initiation or that facilitates transcription termination. Current work is directed towards distinguishing between these possibilities.

Both *crp1* and *crp2* were induced by transposon insertion. We are using the transposons to obtain molecular clones of the CRP1 and CRP2 genes. The clones will be used to further define the roles of the gene products in chloroplast gene expression.

Palo Alto Institute for Molecular Medicine
Mountain View, CA 94043

158. Genetic and Biophysical Studies of the Photosynthetic Reaction Center
D.C. Youvan *$115,430*

Combinatorial mutagenesis and helix swap experiments on the photosynthetic reaction center have been extended to include larger structural motifs than previously reported. The 26 sequence differences found between the L- and M-subunit amphipathic *cd* and transmembrane D helices have been partially symmetrized by using combinatorial mutagenesis techniques. 39 functional mutants were isolated. In experiments involving structural motif swaps, the Q_B pocket has been deleted and replaced by Q_A sequences. This mutant, 'QAQA' is photosynthetic defective, but photosynthetically competent mutants (e.g., A6D1) can be recovered by selection for revertants (occurring near the photo-inactive bacteriopheophytin). All of these mutants are candidates for wrong-way electron transfer and will require extensive biophysical analyses.

Pennsylvania State University
University Park, PA 16802

159. The Characterization of Psychrophilic Microorganisms and Their Potentially Useful Cold-Active Glycosidases
J.E. Brenchley, Department of Molecular & Cell Biology
$172,000 (FY 93 funds/2 years)

Our objective is to characterize psychrophilic and psychrotrophic microorganisms and to purify, study and genetically engineer their cold-active glycosidases. Cold-active enzymes with high activities at low temperatures can be used for converting 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 which carbon sources give the highest enzyme activities and whether isozymes with distinct temperature optima are synthesized during growth at different temperatures; 3) purify model 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. We have isolated a new *Arthrobacter* strain that has three different genes encoding ß-galactosidases. We have demonstrated that one of these is the primary enzyme used for growth on lactose and have purified this enzyme and sequenced its gene. This

enzyme has a temperature optimum approximately 20°C below that of the *E. coli lacZ* ß-galactosidase.

Pennsylvania State University
University Park, PA 16802-4500

160. Light-Energy Transduction in Green Sulfur Bacteria
D.A. Bryant, Department of Molecular & Cell Biology *$150,350 (2 years)*

Green sulfur bacteria are exquisitely adapted for growth at very low light intensities and have reaction centers that are related to Photosystem I of cyanobacteria and higher plants. The long-term objectives of this research program are to develop a detailed understanding of the structure, function, and biogenesis of the light-energy transduction apparatus found in green sulfur bacteria. Chlorosomes, reaction centers, and peripheral electron transport reactions leading to the stable reductants NADPH and ferredoxin will be characterized. The polypeptide composition of the chlorosomes of the moderate thermophile *Chlorobium tepidum* will be determined. The genes encoding these proteins will be cloned, sequenced, and transcriptionally characterized. Proteins will be overproduced in *Escherichia coli* and used for antibody production and structural studies. Methods to allow interposon mutagenesis will be developed. The two 2[4Fe-4S] ferredoxins of *Chlorobium* sp. will be overproduced in *E. coli*, and the ESR and electrochemical properties of the two proteins will be determined. These proteins will be used to study the terminal steps of electron transport with *C. tepidum* reaction centers. Ferredoxin:$NADP^+$ oxidoreductase will be characterized, and the gene encoding this protein will be isolated, sequenced, and overexpressed. Finally, phylogenetic relationships among photosynthetic procaryotes will be explored through DNA sequence analyses of selected components of the transcriptional (RNA polymerase subunits) and translational apparatuses of representative, diverse phototrophs. The proposed studies should greatly expand knowledge of the biochemistry, physiology, and molecular genetics of the green sulfur bacteria and could provide insights into the origins of oxygenic photosynthesis.

Pennsylvania State University
University Park, PA 16802

161. Rapid Regulatory Control of Plant Cell Expansion and Wall Relaxation
D.J. Cosgrove, Department of Biology *$93,120*

Plant cell enlargement is controlled by biochemical processes that modify bonding within the cell wall to induce stress relaxation, which subsequently gives rise to cell water uptake and wall expansion. This project is aimed at elucidating the physical, cellular and molecular mechanisms controlling wall relaxation and cell enlargement. High-resolution analyses of cell expansion and wall relaxation indicate that both processes exhibit complex dynamics. By stimulating plants with low-amplitude pressure sinusoids, we have discovered that plants display resonance, harmonics, noise attenuation and period doublings in their growth

responses to these perturbations. These behaviors are characteristic of a nonlinear feedback system. Our results require two surprising additions to the conventional model of plant cell growth, namely that there is a growth-rate sensor that integrates growth rate over 1 min or less, and also there is 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 developing methods to test for alterations in wall pH, wall redox potential, and wall enzyme activities as potential means for rapid regulation of wall relaxation. Electrical measurements during sinusoidal stimulation show dynamic behaviors in membrane potentials that resemble the growth responses, results that support the idea that changes in ion transport across the plasma membrane take part in the growth feedback. 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

162. Role of Ca^{++}/calmodulin in the Regulation of Microtubules in Higher Plants
R. Cyr, Department of Biology *$85,000*

The cytoskeleton, and in particular its microtubule (Mt) component, participates in several processes that directly affect growth and development in higher plants. Normal cytoskeletal function requires the precise and orderly arrangement of Mts into several cell cycle and developmentally specific arrays. One of these, the cortical array, is notable for its role in somehow directing the deposition of cellulose, the most prominent polymer in the biosphere. Unfortunately, little molecular information is available regarding the formation of these arrays, or the cellular signals to which they respond. It is therefore important to acquire information regarding the molecules which regulate Mts within the different arrays. Experimental data has been obtained to suggest that plant cells use calcium, in the form of a Ca^{++}/calmodulin complex, to affect the dynamics of Mts within the cortical array. Owing to the importance of Ca^{++} as a regulatory ion in higher plants we are probing for a putative Ca^{++}/Mt transduction pathway which may serve to integrate Mt activities within the growing and developing plant cell. Our working hypothesis, derived from biochemical and cell biological data, is that a Ca^{++}/calmodulin complex interacts with a homolog of elongation factor 1-alpha to affect the degree of lateral associations of Mts within the cortical array. 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 16802-4500

163. Characterization of Lignin and Mn Peroxidases from Phanerochaete chrysosporium
M. Tien, Department of Molecular and Cell Biology *$100,880*

Lignin is an aromatic insoluble polymer, nature's plastic, which imparts structural rigidity to woody tissue and protects it from microbial attack. Although resistant to most forms of microbial attack, certain filamentous fungi are capable of degrading it to the level of CO_2. Our studies have focused on the white-rot fungus *Phanerochaete chrysosporium*. The lignin and Mn peroxidase isozymes play key roles in lignin degradation. We are particularly interested in the enzymology of this process; how does a large bulky soluble enzyme degrade an insoluble substrate. The lignin and Mn peroxidase isoenzymes utilize H_2O_2 in oxidizing a large variety of low molecular weight organic (and inorganic Mn^{2+}) substrates by one electron. It has been proposed these small molecular weight substrates act as redox mediators capable of oxidizing lignin from a distance. Our research focuses on determining the structure/function relationships of these enzymes with their low molecular weight substrates and with the actual polymer. We have many site-directed mutants of both enzymes made through heterologous expression in insect tissue culture. Structure/function studies on the native and mutant proteins will assist us in understanding the interaction of the heme active site with the low molecular substrates and with the large insoluble lignin polymer.

University of Pennsylvania
Philadelphia, PA 19104-6018

164. Structural Basis of Signal and Energy Transduction in Plants
A.R. Cashmore, Plant Science Institute *$135,000*

This training program is aimed at training graduate students in both structural and molecular genetic studies in plants. Recent developments in molecular genetics have meant that it has become increasingly plausible to isolate essentially any plant gene of interest. This phenomenon will become more pronounced as interest is heightened in genome sequencing programs. When coupled with conventional genetic studies, these tools are exceedingly powerful and have brought us to a stage with both higher plants and photosynthetic bacteria, as with other systems, where significant advances are beginning to be made in the study of the basic components of many physiological and developmental processes. As the nature of individual gene products are better understood, it is becoming clear that the complexity of many biological processes often reflects unique interactions of gene products. Indeed it could be argued that the almost infinite biological diversity that exists simply reflects complexity that can be readily generated by allowing a finite number of molecules to interact in unique ways. One outcome resulting from this line of thinking is that in order for us to fully understand biological systems, it is imperative to place an appropriate emphasis on structural studies; especially where such studies are concerned with complex macromolecular systems. It is this

philosophy that is behind this training program in Signal and Energy transduction. Training will be provided by members of the Departments of Biochemistry/Biophysics, Biology, and Chemistry. Students will be trained in such a manner that they gain an appreciation of plant biological systems from both a molecular genetic, as well as a structural viewpoint. From such training, students will gain knowledge which will facilitate the beneficial manipulation of plants. Such manipulations will eventually result in the generation of plants with either improved productivity or preferred ecological properties. In addition, it is increasingly likely that by sophisticated molecular genetic manipulations, plants may be generated that have the capacity to produce novel products with valuable properties such as pharmaceuticals or cheap sources of energy.

University of Pennsylvania
Philadelphia, PA 19104

165. Circadian Rhythms in CAB Gene Expression
A.R. Cashmore, Plant Science Institute, Department of Biology *$123,675*

In an effort to identify mutants within the circadian clock, we have isolated 100 independent mutant lines of Arabidopsis that showed reduced sensitivity to photoperiod in the initiation of flowering response. Of these mutant lines, almost half showed in addition a somewhat elongated hypocotyl in white light. Further analysis revealed that at least five of these were selectively insensitive to red light. This red-light insensitivity was less severe than that of phytochrome-deficient elongated hypocotyl mutants, which are virtually blind to red light. Interestingly, the longer petioles and greater leaf area characteristic of the PhyB photoreceptor mutant (*hy3*) was an additional phenotype of some of these early flowering lines.

The mutants described above were thought unlikely to be clock mutants since they flowered somewhat earlier than wild type even under long days. A genuine clock mutant, arrhythmic or with altered period length, would be expected to flower early under short days and at the normal time under long days. We have indeed obtained several such mutants exhibiting no apparent phenotypic alteration with the exception of earlier flowering under short days. Although none of these mutants proved to be arrhythmic with respect to expression of CAB, we are presently analyzing these mutants in greater detail for CAB gene expression over several photoperiods in continuous light, in order to ascertain whether the free-running period length may have been altered.

University of Pennsylvania
Philadelphia, PA 19104-6018

166. Membrane-attached Electron Carriers in Photosynthesis and Respiration
F. Daldal, Department of Biology *$214,000 (FY 93 funds/2 years)*

The overall aim of this project is to identify and characterize at the molecular level all membrane-associated cytochromes (cyt) that act as electron carriers in photosynthesis and

respiration using the facultative photosynthetic bacterium *Rhodobacter capsulatus* as a model system for energy transduction. The existence of a dual photosynthetic electron transfer pathway between the cyt bc_1 complex and the reaction center was first indicated by our photosynthesis-proficient mutants of *R. capsulatus* lacking the soluble carrier cyt c_2. We have now discovered that a novel membrane-associated cytochrome, cyt c_y, which is structurally distinct from and yet functionally similar to cyt c_2, is the molecular basis of this pathway. Mutants lacking cyts c_2, c_y, or the bc_1 complex were utilized to determine the functional roles of these cytochromes. Flash induced electron transfer kinetics in the reaction center, cyt *c* pool, cyt *b* pool and carotenoid band shift indicated that the electron transfer pathways via cyt c_2 or via cyt c_y operate simultaneously in a wild type strain but they have strikingly different kinetics. Cyt c_y pathway appears more prominent under the conditions tested, and the genetic inactivation of both cyts c_2 and c_y demonstrated that no other functionally comparable electron carrier is available in the cell. Further, heme-stained SDS-PAGE and flash spectroscopy experiments indicated that cyt c_y is detectable in strains lacking the bc_1 complex only when grown on minimal growth medium but not on rich medium, suggesting that these components interact with each other. We have also obtained evidence that cyt *cy* carries electrons from the bc_1 complex to the cyt *c* oxidase in respiration. Purification of this latter enzyme led us to discover that it is a novel kind of cyt *c* oxidase which has no Cu_A, no farnesyl side chain in its Cu_B binuclear center, and which contains two *c*-type cytochromes as subunits. Partial amino acid sequences of these subunits revealed significant homology to the high oxygen affinity cyt *c* oxidase from *Bradyrhizobium japonicum* and *Rhizobium meliloti* apparently essential during nodulation.

University of Pennsylvania
Philadelphia, PA 19104-6018

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

J.R. Ecker, Department of Biology *$94,090*

How plants control their growth and differentiation and respond to a variety of external stimuli are central questions in plant biology. Hormones play a crucial role in these processes, affecting virtually every aspect of these processes. The chemically simplest hormone is the gaseous olefin ethylene. Ethylene participates in a number of plant developmental process and stress responses, perhaps the best known of which is fruit ripening. The molecular mechanisms underlying the biosynthesis, perception and transduction of ethylene is the focus of our research program.

The response of etiolated *Arabidopsis* seedlings to ethylene (the triple response) is being used as a model system to study the action of hormones in higher plants. A number of mutants affected in this cell growth response have been identified. These mutations define genes involved in ethylene perception/signal transduction and in the control of ethylene biosynthesis. One of these genes, *CTR1*, encodes a protein kinase that is homologous to a mammalian oncogene, suggesting that some components of signal transduction are conserved in species as divergent as plants and mammals. We are characterizing the

biochemical properties of the CTR1 protein and applying both genetic and molecular approaches to identify proteins that interact with it (regulators and substrates). Studies are in progress to identify and characterize alleles of *CTR1* that result in unregulated kinase activity and extragenic mutations that suppress the constitutive ethylene phenotype of *ctr1*. The results of genetic, molecular and biochemical studies of the CTR1 protein kinase should begin to define the role of this gene in the ethylene action pathway and in the regulation of plant cell growth.

University of Pennsylvania
Philadelphia, PA 19104-6018

168. Structure-Function Analysis of Vacuolar H^+-Pyrophosphatase
P.A. Rea, Department of Biology *$93,000*

Plant cells contain alternate metabolic pathways which utilize nucleotides or inorganic pyrophosphate (PPi) as energy sources. While the full significance of this phenomenon remains to be determined, this pattern of alternate proximate energy sources is exemplified by the presence of two parallel H^+ pumps in the vacuolar membrane. These are the vacuolar H^+-ATPase (V-ATPase; EC 3.6.1.3), an enzyme common to the endomembranes of all characterized eukaryotes, and a vacuolar H^+-translocating inorganic pyrophosphatase (V-PPase; EC 3.6.1.1), which is ubiquitous in plants but otherwise known in only a few phototrophic bacteria. Both enzymes catalyze electrogenic H^+-translocation from the cytosol to vacuole lumen to establish an inside-acid pH difference and inside-positive electrical potential difference which is employed to energize the H^+-coupled, secondary transport of solutes across the vacuolar membrane.

Our research program is directed at understanding the structure and function of the V-PPase at the protein level through the application of biochemical and molecular techniques. The basis of the research underway is our recent success in molecular cloning cDNAs encoding the V-PPase from *Arabidopsis thaliana* and its two isoforms from *Beta vulgaris*, reconstituting partially purified preparations of the enzyme from *Vigna radiata* to generate transport-competent proteoliposomes and our ability to heterologously express cDNAs encoding the V-PPase from *Arabidopsis* in the yeast *Saccharomyces cerevisiae*, to yield membrane fractions active in all of the known core catalytic functions of the enzyme. Rational structure-function analyses of the V-PPase are therefore now feasible through the use of deduced protein sequence data, simplified transport-competent preparations of the pump and a molecularly manipulable organism engineered to expression functional enzyme.

The program of research falls into two main areas: (i) Protein chemical and immunological studies of the overall topology of the V-PPase, through the use of surface-active and lipophilic protein reagents and monospecific antibodies for the enumeration of hydrophilic loops and transmembrane spans. (ii) Molecular studies directed at determining the identity and location of amino acid residues involved in catalysis by *in vitro* mutagenesis to extend and complement our recent protein chemical studies implicating Cys^{634} in cytosolic hydrophilic loop

X as the reactive residue whose alkylation is responsible for substrate (mg_2PPi) protectable, covalent modification and inhibition of the V-PPase by permeant and impermeant maleimides.

In novel origins, membership of a new category of ion translocase and utilization of the limiting case of a high energy phosphate as energy source, make the V-PPase a unique system for examining some of the basic principles underlying phosphoanhydride-energized ion translocation across biological membranes.

Purdue University
West Lafayette, IN 47907

169. Crystallographic Studies of Nitrogenase and Hydrogenase
J.T. Bolin, Department of Biological Sciences *$92,557*

The overall objective of this project is to determine and analyze the crystal structures and functions of enzymes that play key roles in microbiological metabolic processes significantly related to the production and consumption of energy resources. Thus we are investigating the structure and function of nitrogenases, hydrogenases, and carbon monoxide dehydrogenases.

Nitrogenase is the 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. We have achieved the first goal of this project in that we have determined and refined the crystal structure of MoFe-protein, the catalytic component of nitrogenase, at atomic resolution. We are now engaged in studies of the structures of mutant nitrogenase proteins.

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. We have obtained crystals of two all-Fe hydrogenases and are in the process of analyzing diffraction data from these crystals. We are also attempting to obtain crystals of two Ni-dependent hydrogenases.

The carbon monoxide dehydrogenase from the photosynthetic bacterium *Rhodospirillum rubrum* oxidizes CO to CO_2 and directs the electrons derived from this process to the production of H_2 by a CO-induced hydrogenase. We have obtained thin crystals of *R. rubrum* CODH and are currently attempting to improve these crystals to the point where they will support high resolution diffraction studies.

Purdue University
West Lafayette, IN 47907

170. Purification and Molecular Cloning of the Synthases of Cereal (1→3),(1→4)-ß-D-glucan

N.C. Carpita, Department of Botany and Plant Pathology *$180,420 (2 years)*

We will continue our studies of the biosynthesis of (1→3),(1→4)-ß-D-glucans (MGs) by identifying the enzyme or enzymes involved in its synthesis. This cell wall polysaccharide is unique to the cereal grasses and has recently gained attention for its possible role in lowering serum cholesterol. We have successfully enriched membranes of the Golgi apparatus from maize (*Zea mays* L.) by a single-step flotation gradient and used them to synthesize *in vitro* an MG identical in structure to the native cell-wall polymer (Gibeaut, D.M., and N.C. Carpita, 1993, *Proc.Natl.Acad.Sci.* USA **90**,3850-3854). We also observed a callose synthase activity that co-localized with the Golgi apparatus and did not require calcium activation. Our improved procedures for isolation of the Golgi apparatus coupled with the analytical advancements for determination of structure will be used to investigate some of the factors involved in the synthesis of this polymer *in vitro*. We propose to use several analogs of UDP-Glc to photoaffinity-label proteins associated with the MG and callose synthases that are localized to the Golgi apparatus. Our ultimate goal is to use the antisera and peptide sequence information to identify from expression libraries the cDNAs of maize MG and Golgi apparatus-specific callose synthases.

Purdue University
West Lafayette, IN 47907-1153

171. Modification of Lignin Composition in Plants by Manipulation of ferulate-5-hydroxylase Expression

C.C.S. Chapple, Department of Biochemistry *$285,709 (2 years)*

Our goal is to understand key factors that determine the quality and quantity of lignin in plants, and ultimately to address this economically important issue by the directed manipulation of lignification. The resistance of lignin to chemical and biochemical degradation is a significant barrier to the utilization of lignocellulosic materials. Lignin in angiosperms is largely derived from ferulic acid and sinapic acid, and a key enzyme catalyzing this conversion is ferulate-5-hydroxylase (F5H) a cytochrome P-450-dependent monooxygenase. We propose that this enzyme is an important regulatory site governing lignin composition. To evaluate this hypothesis we have initiated the cloning and characterization of the F5H gene from Arabidopsis using a mutant of Arabidopsis that is defective in F5H.

A T-DNA-tagged allele of the *fah1* mutant has been identified. Plasmid rescue of DNA flanking the right border of the T-DNA insert yielded a fragment of Arabidopsis DNA that appears to encode a cytochrome P450-dependent monooxygenase, potentially the structural gene for ferulate-5-hydroxylase. This fragment has been used to clone cDNA and genomic clones for the putative F5H gene, and these clones are currently being sequenced in

preparation for experiments aimed at complementing the *fah1* mutation by *Agrobacterium*-mediated transformation.

We now intend to overexpress F5H in wild type and mutant Arabidopsis to manipulate lignin composition in a model system, and to study the impact of changes in F5H expression on the chemical nature of the lignin produced in the mutant and transgenic plants.

Purdue University
West Lafayette, IN 47907

172. Analysis of the PSII Proteins MSP and CP43'
L.A. Sherman, Department of Biological Sciences *$89,240*

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 will analyze site-directed and random mutants in the *psbO* gene and other Photosystem II genes to better understand the mechanism of O_2-evolution. We are studying the involvement of super-reduced states and a 5-step O_2-evolving mechanism as an explanation for anomalies that have long been associated with the O_2-flash yield experiments. During this next period, we will screen for mutations using a digital imaging spectrometer (DIS), developed by D. Youvan. This device permits the analysis of 300 or more colonies per plate to rapidly screen for photosynthesis mutants. We will perform saturation mutagenesis and isolate low or high fluorescence mutations.

We will continue our studies of membrane assembly and protein localization. We have developed techniques to carefully analyze chl-proteins on gels and to localize membrane proteins by EM immunocytochemistry. We have determined that there is a radial asymmetry in *Synechococcus* sp. PCC7942 that results in a high percentage of PSI and ATPase in the outermost thylakoid, whereas PSII and cyt b_6/f are located more evenly throughout the thylakoids. With these techniques, we will search for contact zones between the thylakoid and the cytoplasmic membranes; these connections may represent membrane insertion sites.

Rice University
Houston, TX 77251

173. Genetic and Biochemical Analysis of Solvent Formation in Clostridium acetobutylicum
G.N. Bennett and F.B. Rudolph, Department of Biochemistry and Cell Biology *$90,210*

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 toward production of desired products. Two factors that limit butanol production in continuous cultures are: 1) The degeneration of the culture, with an increase in the proportion of cells which are incapable of solvent production. Currently isolated degenerate 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, loss of regulatory element or an increase in general mutagenesis. 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. These focus on hydrogenase and ferredoxin and rubredoxin and their oxidoreductases.

University of Rochester
Rochester, NY 14627-0166

174. Synergism and Interaction Between Clostridium thermocellum Major Cellulosome Components, CelS and CelL

J.H.D. Wu, Department of Chemical Engineering *$73,672*

Clostridium thermocellum produces an extracellular cellulase system highly active on crystalline cellulose. The cellulase activity resides mainly in an extremely complicated protein aggregate called cellulosome. We have proposed a novel anchor-enzyme model involving two major cellulosome subunits, CelL (M_r=250,000) and CelS (M_r=82,000), which degrade crystalline cellulose synergistically. The model depicts that CelL functions as an anchor on the cellulose surface for CelS, the major catalytic subunit. Molecular cloning of the *celL* and *celS* genes have revealed remarkable structural features. CelL consists of nine repeated domains separated by a non-repeated domain. The *celS* gene shows no global homology to the known cellulase genes and therefore belongs to a new cellulase family.

The overall goal of this project is to study the catalytic activities, protein-protein interactions, enzyme-substrate interactions, and synergism in the cellulosome by focusing on CelL and CelS. The *celS* gene has been expressed in *E. coli*. The rCelS displays activities typical of an exoglucanase. Its mode of action and enzymatic properties will be further characterized. The genes coding for the repeated and non-repeated domains of CelL have also been subcloned and expressed in *E. coli*, respectively. These two classes of domains are being characterized for the catalytic, subunit binding and cellulose binding activities. Finally, the interactions between CelS and CelL domains will be studied to determine the structure and the stoichiometry of the complex. The study will shed light into this extremely unconventional, intriguing, and sophisticated enzymatic process of cellulose degradation.

Rockefeller University
New York, NY 10021

175. Characterization of the Systemic Acquired Resistance Immediate-Early Response to Salicylic Acid
N.-H. Chua, Laboratory of Plant Molecular Biology *$180,420 (2 years)*

Salicylic acid (SA) has been shown to be an endogenous signal molecule in the plant defense response to pathogens. Recently, we have characterized a *cis* element in the promoter of the Cauliflower Mosaic Virus 35S gene which is SA-inducible in plants. The striking feature of induction is that it is rapid and transient, relative to the later induction of the SA-inducible pathogenesis-related (PR) 1a protein gene. Furthermore, SA induction of the 35S promoter is insensitive to the protein synthesis inhibitor, cycloheximide, whereas SA induction of Pr1a is blocked by cycloheximide treatment. These and additional features of the induction are consistent with a mechanism of immediate-early transcription.

In an effort to further examine the immediate early responses of plant cells to SA, we have undertaken a screen for plant genes regulated in the same manner as the 35S gene. By using the PCR-based method of differential display, we have identified several immediate-early SA-responsive genes, which are now being characterized. It is expected that some of the genes identified in this study may participate in the continuing pathway leading to the coordinate induction of PR genes in the defense response, as well as other SA-inducible functions. Ultimately, we may be able to order a cascade of gene activations over the entire period of the plant defense response.

Rutgers University
New Brunswick, NJ 08903-0231

176. The Role of Alternative (Cyanide-Insensitive) Respiration in Plants
I. Raskin, AgBiotech Center *$85,000*

Plants are generally considered poikiolotherms (organisms whose temperature is determined by the environment). Thermogenic plants, which can generate large amounts of heat in their inflorescences 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 organismal level, they still do so at the mitochondrial level. 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.

In addition to chilling, pathogenesis is another stress associated with increases in both cyanide-sensitive and cyanide-insensitive respiration. Increased respiration in the infected tissues is associated with a substantial increase in heat production. We will test the hypothesis that free radicals generated as a result of the increased respiration in plant leaves undergoing a hypersensitive response initiate the cascade of molecular events which directly leads to the increased expression of defense-related proteins. We will also assess whether salicylic acid, a known inducer of plant respiration, is the cause or the result of the respiratory burst and free radical production observed during the incompatible plant-pathogen interaction.

Rutgers University
Piscataway, NJ 08855-0759

177. Corn Storage Protein: A Molecular Genetic Model
J. Messing, Waksman Institute *$105,730*

Our studies have concentrated on the maize 27-kDa storage protein gene of the γ zein class because its borders have been determined from DNA insertions in the 5' and 3' flanking regions, different deletion end points and corresponding expression levels *in vivo*. We have characterized several DNA binding sites in the promoter region, including the -300 element that we think is important for the transcriptional activation of this and other storage protein genes. In the 3' region, we have focused on the 3' end processing of the mRNA. Since it has been known for many years that plant mRNAs deviate from animal mRNAs in their poly-A sites, any future studies on mRNA accumulation will need a better understanding of the differences between plant and animal 3' end mRNA processing. We have now shown that the preferential poly-A site in the 27-kDa zein gene is AATGAA rather than AATAAA, the 3' proximal site is used, but can be substituted by a second upstream AATGAA and that the GT element, found 3' of the processing site, is not required in contrast to animal genes. On the other hand, upstream sequences and their spatial arrangement are critical for the selection of the poly-A site and efficient 3' end processing. Furthermore, these signals are not tissue specific and are conserved between tobacco and maize cells.

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

178. Signal Transduction Pathways that Regulate CAB Gene Expression
J. Chory, Plant Biology Laboratory *$99,910*

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 *cab*3 promoter-marker gene chimeras to select for mutants in which the light-regulated *cab*3 promoter is aberrantly expressed at high levels in the dark, at low levels in the light, 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 at least 3 complementation groups. A subset of the *doc* mutations also affect the levels of *rbc*S mRNAs in dark-grown mutant seedlings. Thus, the *doc* mutations identify a branch point in which the control of *cab* gene expression can be genetically separated from *rbc*S expression. In addition, at least two loci have been identified, mutations which result in light-grown seedlings that express *cab* and *rbc*S mRNAs at only 10% of wild-type levels. In *gun* (***g***enomes ***un***coupled) mutants, the expression of *cab* and *rbc*S genes has become uncoupled from the expression of photosynthetic chloroplast genes. Detailed biochemical and genetical characterization of these signal transduction mutants is in progress. Experiments are underway to clone the *DOC1* and *GUN1* loci.

The Scripps Research Institute
La Jolla, CA 92037

179. Genetic Engineering with a Gene Encoding a Soybean Storage Protein
R.N. Beachy, Department of Cell Biology *$96,999*

The ß-conglycinins are soybean storage proteins encoded by genes that are tightly regulated both spatially and temporally. We are studying the DNA sequences that lie both upstream and distal to the coding sequences of the α' and ß-genes of the ß-conglycinins in our research to identify those sequences that are responsible for the regulated expression of these genes. Although the α' and ß-genes are members of the same gene family, they exhibit different patterns of gene expression. The Soybean Embryo Factors (SEF) are identified as the nuclear proteins that bind specifically to sequences, i.e., the SEF binding sites, that are upstream of the α' and ß-genes. In ongoing experiments to identify DNA sequences that contribute to the patterns of expression of the α' and ß-subunit genes we constructed a series of chimeric genes comprising different fragments of the promoters of each gene as well as 3' distal sequences of the genes combined with the *uidA* reporter gene for expression in transgenic plants. Analysis of the transgenic plants is currently in progress and will be complete during 1994.

Previous attempts to purify the SEF proteins by standard chromatographic and affinity techniques were unsuccessful and a more direct approach has been taken, namely developing cloned cDNAs that encode proteins that bind to DNA sequences upstream of the α' and ß-gene promoters. After expending considerable effort on screening our lambda phage expression libraries with no clear success, we have used a yeast single plasmid system in which to express cDNAs derived from mRNAs from soybean seeds. In this system, sequences comprising multimers of SEF-binding sites are linked to a yeast core promoter. Expression of chimeric genes comprising the cloned cDNA with the activator for the yeast

promoter will select colonies that contain potential DNA binding proteins of the cis sequences that regulate expression of the soybean genes. To date we have identified two cloned sequences that potentially encode DNA binding proteins, and are characterizing the clones and their encoded proteins by *in vitro* assays.

The Scripps Research Institute
La Jolla, CA 92037

180. Targeting Pathway for Plasma Membrane Proteins in Plants
J. Harper, Department of Cell Biology *$182,360 (2 years)*

In eukaryotic cells, specialized membrane proteins are required for the function of the plasma membrane. One important function of this membrane system is to transport nutrients into the cell. Nutrient transport is a very active process in some cell types, such as root epidermal cells engaged in fertilizer uptake from the soil. In plant cells, many transport systems are driven by the energy of a proton gradient. A proton gradient is created across the plasma membrane by a proton pump which pumps protons out of the cell. The potential energy of this gradient is then used by proton-coupled co-transporters to bring nutrients such as phosphate and potassium into the cell.

In plants, it is not known how specific pumps and transporters are targeted to their correct location at the plasma membrane. Understanding this pathway is critical to designing new transporters for expression in plants -- an unexplored frontier which may permit plants to be engineered with new solute uptake systems useful to crop improvement and bioremediation.

The long range objective of the proposed research is to understand the rules which govern the targeting of integral membrane proteins to the plasma membrane. The hypothesis to be tested is that plasma membrane targeting requires specific information. The approach is to both delete and add targeting information to a plasma membrane proton pump and an *E. coli* lactose transporter and determine their fate in transgenic *Arabidopsis* plants. The purpose is to determine the "default" targeting location of a non-plant membrane protein, and to determine what information is required to redirect its targeting to the plasma membrane.

The Scripps Research Institute
La Jolla, CA 92037

181. Nuclear Genes Regulating Translation of Organelle mRNAs
S. Mayfield, Department of Cell Biology *$182,000 (FY 93 funds/2 years)*

The general aims of this project are to 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 five proteins that bind with high affinity and specificity to the 5' UTR of the psbA mRNA. The binding of these proteins to their target RNA is modulated *in vivo* in response

to light. We have identified two biochemical mechanisms which regulate the binding of proteins to the psbA RNA *in vitro*; redox potential and ADP-dependent phosphorylation. Both of these mechanisms are potential modulators of RNA binding *in vivo* in response to light as both redox potential and ADP concentration fluctuate in plants in response to light via photosynthesis. We have generated antisera for several members of the psbA binding complex and have cloned a cDNA for one member of the complex (a 47 kDA protein, RB47). These reagents will be used to characterize the molecular mechanism by which these proteins and RNAs interact to regulate translation. The analysis of translational regulation in *C. reinhardtii* will help us understand basic principles in nuclear/organelle interaction in coordinate gene expression, and provide insight into fundamental aspects of translational regulation, an important but not well understood aspect of gene regulation in plants.

University of South Carolina
Columbia, SC 29208

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

L. Marton, Department of Biological Sciences *$90,210*

Genetic manipulation of plants often involves introducing homologous or partly homologous genes. Ectopic introduction of homologous sequences into plant genomes may trigger epigenetic changes, 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). A binary plant transformation/targeting vector family based on the *nia2* nitrate reductase (NR) gene of *Arabidopsis thaliana* has been developed with the corresponding single target gene recipient system, that is a G5 x WT heterozygote plant (G5 is a NR^- mutant with a major [<25 kb] deletion). The aim is to simultaneously analyze the ratios of gene replacement, random insertion, and other mutations occurring within the target locus during transformation with different constructs.

The core HGT construct with 5' and 3' deletions of the *nia2* gene, combined with four newly developed negative selection markers is being used for transformation of explants from sustained root cultures derived from a G5 x WT plant. There are two basic approaches in progress for obtaining HGT events: (i) isolation of chlorate resistant (that is NR^-) lines first, and further analysis of them; (ii) selection for the positive selection marker, which is within the *nia2* homologous region, and against the negative selection marker combinations outside the homologous region of the donor construct. Classification of clones for homologous gene targeting, random insertion, and other mutations are in progress.

Southern Illinois University
Carbondale, IL 62901

183. Regulation of Alcohol Fermentation by Escherichia coli
D.P. Clark, Department of Microbiology *$187,986 (2 years)*

The purpose of this project is to elucidate the way in which the synthesis of ethanol and related fermentation products are regulated in the facultative anaerobe *Escherichia coli*. We are also investigating the roles of certain genes which are induced during fermentative growth. Structural and regulatory mutations affecting the expression of the fermentative alcohol dehydrogenase have been isolated. The *adhE* 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 conditions appears to play the major role in regulating the *adhE* gene. The protein encoded by the *adhE* 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 also been sequenced and although clearly homologous to that of *E. coli*, shows significant differences. Corresponding differences in kinetic behavior of the ADH activity are being investigated. The *ldhA* gene, encoding the fermentative lactate dehydrogenase has also been cloned and sequenced. We have also sequenced the regulatory region and have started to construct gene fusions between *ldh* and the *cat* and *lacZ* genes. The mechanism of *ldhA* induction in response to a acidic conditions will be investigated using these fusions. Many anaerobically induced gene fusions can be switched on in air in the presence of cyanide or chelating agents. Some at least of these effects are due to the Arc AB, dual components regulatory system.

Stanford University
Stanford, CA 94305-5025

184. The Effect of Oligosaccharides on Glycoprotein Stability
C. Khosla, Department of Chemical Engineering (formerly C.F. Goochee) *$95,060*

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

A 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 glycoprotein(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

185. Nodulation Genes and Factors in Rhizobium-Legume Symbiosis
S. R. Long, Department of Biological Sciences *$214,549*

Our work concerns the nitrogen fixing symbiosis of *Rhizobium meliloti* and its legume host, alfalfa. Using biochemistry and genetics, we are studying signals important in early stages of the bacteria-plant interaction. This work may reveal limiting factors for establishment of symbiosis, which in turn may be useful in improving or extending symbiotic nitrogen fixation and eventually decreasing the use of energy intensive chemical nitrogen fertilizers. We are studying the bacterial signals, called Nod factors, that cause the plant host to form symbiotic root nodules, and are examining the initial period of the host response. Our work on the signals includes optimizing preparative procedures, and trying to determine biosynthetic pathways. 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 an affinity bead method for isolation of these morphogenic Nod factors from bacterial culture supernatants. We have established that some nod genes encode enzymes that synthesize Nod factor. We have recently 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 an 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

186. Biochemical and Molecular Characterization of Enzymes for Cell Wall Synthesis

P.M. Ray, Department of Biological Sciences *$197,880 (2 years)*

Our laboratory is attempting to identify polypeptides responsible for enzymatic synthesis of cell wall polysaccharides (sugar polymers), which are the source of most of the renewable fuels available to mankind. We have targeted two such enzymes: plasma membrane-associated glucan synthase-II (GS-II) and Golgi-associated GS-I. By a complex purification procedure, together with direct photolabeling and antibody evidence, we can associate GS-II activity of pea seedlings with 2 polypeptides of 55 and 70 kDa. Separately, by affinity chromatography we have isolated in pure form a polypeptide doublet of about 40 kDa which various evidence suggests is part of the Golgi polysaccharide synthase system, which forms cell wall matrix (i.e., non-cellulosic) polysaccharides. Our current goal is to clone the genes corresponding to the 55-kDa, 70-kDa and 40-kDa polypeptides, and use the cloned sequences or their gene products in tests designed to show whether they actually are polysaccharide synthase components and, if so, to get information on how they act and are regulated.

By screening two pea cDNA expression libraries using antisera against the 40, 55, or 70-kDa polypeptides we have isolated several antibody-positive clones for each of these polypeptides. These clones are currently being characterized. One way of authenticating their relationship to the polypeptides of interest is of course to compare the amino acid sequences that their open reading frames specify, with sequences from the 40, 55, or 70-kDa polypeptides. This has become complicated, for different reasons in the case of the different polypeptides. For the 55, sequence data base search suggests that the polypeptide preparation we used as antigen and as sequencing substrate may contain more than one kind of polypeptide. The possibility of such contamination arises because our purification procedure for the intractable 55- and 70-kDa polypeptides has a very low yield and cannot be scaled up to produce the amounts of purified polypeptides that would be needed for sequencing or raising antiserum. For these purposes we instead used 55 and 70-kDa bands from gels run simply from isolated plasma membrane material. One way by which we hope to distinguish contaminants from genuine subunits of GS-II is to use each of our expression clones to adsorb, from the antiserum that was used to screen for that clone, whatever antibodies are specially directed against that clone's gene product. After being eluted, these antibodies will be tested for ability to immunoadsorb GS-II activity, or to recognize on a Western blot the appropriate polypeptide from our highly purified GS-II preparation. Positive results will identify a clone for a GS-II subunit.

University of Tennessee
Knoxville, TN 37996

187. Plant Recognition of Bradyrhizobium japonicum Nod factors
G. Stacey, Department of Microbiology *$86,330*

We are studying the nitrogen-fixing symbiosis between *Bradyrhizobium japonicum* and soybean. *B. japonicum* infects soybean roots and induces the formation of a nodule, a new organ in which the bacterium resides. Organogenesis of the nodule is induced by lipo-oligosaccharides synthesized by the products of the bacterial nodulation genes. We have chemically characterized 10 different substituted lipo-chitose nodulation signals produced by *B. japonicum* and *B. elkanii*, also a symbiont of soybean. Recently, we have shown that the presence of 2-0-methylfucose on the terminal, reducing N-acetylglucosamine residue is critical for biological activity. We have also identified glycerol substituted nodulation signals that we believe may represent biosynthetic intermediates. Data indicate that the production of a variety of nodulation signals may be important for the interaction of the bacterium with a variety of host species and may also play an important role in competition between strains for nodulation of a single host. We now have a variety of chemically synthesized nodulation signals with which to test these hypotheses. These molecules are biologically active proving that the structures previously determined are correct and do account for the activity seen. Future work will focus on a molecular characterization of the recognition and response of 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

188. Regulation of Chloroplast Number and DNA Synthesis in Higher Plants
J.E. Mullet, Department of Biochemistry and Biophysics *$78,819*

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

189. Characterization of a 1,4-ß-D-glucan synthase from Dictyostelium discoideum
R.L. Blanton, Department of Biological Sciences

$69,354 (FY 92 funds/3 years)

Progress in the study of eukaryotic cellulose synthesis continues to be slow. Using the cellular slime mold *Dictyostelium discoideum* as a model organism for eukaryotic cellulose synthesis has several advantages: (1) there exists an *in vitro* assay for the cellulose synthase in which the product has been extensively characterized; (2) the cellulose synthase activity is inducible; and (3) molecular methods such as gene disruption can be applied to the cellulose synthase problem.

In collaboration with Dr. R.R. Drake (University of Arkansas School for Medical Sciences) we have used a UDPG photoaffinity probe to identify UDPG-binding proteins in crude membrane preparations. We identified a few UDPG-binding polypeptides that are developmentally regulated in a pattern similar to that of the cellulose synthase activity. These will be pursued in further studies.

We have purified *D. discoideum* plasma membranes in an effort to determine the membrane fraction localization of the cellulose synthase activity. The initial data suggest that the enzyme activity is associated with the plasma membrane fraction. In the course of this study, we discovered that membranes from cells disrupted in the presence of Ca^{2+} were inactive. This observation will be pursued to determine if there is a Ca^{2+}-dependent inhibitor of the cellulose synthase.

We have renewed our genetic efforts by applying a new method for generating tagged mutants in *Dictyostelium*. Although the screens for cellulose-deficient mutants will still be difficult, this new method has the advantage of allowing the isolation of the affected gene in any mutant.

Texas Tech University
Lubbock, TX 79409

190. Ferredoxin-Linked Chloroplast Enzymes
D.B. Knaff, Department of Chemistry and Biochemistry

$172,000 (FY 93 funds/2 years)

Oxidation-reduction titrations have been performed on the two prosthetic groups of the ferredoxin-dependent nitrite reductase of spinach leaves using two different

electrochemical techniques. E_m valves of -290 mV for the siroheme and -365 mV for the [4Fe-4S] cluster were determined. The latter value, which is significantly more positive than those measured previously and is likely to be free from artifacts that affected the earlier determinations, places the [4Fe-4S] cluster midway on the electrochemical scale between the siroheme site for nitrite reduction and ferredoxin, the physiological donor to the enzyme. Laser flash photolysis measurements have provided the first evidence for electron flow between the two prosthetic groups of the enzyme and have provided support for the hypothesis that electrostatic interactions between ferredoxin and nitrite reductase play an important role in the reaction mechanism. Chemical modification studies using N-bromosuccimide point to the participation of a single tryptophan in the electron transfer reaction catalyzed by nitrite reductase. The complete nucleotide sequence of a cDNA clone encoding a second ferredoxin-dependent, spinach chloroplast enzyme glutamate synthase - was determined and portions of the predicted amino acid sequence confirmed by amino acid sequencing of the purified enzyme.

University of Texas
Austin, TX 78713-7640

191. Molecular, Genetic, and Biochemical Analysis of Cellulose Synthesis in Arabidopsis thaliana

R.M. Brown, Jr., and K. Sathasivan, Department of Botany *$166,840 (2 years)*

Cellulose, the most abundant biopolymer, is a major constituent of cell walls (greater than 40%). It is structurally very important to plant cell growth, development, and defense response against biotic and abiotic stresses. Economically, cellulose is a major renewable industrial product (cotton and trees) and energy source (wood). Yet, the fundamental biological mechanisms underlying cellulose biosynthesis, assembly, and regulation are vastly unknown. We are investigating the biochemistry and molecular biology of cellulose biosynthesis using *Arabidopsis thaliana* as the model system because of the small genome size and the extensive genetic databases now available. Considering the complexity of the problem in studying a heteromeric, multi-subunit membrane protein--cellulose synthase--we are using a multidisciplinary approach (biochemical and molecular) to advance our basic knowledge on cellulose biosynthesis. Recent breakthroughs in the achievement of *in vitro* cellulose synthesis from the cotton fiber in our laboratory is being used as the basis for the biochemical investigation. Preliminary results have demonstrated a successful *in vitro* cellulose product synthesized from *Arabidopsis* plasma membrane preparations. We are using actively growing *Arabidopsis* plants for isolation and characterization of the polypeptides involved in cellulose biosynthesis. Antibodies will be prepared against these polypeptides and used to clone the genes and to characterize gene products. The n-terminal or internal amino acid sequences, chemically determined from these polypeptides, will be used for the molecular cloning of the corresponding genes. In addition, T-DNA-tagged *Arabidopsis* mutants are being screened to identify and analyze the genetic mutations impairing cellulose biosynthesis and its regulation. These results should unravel the biological machinery in cellulose biosynthesis and reveal the genetic control of the regulation and assembly of this

important natural biopolymer. This advancement in knowledge should facilitate crop improvement and better energy utilization for the future.

University of Utah
Salt Lake City, UT 84112

192. The Plant Mitochondrial mat-r Gene/nad1 Gene Complex
D.R. Wolstenholme, Department of Biology *$91,180*

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 close to the 3' end of all introns that are *trans*-spliced. Further analyses of cDNAs are 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. Run-off transcripts of maize cDNAs will be employed to determine whether, *in vitro*, the continuous intron can self-*cis*-splice, and the discontinuous introns can self-*trans*-splice. Transcripts of maize and soybean *mat-r* gene-containing cDNAs will be used in an *in vitro* rabbit reticulocyte lysate translation system to synthesize MAT-R proteins. 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

193. Enzymology of Acetone-Butanol-Isopropanol Formation
J.-S. Chen, Department of Biochemistry and Anaerobic Microbiology
$175,807 (2 years)

Several species of anaerobic bacteria within the genus *Clostridium* produce acetone, butanol, ethanol and isopropanol, which are important solvents as well as chemical feedstocks and fuel additives. The goal of the project is to understand the fundamental properties of the solvent-forming fermentation so that it will be possible to prevent degeneration of useful traits, 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 strategies for regulating the expression of solvent-production genes and the flow of metabolites. We have purified and

characterized the key enzymes required for solvent formation by *Clostridium beijerinckii*, a species producing isopropanol in addition to acetone and butanol. Solvent-producing clostridia have a multiplicity of aldehyde and alcohol dehydrogenases. Our present study has an emphasis on these dehydrogenases. Four distinct primary alcohol dehydrogenases (ADHs) have been identified, and three of them are isozymes that can use both NADH and NADPH as the coenzyme. These isozymes have been purified and they consist of two homodimers (α_2 and $ß_2$) and a heterodimer ($\alpha ß$). The role of the ADH isozymes in solvent production is being examined. Isopropanol-producing *C. beijerinckii* contains a primary/secondary ADH that is equally active toward aldehydes and ketones. The structural gene (*adh*) for the primary/secondary ADH has been cloned, sequenced, and expressed in *Escherichia coli*. Immediately upstream to the *adh* gene is a putative gene (*stc*), which may encode a signal-transducing transcription regulator. We are using the cloned *adh* gene as a reporter gene to study the control elements for solvent production.

Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

194. Enzymological Studies of One-Carbon Reactions in the Pathway of Acetate Utilization by Methanogenic Bacteria

J.G. Ferry, Department of Anaerobic Microbiology *$91,180*

Several enzymes in the pathway of acetate conversion to CH_4 and CO_2 have been purified from *Methanosarcina thermophila*. The enzyme mechanisms 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 overproduced in *Escherichia coli* and purified to homogeneity; site-directed mutagenesis studies are in progress to identify active sites. Cloning and sequencing of the genes for the two subunits of the cobalt/iron-sulfur component of the CO dehydrogenase complex have been accomplished. The genes have been hyperexpressed, and the subunits overproduced. Purification and biochemical characterizations of the independently-overproduced subunits are in progress. Ferredoxin is an electron acceptor for the nickel/iron-sulfur component of the complex which cleaves acetyl-CoA and oxidizes the carbonyl group to CO_2. This ferredoxin has been further characterized by determining the properties of the iron-sulfur centers. A second ferredoxin gene has been discovered. The function of this ferredoxin is unknown but the gene is transcribed only when cells are grown on methanol. Experiments are in progress to overproduce the second ferredoxin for biochemical characterization and to determine the physiological function during methanol metabolism. The gene encoding carbonic anhydrase has been cloned and sequenced, and the enzyme overproduced. Biochemical characterizations of the overproduced carbonic anhydrase are in progress.

University of Virginia
Charlottesville, VA 22901

195. Structural Domains in NADPH:Protochlorophyllide Oxidoreductases Involved in Catalysis and Substrate Binding

M.P. Timko, Department of Biology *$176,540 (2 years)*

Chlorophylls play a fundamental role in the energy absorption and transduction activities of all photosynthetic organisms. The formation of these compounds is regulated in part by the light-dependent reduction of protochlorophyllide to chlorophyllide catalyzed in most plants by a nuclear-encoded, plastid-localized enzyme known as NADPH:protochlorophyllide oxidoreductase. Protochlorophyllide reductase requires reduced pyridine nucleotides (NADPH) and stoichiometric amounts of light quanta for enzymatic activity. Despite its critical regulatory role in chlorophyll synthesis and its function as a photoreceptor involved in the control of chloroplast development and overall plant growth and differentiation, little is known about the structural domains in the protein required for catalysis, NADPH and Pchlide binding, or association with the thylakoid membrane. We have developed a novel light-dependent expression system for the mature pea protochlorophyllide reductase protein based on functional complementation of protochlorophyllide reduction mutants in the photosynthetic bacterium *Rhodobacter capsulatus*. Using this expression system we are evaluating clustered charged-to-alanine scanning mutants and site-directed mutants of the enzyme for their ability to catalyze light-dependent protochlorophyllide reduction. Our goal is to identify mutant enzymes exhibiting altered specific activity, substrate binding and catalytic efficiency, and the ability of the enzyme to associate properly with the thylakoid membrane. A second aspect of our studies is to establish conditions for the production of high quality crystals of the protochlorophyllide reductase protein and work toward solving its three-dimensional structure. Information on the molecular architecture of the protein will enhance both our current structure-function studies as well as direct subsequent mutational analyses. When completed, these combined studies will provide significant information on one of the most crucial biosynthetic steps in the development of photosynthetic organisms.

Washington State University
Pullman, WA 99164-6340

196. Membrane Function in Lipid Mutants of Arabidopsis

J. Browse, Institute of Biological Chemistry *$101,850*

Our investigations of the biochemistry of wild type and mutant plants have provided us with new information about the enzymology and regulation of lipid metabolism in higher plants. They have revealed, for example, that there is a significant two-way exchange of lipid molecules between the chloroplast and other membrane sites in the cell. They also demonstrate the versatility in metabolism that allows flux of carbon through the two pathways of lipid synthesis to be adjusted in response to a mutation in one pathway or the other. Several of the mutants have striking phenotypes. For example, the *fab2* mutant has a profound dwarf growth habit that is a consequence of increased levels of stearate in these

plants. Dwarf growth in *fab2* results from cell specific changes in cell expansion and maturation processes. These cellular changes occur without distorting the chronology of development, or the metabolic integrity of the organism. A second site suppressor mutation restores both normal fatty acid composition and normal body morphology, causally linking the two phenotypes. Growth of the *fab2* mutant at high temperature substantially corrects the morphological phenotype without altering the fatty acid composition, suggesting that the aberrant morphology is directly caused by an alteration in membrane structure which in turn is a consequence of the increased levels of stearate.

Washington State University
Pullman, WA 99164-6340

197. Regulation of Terpene Metabolism
R. Croteau, Institute of Biological Chemistry *$109,125*

Terpenoid oils, resins and waxes from plants are important renewable resources with a range of industrial, pharmaceutical, food and industrial uses. 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 in part 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 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 used to examine transcriptional and translational control of metabolism. A method for the isolation of viable oil glands is combined with an HPLC-based protocol for metabolite separation and quantification to determine flux through the various branches of the pathway and measure intracellular concentrations of key intermediates, thereby revealing other control points of the biosynthetic sequences. Results from this project will target regulatory steps for genetic engineering to increase the yields and types of terpenoid natural products that can be made available for industrial exploitation.

Washington State University
Pullman, WA 99164

198. Carbon Metabolism in Symbiotic Nitrogen Fixation
M.L. Kahn, Institute of Biological Chemistry *$162,000 (FY 93 funds/2 years)*

Combined nitrogen is the nutrient that most often limits plant growth. For this reason, nitrogen fertilizer is an important input for increasing crop yield. However, producing nitrogen

fertilizer is expensive and energy intensive and excess fertilizer can increase nitrate in groundwater and soil acidity. Some plants are able to obtain nitrogen through symbiotic associations with nitrogen-fixing bacteria. The plant exchanges photosynthetically derived carbon compounds for ammonia produced by the bacteria. 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 are investigating *R. meliloti* TCA cycle mutants, including those with defects in citrate synthase, isocitrate dehydrogenase and oxoglutarate dehydrogenase, and are developing conditional TCA cycle mutants to probe the relationship between the TCA cycle and nodule development and metabolism. We are also investigating the molecular genetics of plant NADP-isocitrate dehydrogenases by studying several cDNAs we have cloned that code for various forms of the enzyme.

Washington State University
Pullman, WA 99164-6340

199. A Comprehensive Approach to Elucidation of Lignification at the Plasma Membrane/Cell Wall Interface
N.G. Lewis, Institute of Biological Chemistry *$350,403 (2 years)*

In woody plants, and presumably also in cereals/grasses, there are a series of cell wall associated oxidases and peroxidases which catalyze a continuum of phenylpropanoid coupling reactions at the plasma membrane/cell wall interface leading to the lignins. Recent evidence strongly suggests a highly ordered sequence of polymer assembly. We are purifying, cloning and overexpressing these oxidases and peroxidases in *Pinus taeda/Forsythia intermedia* in order to systematically define both the mode of catalytic action and the sequential steps involved; the effects of amplifying/suppressing gene expression of each protein on lignin formation and structure will also be determined. As an additional goal, the fate of nitrogen during phenylalanine deamination will be examined.

Washington State University
Pullman, WA 99164-6340

200. Interdisciplinary Plant Biochemistry Research and Training Center
N.G. Lewis, Institute of Biological Chemistry *$407,000 (18 months)*

Fundamental plant biochemistry has not kept pace with the demands of molecular biology, and the store of current biochemical knowledge is being rapidly depleted. United States biotechnology companies warn that, very soon, future biotechnological developments will become critically dependent upon new (targeted) discoveries in plant biochemistry.

Washington State University recognizes this urgent need, and its broader impact upon the U.S. research enterprise. To respond effectively to this challenge, a Plant Biochemistry Research and Training Center will be established which interfaces its biochemistry program with the major plant sciences. Although the rigorous training of graduate and undergraduate students will be the major emphasis of this endeavor, three research areas of strategic importance will be targeted; these include plant cell wall formation (e.g., how fiber formation occurs, and how novel biopolymers are formed), regulation of biochemical pathways (e.g., to enhance productivity of plant derived substances for alternate energy sources, food reserves, pharmaceuticals and fine chemicals), and signal transduction mechanisms (e.g., to offset or reduce the need for pesticides, etc.).

Washington State University
Pullman, WA 99164-6340

201. Enhancement of Photoassimilate Utilization by Manipulation of the ADPglucose Pyrophosphorylase Genes

T.W. Okita, Institute of Biological Chemistry *$84,390*

The goal of this project is to increase the conversion of photosynthate into starch via manipulation of ADPglucose pyrophosphorylase (ADPG-PP), a key regulatory enzyme of starch biosynthesis. Temporal and spatial expression of these genes encoding each subunit as well as their correlation to starch biosynthesis have been elucidated. Our analyses of transcript and antigen levels indicates that the expression of the ADPG-PP genes is primarily under transcriptional control in developing tubers. In contrast, small subunit transcript levels are subjected to pronounced post-transcriptional control in leaf tissue. This post-transcriptional control is also evident during photoperiod and during the sucrose-induced expression of these genes in leaf tissue. Despite the dramatic increases in transcript levels and, in turn, starch levels, antigen and enzyme activity levels of either subunit remained either constant or decreased under these conditions. We conclude based on these results that the allosteric control of ADPG-PP plays a dominant role under these conditions. Using a bacterial expression system, each of the subunit genes have been subjected to random chemical mutagenesis. The resulting mutants were divided into six classes based on their capacity to complement the function of *glg*C, which encodes the bacterial ADPG-PP, and levels of measured enzyme activity under saturating catalytic and allosteric conditions. Analysis of these mutants should provide insights on the structure/function relationships of the tuber ADPG-PP and possibly the means to genetically manipulate starch levels in storage organs.

Washington University
St. Louis, MO 63130

202. Processing and Targeting of the Thiol Protease, Aleurain
J.C. Rogers, Department of Biology *$90,210*

Clathrin-coated vesicles are known to transport proteins from the Golgi to the vacuole in plant cells. The mechanism(s) by which proteins are sorted into this pathway is not known. We have identified an integral membrane protein of 80 kDa, extracted from clathrin coated vesicles of developing pea (*Pisum sativum* L.) cotyledons, that bound at neutral pH to an affinity column prepared with the N-terminal targeting determinant of the vacuolar thiol protease, proaleurain, and eluted when the pH was lowered to 4. The protein was not retained on a control column prepared with the N-terminal sequence of a homologous, secreted thiol protease, endopeptidase-B. *In vitro* studies demonstrated a binding constant of 37 nM between the 80 kDa protein and the proaleurain targeting determinant. A peptide with a vacuolar targeting determinant from prosporamin weakly competed for binding to the 80 kDa protein, while a peptide carrying a single amino acid substitution known to abolish prosporamin vacuolar targeting had no measurable binding affinity for the protein. The binding protein is a glycoprotein with a transmembrane orientation in which the carboxy-terminus is exposed to the cytoplasm. The binding domain is located in the N-terminal luminal portion of the protein. These properties of the binding protein are consistent with the function of a receptor that would select proteins in the trans-Golgi for sorting to clathrin-coated vesicles and delivery to the vacuole. Work to clone a cDNA for this potential vacuolar targeting receptor is underway.

Washington University
St. Louis, MO 63130

203. Plant Cell Wall Architecture
J.E. Varner, Biology Department *$134,046 (2 years)*

We continue our interest in the biosynthesis of wall components and in the assembly of these components into walls. We are especially interested in xylogenesis. This process involves hormonal induction, microtubule reorganization, microtubule-oriented secondary cell wall thickening, lignin deposition and breakdown of cytoplasm. We are using the *in vitro* xylogenesis system from isolated *Zinnia* mesophyll cells in conjunction with whole plants. By subtractive hybridization methods we have obtained several different cDNA clones thought to represent genes transcribed frequently during xylogenesis. These genes include a putative lipid transfer protein, adenylate kinase, a proteinase, an O-methyltransferase, a proline-rich protein, a ribonuclease, cinnamate hydroxylase and S-adenosylmethionine synthase. By tissue print hybridization we have found the following patterns of gene expression *in planta*: the putative lipid transfer protein is expressed in the cambial regions; the O-methyltransferase in cells undergoing lignification; and the ribonuclease and proteinase in the maturing tracheary elements. Biochemical assays on extracts of the cultured cells show that the O-methyltransferase activity increases coincident with lignification. Biochemical assays in the

cell culture medium show an increased production of hydrogen peroxide that is coincident with lignification.

Wayne State University
Detroit, MI 48201

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

R. Needleman, Department of Biochemistry *$82,103*

Our goal is to understand how the light activated proton pump bacteriorhodopsin (BR), transports protons. To achieve this we have expressed mutant bacteriorhodopsins constructed by site-directed mutagenesis in the natural host *H. halobium*, and in collaboration with Janos Lanyi (University of California at Irvine), we have used these BR mutants to evaluate the role of particular amino acids in proton translocation. During the last year we have concentrated on developing an improved expression system and on defining the residues involved in proton uptake from the cytoplasmic surface. Previous *E. coli-H. halobium* shuttle vectors used mevinolin resistance as a selective marker. Mevinolin is expensive and works poorly in liquid media. The new vector system uses novobiocin resistance as the selective marker and the replication origin from the *H. volcanii* plasmid pUBP2 to allow plasmid maintenance in *H. halobium*. The current picture of the BR photocycle is that aspartate-96 is the direct acceptor of the proton on the cytoplasmic side; this proton is subsequently transferred to the Schiff base. However, we have observed proton uptake from the mutant aspartate-82→glutamine without reprotonation of aspartate-96, suggesting that aspartate-96 is not the direct proton acceptor. Water hydrogen bonded to aspartate-96 and threonine-46 is a likely candidate for the direct proton acceptor and threonine-46 and arginine-227 interact with aspartate-96 to influence the reprotonation of the Schiff base. We are continuing this work with the hope of developing an improved system for the random mutagenesis of BR and further defining the amino acids involved in proton translocation.

University of Wisconsin
Madison, WI 53706-1598

205. Regulation of Extracellular Polygalacturonase Production in Pseudomonas solanacearum

C. Allen, Department of Plant Pathology *$68,996*

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 during 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 levels 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. Expression of *pehR* is repressed by *phcA*, a previously described global regulator of many virulence functions, since *pehR* expression increases twelvefold in a *phcA* mutant background. Like *phcA* mutants, strains overexpressing *pehR* overproduce PG and exhibit increased motility. 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 53706-1381

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

A.B. Bleecker, Department of Botany *$183,912 (2 years)*

Receptor protein kinases transmit signals from the plasma membrane to intracellular targets in animal cells, and are crucial to the growth and development of the cell. We have identified a class of protein kinases in the plant *Arabidopsis thaliana* with biochemical characteristics similar to those of the animal receptor protein kinases. These plant protein kinases are membrane-associated, glycosylated, and have a similar cation requirement to the animal receptor kinases. We have also cloned a receptor-like protein kinase, designated TMK1, from Arabidopsis. This kinase is being studied by combining biochemical, molecular and genetic approaches. The native form of the TMK1 protein in plants is membrane-associated, glycosylated, and has a molecular mass of 120 kDa. Immunochemical analysis and studies using the kinase promoter with the GUS reporter gene system indicate that TMK1 is expressed in all plant tissues except the receptacle and ovary of the flower. 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. A protein:protein interaction between TMK1 molecules has been demonstrated using the yeast two-hybrid system, and is suggestive that dimerization may play an important role in signal transduction by receptor-like protein kinases in plants. Transgenic plants are now being produced that either overexpress, underexpress, 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

207. Enzymology of Biological Nitrogen Fixation
R.H. Burris, Department of Biochemistry *$102,108 (FY 93 funds/2 years)*

Nitrogenase activity is regulated by reversible ADP-ribosylation in response to NH_4^+ and anaerobic conditions in *Azospirillum brasilense*. DRAT (dinitrogenase reductase ADP-ribosyl transferase) catalyzes ADP-ribosylation, and DRAG (dinitrogenase reductase activating glycohydrolase) reactivates dinitrogenase reductase. The effect of mutations in *ntrBC* on this regulation was examined. Whereas NH_4^+ addition to *ntrBC* mutants caused a partial loss of nitrogenase activity, the effect was substantially less than that seen in *ntr*$^+$ strains. In contrast, nitrogenase activity in these mutants was normally regulated in response to anaerobic conditions. The analysis of mutants lacking both the NTRB/NTRC system and DRAG suggested that the primary effect of the *ntrBC* mutations was to alter the regulation of DRAG activity. Although *nif* expression in the *ntr* mutants appeared normal, as judged by activity, glutamine synthetase activity was significantly lower in *ntrBC* mutants than in the wild type. It may be that this lower glutamine synthetase activity delays the transduction of the NH_4^+ signal necessary for the inactivation of DRAG, resulting in a reduced response of nitrogenase activity to NH_4^+. Our data suggest that different environmental stimuli use independent signal pathways to affect the DRAG/DRAT system.

University of Wisconsin
Madison, WI 53706

208. Molecular Genetics of Ligninase Expression
D. Cullen and T.K. Kirk, Department of Bacteriology *$98,940*

Lignin depolymerization is catalyzed by extracellular enzymes of white rot fungi 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, genes encoding GLOX and several LiP isozymes were cloned and sequenced. Using pulse field electrophoretic separation of chromosomes together with meiotic recombination analysis, detailed physical and genetic maps of the *P. chrysosporium* genome have been constructed. The ten known lignin peroxidases have been mapped to three separate linkage groups, all of which are unlinked to the GLOX gene. LiP and GLOX genes have been shown to be regulated at the transcriptional level in *P. chrysosporium*, and the expression of LiP genes is dramatically altered in response to culture conditions. Transcript analysis is being extended to complex substrates such as wood and soil. Through these investigations, we hope to establish the roles and interactions of specific genes in ligninolysis, in organopollutant degradation, and in biomechanical pulping. The GLOX and LiP genes have been expressed

in *Aspergillus* under the control of the glucoamylase promoter. Heterologous expression of recombinant enzymes will facilitate basic biochemical investigation and aid the development of processes such as biological bleaching of pulps, effluent treatments, and biomechanical pulping.

University of Wisconsin
Madison, WI 53706

209. Identification of the Primary Mechanism for Fungal Lignin Degradation
K.E. Hammel, Department of Bacteriology *$145,694 (2 years)*

Fungal ligninolysis is an essential link in the terrestrial carbon cycle, and is generally thought to be catalyzed by highly oxidizing extracellular peroxidases known as lignin peroxidases (LiPs). However, there is a major problem with the LiP hypothesis, in that certain aggressive white rot fungi that selectively delignify wood have been reported to lack LiP activity. Nevertheless, LiPs are the only enzymes presently known to cleave the recalcitrant nonphenolic linkages that predominate in lignin, and even those white rot fungi that appear to lack LiPs possess LiP-like genes. To resolve this problem, we need to determine whether white rot fungi that are evident nonproducers of LiP do in fact depend on this enzyme for ligninolysis in their natural environment, wood. Specific goals of this project are (1) to infiltrate (14)C-labeled model compounds that represent the principal arylglycerol-2-aryl ether of lignin into wood specimens, and then to determine what cleavage products are formed in situ by white rot fungi when they colonize the wood; and (2) to perform similar infiltration experiments with (13)C-labeled polymeric lignin models, and then to determine by (13)C NMR spectrometry what functional group modifications are introduced into the polymer by the fungi. Preliminary data show that one reported nonproducer of LiP, *Ceriporiopsis subvermispora*, is able to degrade nonphenolic lignin structures in wood as rapidly and extensively as known LiP producers do, but that LiP is not involved in the degradative pathway.

University of Wisconsin
Madison, WI 53706

210. Organization of the R Chromosome in Maize
J. Kermicle, Laboratory of Genetics *$162,000 (FY 93 funds/2 years)*

The R region is under study with a view to determining the number, kind, and arrangement of components involved in the control of anthocyanin pigmentation. Due to chromosome segment duplication, R is organized on a modular basis. The number and orientation of segments differ between alleles. The *R* gene in each segment functions as a separate complementation group, coding for a polypeptide of the *myc* family of transcriptional activators. *R* genes are distinguished by 5' control regions having particular tissue-specific effects. Intragenic recombination serves to place differences between genes relative to sites of recessive mutation. Molecular characterization provides detail concerning the physical structure of regions of particular functional significance, such as those involved in tissue-

specific action, parental imprinting, 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

211. Carbon Monoxide Metabolism by Photosynthetic Bacteria
P.W. Ludden and G.P. Roberts, Departments of Biochemistry & Bacteriology *$89,240*

This project focuses on the biochemistry and physiology of the carbon monoxide dehydrogenase system of *Rhodospirillum rubrum*. The carbon monoxide dehydrogenase (CODH) system from *R. rubrum* carries out the oxidation of CO and production of H_2 via an associated hydrogenase. CODH is a nickel-, iron-, sulfur-enzyme that can be produced in an apo form (lacking nickel); a mutation in a gene downstream from the *cooS* (CODH) gene renders the organism unable to accumulate nickel. Crystals of the holoenzyme have been obtained in collaboration with other workers, and the determination of the crystal structure of the CODH enzyme is a high priority. Because this enzyme contains a novel Ni-Fe cluster, the determination of the prosthetic groups associated with CODH will be an important step in understanding the mechanism of action of CODH. The CO-induced hydrogenase has been significantly purified and antibodies to the large subunit of this hydrogenase have been obtained and used to detect the hydrogenase at various steps of purification. A region downstream of *cooS* has been isolated, and mutations in this region result in lack of synthesis of the *cooS, cooF*, and the *cooH* gene products in response to CO. The hypothesis is that this region encodes a regulatory gene product which is involved in regulating synthesis of the *coo* gene products in response to carbon monoxide.

University of Wisconsin
Madison, WI 53706

212. Starch Synthesis in the Maize Endosperm as Affected by Starch-Synthesizing Mutants
O.E. Nelson, Department of Genetics *$57,286*

An estimate of recombination between the *glucosidase transferasel (glt1)* locus *sugaryi (su1)* was made using *su1* and *Gametophyte factor1 (Ga1)* as markers. The *glt1* locus is about three crossover units from *su1*, and may be proximal to it although that conclusion is still tentative. A third mutant, *glt1-9201*, has been isolated from a population of plants grown from EMS-treated seed. As is the case with *glt1-9101*, the other allele isolated following EMS treatment, *glt1-9201* is very shrunken, much more so than *glt1-1*, which resulted from the insertion of a transposable element. By growing another generation from nonmutant seed of W64A X W182E (EMS-treated) X *glt1-1*, a large number of mutants affecting seed development have been isolated. A high proportion of these appear to be starch-synthesizing mutants and are being investigated. We also have evidence that there is a third enzyme in

developing maize endosperms that can catalyze a first step in converting sucrose into a substrate for starch synthesis. This is a sucrose phosphorylase (EC 2.4.1.7), which catalyzes the reaction, Sucrose + Pi <---> Glc-1-P = Fru. The deep orange color of the kernels of the genotype in which *glt1-1* occurred appears to depend on a single factor that is linked to *glt1*. The effect of this factor is to elevate the content of all the carotinoid components equally.

University of Wisconsin
Madison, WI 53706

213. Feedback Regulation of Photosynthetic Processes
T.D. Sharkey, Department of Botany *$172,000 (FY 93 funds/2 years)*

When light and carbon dioxide are in ample supply, photosynthesis becomes limited by the capacity for starch and sucrose synthesis, the primary end products of photosynthesis in leaves. The mechanisms of end-product-synthesis limitations have been worked out as a result of this research. Two important links in the feedback chain are (1) energy dependent fluorescence quenching of chlorophyll fluorescence indicating dissipation of light energy so that the rate of electron transport matches the rate of end product synthesis and (2) decreased activity (decarbamylation) of the CO_2-fixing enzyme rubisco so that its activity matches the rate of end product synthesis. A naturally occurring mutant of *Flaveria linearis* lacking most cytosolic fructose bisphosphatase (FBPase) activity and transgenic tomato and potato plants are now being studied to determine how manipulation of end product synthesis affects plant growth and yield. The ability of plants to respond to elevated CO_2 appears to depend upon a high capacity for end product synthesis. This provides information for genetic engineers designing plants for the future. Planned work includes an exploration of the relationship between manipulations of end product synthesis rate and plant growth rate. Anticipated mechanistic studies include (1) a RFLP analysis of the linkage between reduced FBPase activity and the FBPase gene and (2) an analysis of rubisco activase protein and mRNA levels when plants are grown for extended periods in feedback conditions.

University of Wisconsin
Madison, WI 53706

214. Molecular Mechanism of Energy Transduction by Plant Membrane Proteins
M.R. Sussman, Department of Horticulture *$205,640 (2 years)*

Proton pumps are membrane-bound proteins that act as biological energy transducers. The plasma membrane of plants and fungi contains an ATP-coupled proton pump (H^+-ATPase) that converts chemical into electrical energy. This enzyme is essential for the growth of plants and fungi and provides the driving force used to catalyze the uptake and accumulation of solutes. The plant/fungal plasma membrane H^+-ATPase contains a single polypeptide of Mr=100,000. Its simple polypeptide structure makes this enzyme an attractive candidate for studying the molecular basis of energy transduction. A major aim of this project is to identify aspects of the enzymes' primary structure that are essential for converting chemical into electrical energy. DNA cloning and sequencing techniques are being used to obtain the

complete animo acid sequence for ATPase structural genes present in *Arabidopsis thaliana*, a model 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 these isoforms present in the nuclear genome. Expression of the genes is being studied using Northern blots and 'epitope-tagging' to produce isoform-specific polypeptide probes. 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 53706-1590

215. Analysis of Structural Domains Required for Phytochrome Function by In Vitro Mutagenesis
R.D. Vierstra, Department of Horticulture *$100,880*

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 phytochrome 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 in oat phytochrome A including: the minimal domains necessary for chromophore attachment (residues 70-399) and assembly of the holoprotein into a red/far-red light photoreversible photoreceptor (1-672); a site necessary for dimerization (919-1093); and two domains essential for full biological activity (7-69 and 1094-1129). The two domains involved in biological activity are of interest because they have been reported to undergo conformational changes during Pr/Pfr transformations. Further refined mapping to locate critical amino acids within phytochromes A and B is in progress, especially with respect to the N- and C-terminal domains required for biological activity. 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 and will hopefully lead to an understanding of its mode of action.

Worcester Foundation for Experimental Biology
Shrewsbury, MA 01545

216. Novel Biomaterials: Genetically Engineered Pores
H. Bayley *$40,740**

A selection of nanometer-scale pores is being constructed by genetic manipulation and targeted chemical modification of α-hemolysin (αHL), a protein secreted by the bacterium *Staphylococcus aureus*. The single polypeptide chain of 293 amino acids forms oligomeric pores in membranes ~11 Å in internal diameter. Our recent studies suggest that the pore is a heptamer. By analyzing the properties of mutant and chemically modified αHLs, a working model for assembly has been devised. Monomeric αHL binds to lipid bilayers and undergoes a conformational change involving the central glycine-rich loop, which permits the formation of an oligomeric prepore complex. The open pore is formed when subunits in this complex undergo a second conformational change, involving an N-terminal segment of the polypeptide. The central loop lines a section of the transmembrane channel in the assembled pore. These mechanistic findings are allowing point mutagenesis, combinatorial mutagenesis and targeted chemical modification to be 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. For example, αHL polypeptides that can be activated by specific proteases or reversibly inactivated by divalent cations have been made. Ultimately, the new pores will be used to confer novel permeability properties upon materials such as thin films, which might then 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).

Xavier University of Louisiana
New Orleans, LA 70125

217. Molecular Characterization of Bacterial Respiration on Minerals
R. Blake II, College of Pharmacy *$86,330*

Aerobic respiration on reduced iron is a principal metabolic activity exhibited by certain chemolithotrophic bacterial that inhabit ore-bearing geological formations exposed to the atmosphere. 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 two 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. Laser Doppler

velocimetry and other physical methods commonly employed to characterize colloidal particles are currently under investigation as a means to study the mechanisms, 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.

Xavier University of Louisiana
New Orleans, LA 70125

218. Biochemistry of Dissimilatory Sulfur Oxidation
R. Blake II, College of Pharmacy *$86,067*

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 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. Another aim is to exploit laser Doppler velocimetry and other physical methods commonly employed to characterize colloidal particles to investigate the mechanism whereby these bacteria adsorb to insoluble, elemental sulfur. 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.

Yale University
New Haven, CT 06520-8104

219. Molecular Genetics of the R Supergene Family of Maize
S.L. Dellaporta, Department of Biology *$91,180*

The *R-r* complex of maize 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 is comprised of a simple *P* component, which pigments plant parts, and a *S* subcomplex separated by approximately 190 kb. The *S* subcomplex consists of a truncated *q* component and two *S* genes (*S1* and *S2*) arranged in head-to-head orientation. Sequence analysis of chromosomal breakpoints in the *S* subcomplex indicate that the breakpoint sequences show characteristics of remnants of a

transposable element, *doppia*, at these locations suggesting that transposon-mediated rearrangements may catalyze the formation of the extant subcomplex. Meiotic instability of the *S* complex results in loss of seed color (*S* activity). These events have been characterized at the molecular level and shown to result by deletion of the intragenic region separating the two *S* genes. Two of these deletion breakpoints correspond to the ends of *doppia* sequences which suggests that the transposon continues to exhibit instability. Meiotic instability of the simple *P* gene is the result of insertions into the coding region of *P*. Finally, studies have been initiated to investigate the epigenetic behavior of selected *R* alleles. In heteroallelic combinations, certain *R* alleles have the ability to alter the tissue-specific pattern of gene expression in a stable and heritable fashion. Our current investigations include an examination of the genetic requirements for heteroallelic interactions at *R*.

Yale University
New Haven, CT 06511-7444

220. Spatial Regulation of C4 Genes in C3, C4, and C3/C4 Intermediate Flaveria Species

T. Nelson, Biology Department *$200,000 (FY 93 funds/2 years)*

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

Yale University
New Haven, CT 06520-8103

221. Organization and Control of Genes for Phenolic Catabolism in Rhizobium and Bradyrhizobium

D. Parke and L.N. Ornston, Department of Biology *$88,000*

The nitrogen-fixing plant symbiotic bacteria *Rhizobium* and *Bradyrhizobium* grow at the expense of diverse phenolics which originate from lignin and plant root exudates. Most of these phenolic growth substrates are degraded via the ß-ketoadipate pathway. Specific ß-ketoadipate pathway gene probes from *Agrobacterium*, a bacterium related to *Rhizobium*, have been created in order to study the organization of genes for phenolic catabolism and to localize them to plasmids, megaplasmids or the chromosome in strains of *Rhizobium* and *Bradyrhizobium*. A novel agrobacterial regulatory gene, *pcaQ*, was cloned and ß-carboxy-*cis,cis*-muconate was identified as a co-inducer. PcaQ was found to be a member of the LysR family of transcriptional activators. Southern hybridization has revealed that *pcaQ* has homologues in rhizobia, and their functional significance in phenolic catabolism is being assessed. Contrasting with inducible regulation in *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 *pcaIJ* genes, is expressed at high levels in saprophytic and symbiotic *Bradyrhizobium*; the substrate of this enzyme supports growth of *Bradyrhizobium*. The *pcaIJ* genes from *Bradyrhizobium japonicum* will be cloned in order to explore the selective value of their high level of constitutive expression. A mutant strain of *B. japonicum* blocked in the catabolism of phenolics and hydroaromatics has been isolated and used to define its pathways of aromatic catabolism. Additional mutant strains will be constructed in order to investigate the selective value of aromatic catabolism to rhizosphere competition and survival in the soil.

Yale University
New Haven, CT 06510

222. Electroenzymology of Plant and Fungal Vacuoles

C.L. Slayman, Department of Cellular and Molecular Physiology
$232,000 (FY 93 funds/2 years)

These experiments are directed principally toward molecular/functional investigation of critical vacuolar membrane proteins in the yeast *Saccharomyces cerevisiae* and the model plant *Arabidopsis thaliana*. Current work includes kinetic characterization of cation channels: particularly YVC1, a calcium-activated channel in yeast tonoplast capable of modulating cytoplasmic calcium levels, and itself controlled by redox potential, membrane voltage, and calcium-calmodulin. Detailed analysis of YPK1 behavior is based on electrical kinetic measurements obtained by voltage-clamping of isolated vacuolar membrane patches and of bilayer membranes doped with partially purified channel protein. Purification of the protein is being carried out by calmodulin affinity chromatography, and cloning of the structural gene

is being attempted via a PCR strategy using consensus sequences for "channel" and for calmodulin binding. Electrical-kinetic characterization of the primary vacuolar ion pump in *Saccharomyces*, the oligomeric V-type H^+-ATPase, is also being initiated since the constituent polypeptides have been intensely studied (cloned, sequenced, mutated) in several other laboratories. The objective of the new experiments is structure-function analysis based on chimeric peptide oligomers.

Complementary experiments, mostly involving electrical-kinetic measurements are also being carried out on pumps and channels in the plasma membranes of *Saccharomyces, Neurospora*, and *Arabidopsis*, including the (P-type) H^+-ATPase, outward rectifying K^+-channels (YPK1 in *Saccharomyces*), an inward rectifying K^+-channel from *Arabidopsis*, and an inward rectifying current associated with two genes for K^+ uptake in yeast.

Yale University
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223. Transfer RNA Involvement in Chlorophyll Biosynthesis
D. Söll, Department of Molecular Biophysics and Biochemistry
$212,000 (FY 93 funds/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.

To further examine ALA formation in plants, we have isolated genes of *Arabidopsis thaliana* that encode the enzymes of the C5 pathway, via functional complementation of mutations in the corresponding genes of *E. coli*. In *Arabidopsis*, there appear to be two loci, designated HEMA1 and HEMA2, that encode GluTR, whereas GSA1, the GSA-2,1-aminomutase gene,

exists as a single copy. We have shown that HEMA and GSA1 expression is stimulated by light in dark-adapted plants. As an initial step in elucidating the molecular basis of light regulation, we have constructed fusions of the GUS reporter gene with HEMA1 and GSA1 promoters and deletions thereof, and are currently analyzing their expression in protoplasts, and as a stable genomic insertion in transgenic plants.

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.

Abs. 4 Sites of Rubisco Activase Interaction with Rubisco
W.L. Ogren and A.R. Portis, Jr., U.S. Department of Agriculture

Abs. 5 Antenna Organization in Green Photosynthetic Bacteria
R.E. Blankenship, Arizona State University

Abs. 6 The Chlorophyll-Binding Protein CP47 in Photosystem II
W.F.J. Vermaas, Arizona State University

Abs. 15 Molecular Bases of Photoadaptation in Unicellular, Eucaryotic Algae
P.G. Falkowski and J. LaRoche, Brookhaven National Laboratory

Abs. 16 Regulation of Energy Conversion in Photosynthesis
G. Hind, Brookhaven National Laboratory

Abs. 34 Membrane Bioenergetics of Salt Tolerant Organisms
J.K. Lanyi, University of California - Irvine
(also listed under Extremophilic Microbes)

2. PLANT CELL WALL DEVELOPMENT

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.

Abs. 170 Purification and Molecular Cloning of the Synthases of Cereal (1→3),(1→4)-ß-D-glucan
N.C. Carpita, Purdue University

Abs. 171 Modification of Lignin Composition in Plants by Manipulation of ferulate-5-hydroxylase Expression
C.C.S. Chapple, Purdue University

Abs. 186 Biochemical and Molecular Characterization of Enzymes for Cell Wall Synthesis
P.M. Ray, Stanford University

Abs. 189 Characterization of a 1,4-ß-D-Glucan Synthase from *Dictyostelium discoideum*
R.L. Blanton, Texas Tech University

Abs. 191 Molecular, Genetic, and Biochemical Analysis of Cellulose Synthesis in *Arabidopsis thaliana*
R.M. Brown, Jr., and K. Sathasivan, University of Texas

Abs. 199 A Comprehensive Approach to Elucidation of Lignification at the Plasma Membrane/Cell Wall Interface
N.G. Lewis, Washington State University

Abs. 203 Plant Cell Wall Architecture
J.E. Varner, Washington University

3. PLANT RESPIRATION/NUTRITION/ION TRANSPORT

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.

Abs. 10 Molecular Characterization of the Role of a Calcium Channel in Plant Development
K.S. Schumaker, University of Arizona

4. PLANT PIGMENT 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.

Abs. 145 Biosynthesis of Hydrocarbons
P.E. Kolattukudy, Ohio State University

Abs. 195 Structural Domains in NADPH:Photochlorophyllide Oxidoreductases Involved in Catalysis and Substrate Binding
M.P. Timko, University of Virginia

Abs. 196 Membrane Function in Lipid Mutants of Arabidopsis
J. Browse, Washington State University
(also listed under Plant Stress)

Abs. 197 Regulation of Terpene Metabolism
R. Croteau, Washington State University

Abs. 200 Interdisciplinary Plant Biochemistry Research and Training Center
N.G. Lewis, Washington State University

Abs. 201 Enhancement of Photoassimilate Utilization by Manipulation of the ADPglucose Pyrophosphorylase Genes
T.W. Okita, Washington State University

Abs. 223 Transfer RNA Involvement in Chlorophyll Biosynthesis
D. Söll, Yale University

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.

Abs. 2 Metabolic Regulation of the Plant Hormone indole-3-acetic Acid
J.D. Cohen and J.P. Slovin, U.S. Department of Agriculture

Abs. 9 Role of Zein Proteins in Structure and Assembly of Protein Bodies and Endosperm Texture
B.A. Larkins, University of Arizona

Abs. 20 Molecular and Genetic Analysis of LEAFY, a Gene Controlling Floral Induction and Flower Development in *Arabidopsis thaliana*
E. Meyerowitz, California Institute of Technology

Abs. 206 Biochemical and Molecular Analysis of a Transmembrane Protein Kinase from *Arabidopsis thaliana*
A.B. Bleecker, University of Wisconsin

Abs. 215 Analysis of Structural Domains Required For Phytochrome Function By *In Vitro* Mutagenesis
R.D. Vierstra, University of Wisconsin

6. PLANT GENETIC REGULATION AND GENETIC MECHANISMS

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.

Abs. 13 Differential Regulation of Plastid mRNA Stability
D.B. Stern, Boyce Thompson Institute for Plant Research, Inc.

Abs. 14 Plant Molecular Genetics
B. Burr and F.A. Burr, Brookhaven National Laboratory

Abs. 22 Regulation of Tomato Fruit Growth by MVA and GTP-Binding Proteins
W. Gruissem, University of California - Berkeley

Abs. 41 Production of Lipophilic Materials from Plants
C.R. Somerville, Carnegie Institution of Washington
(also listed under Plant Pigment Metabolism)

Abs. 46 The Suppression of Mutations Generated by *Mu* Transposons in Maize
R.A. Martienssen and V. Sundaresan, Cold Spring Harbor Laboratory

Abs. 50 Mechanisms and Genetic Control of Interspecific Crossing Barriers in *Lycopersicon*
M.A. Mutschler, Cornell University and S. McCormick, USDA

Abs. 70 Why Do Plants Have Two Pathways of Polyamine Synthesis?
R.L. Malmberg, University of Georgia

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.

Abs. 28 Genetic Characterization of *Lophopyrum elongatum* Salt Tolerance and Associated Ion Regulation as Expressed in Bread Wheat
J. Dvorak, D.W. Rains and E. Epstein, University of California - Davis

Abs. 53 Effects of Freezing and Cold Acclimation on the Plasma Membrane of Isolated Cereal Protoplasts
P.L. Steponkus, Cornell University

Abs. 57 Metabolic Mechanisms of Plant Growth at Low Water Potentials
J.S. Boyer, University of Delaware

Abs. 196 Membrane Function in Lipid Mutants of *Arabidopsis*
J. Browse, Washington State University
(also listed under Plant Pigment Metabolism)

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.

Abs. 11 Phytoalexin Detoxification Genes and Gene Products: Implications for the Evolution of Host Specific Traits for Pathogenicity
H.D. VanEtten, University of Arizona

Abs. 23 Determination of Environmental Stress Tolerance by Bacteria on Leaves
S.E. Lindow, University of California - Berkeley

Abs. 25 Molecular Cloning and Characterization of the Arabidopsis thaliana RPS2 Disease Resistance Locus
B.J. Staskawicz, University of California - Berkeley

Abs. 31 Cellular and Molecular Characterization of Vascular Plasmodesmata
W.J. Lucas, University of California - Davis
(also listed under Plant Growth and Development)

Abs. 42 Molecular Basis of Disease Resistance
S.C. Somerville, Carnegie Institution of Washington

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.

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.

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

Abs. 55 Studies of the Genetic Regulation of the *Thermomonospora fusca* Cellulase Complex
D.B. Wilson, Cornell University

Abs. 69 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, University of Georgia
(also listed under Fermentative Microbial Metabolism)

Abs. 75 Hemicellulases from Anaerobic Thermophiles
J. Wiegel, University of Georgia
(also listed under Extremophilic Microbes)

Abs. 108 Cellulose Fermentation by Nitrogen-Fixing Anaerobic Bacteria
S. Leschine, University of Massachusetts
(also listed under Nitrogen Fixation and Plant-Microbial Symbiosis)

Abs. 120 Xylan-Degrading Enzymes of Cytophaga xylanolytica
J.A. Breznak, Michigan State University

Abs. 125 Physiology and Molecular Biology of Lignolytic Enzyme System in Selected Wood-Rotting Fungi
C.A. Reddy, Michigan State University

Abs. 153 Biochemical Genetics of Lignin Degradation by *Phanerochaete chrysosporium*
M.H. Gold, Oregon Graduate Institute of Science and Technology

Abs. 154 Oxidative Enzymes Involved in Fungal Cellulose Degradation
V. Renganathan, Oregon Graduate Institute of Science and Technology

Abs. 163 Characterization of Lignin and Mn Peroxidases from *Phanerochaete chrysosporium*
M. Tien, Pennsylvania State University

Abs. 174 Synergism and Interaction Between *Clostridium thermocellum* Major Cellulosome Components, Ce1S and Ce1L
J.H.D. Wu, University of Rochester

Abs. 194 Enzymological Studies of One-Carbon Reactions in the Pathway of Acetate Utilization by Methanogenic Bacteria
J.G. Ferry, Virginia Polytechnic Institute and State University

Abs. 208 Molecular Genetics of Ligninase Expression
D. Cullen and T.K. Kirk, University of Wisconsin

Abs. 209 Identification of the Primary Mechanism for Fungal Lignin Degradation
K.E. Hammel, University of Wisconsin

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.

Abs. 48 Anaerobic Metabolism of Aromatic Compounds by Phototrophic Bacteria: Biochemical Aspects
J. Gibson, Cornell University

Abs. 61 Ethanologenic Enzymes of *Zymomonas mobilis*
L.O. Ingram, University of Florida

Abs. 69 Microbiology and Biochemistry and Anaerobic Fermentations: The Conversion of Complex Organic Materials to Simple Gases
L.G. Ljungdal, H.D. Peck, Jr., A. Przybyla, and J. Wiegel, University of Georgia
(also listed under Lignin-Polysaccharide Breakdown)

Abs. 79 Genetics of Solvent-Producing Clostridia
H.P. Blaschek, University of Illinois

Abs. 92 Molecular Biology of Anaerobic Aromatic Biodegradation
C.S. Harwood, University of Iowa

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.

Abs. 148 Structure and Regulation of Methanogen Genes
J.N. Reeve, Ohio State University

Abs. 211 Carbon Monoxide Metabolism by Photosynthetic Bacteria
P.W. Ludden and G.P. Roberts, University of Wisconsin

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.

Abs. 34 Membrane Bioenergetics of Salt Tolerant Organisms
J.K. Lanyi, University of California - Irvine
(also listed under Photosynthesis)

Abs. 47 Sugar Transport and Metabolism in *Thermotoga*
A.H. Romano and K.M. Noll, University of Connecticut

Abs. 63 The Metabolism of Hydrogen by Extremely Thermophilic Bacteria
M.W.W. Adams, University of Georgia

Abs. 75 Hemicellulases from Anaerobic Thermophiles
J. Wiegel, University of Georgia
(also listed under Lignin-Polysaccharide Breakdown)

Abs. 82 Mechanism of Proton Pumping in Bacteriorhodopsin
T.G. Ebrey, University of Illinois

Abs. 89 Phylogenetic Analysis of Hyperthermophilic Natural Populations Using Ribosomal RNA Sequences
N.R. Pace, Indiana University

Abs. 105 Structure and Regulation of L-Glutamate Dehydrogenase from Hyperthermophilic *Archaea* (Archaebacteria)
F.T. Robb, University of Maryland

Abs. 135 The Respiratory Chain of Alkaliphilic Bacteria
T.A. Krulwich, Mount Sinai School of Medicine

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.

15. MATERIALS BIOSYNTHESIS

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.

INVESTIGATOR INDEX