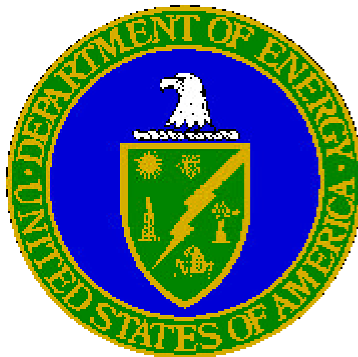
Summaries of FY 2000 Activities

Energy Biosciences

September 2001



Energy Biosciences Program Overview

The mission of the Energy Biosciences program is to support research that advances the fundamental knowledge necessary for the future development of biotechnologies related to the Department of Energy's mission. Departmental objectives include effective and efficient energy production, energy conservation, environmental restoration, and waste management. The Energy Biosciences program emphasizes research in the microbiological and plant sciences, as these understudied areas offer numerous scientific opportunities to dramatically influence environmentally sensible energy production and conservation. The research supported is focused on the basic mechanisms affecting plant productivity, conversion of biomass and other organic materials into fuels and chemicals by microbial systems, and the ability of biological systems to replace energy-intensive or pollutant-producing processes. The program also addresses the increasing number of new opportunities arising at the interface of biology with other basic energy-related sciences such as biosynthesis of novel materials and the influence of soil organisms on geological processes.

The Energy Biosciences program supports research at the very fundamental level in an effort to support as broad a scientific foundation for the Department's technology development efforts as possible. The Department currently expends considerable effort through its Offices of Energy Efficiency and Renewable Energy, Fossil Energy, and Environmental Management to develop technologies based on available fundamental knowledge. The biological research supported by the Energy Biosciences program includes basic research on plants, algae and photosynthetic bacteria with emphases on photosynthetic mechanisms and bioenergetics, control of plant growth and development, genetic transmission and expression, and plant cell wall structure and function. Support in these areas seeks to define and understand the biological mechanisms that effectively transduce light energy into chemical energy, to identify the biochemical pathways and genetic regulatory mechanisms for the energy efficient biosynthesis of potential fuels and petroleum-replacing compounds, and to elucidate the capacity of plants to remediate contaminated environments by transporting and detoxifying toxic substances. Another area covered by the program is fermentative (and related) microbiology, which includes support of basic research on fungal and bacterial metabolism and physiology with emphases on anaerobic bacteria, bacteria that live in extremely harsh environments and biopolymer degradation by fungi and bacteria. Support in these areas seeks to provide the capability of converting organic and inorganic compounds, such as cellulose and sulfur-containing coal, in a rapid and energy efficient manner. Several DOE technology programs are interested in the potential to integrate biological-based systems into industrial processes.

The program's efforts in supporting interdisciplinary activities at the boundaries of biology and other disciplines represented within Basic Energy Sciences include: biocatalytic mechanisms, biomaterials and materials biosynthesis, interactions between plant/microbes with mineral particles, interface of photobiology and photochemistry, and bioengineering. The program also interacts extensively with other Federal agencies to coordinate and cooperate in areas of joint interest. The most prominent of these efforts has been the three-agency plant science activities in partnership with the National Science Foundation (NSF) and the U.S. Department of Agriculture (USDA). This partnership, initiated in 1987 under the auspices of the Office of Science and Technology Policy, focuses on multi-institutional research coordinating group awards and interdisciplinary research training group awards. The three agency programs have done much to stimulate plant science research nationwide on university campuses.

The Energy Biosciences program has also joined with NSF and USDA to fund groups to sequence, at a large scale, the genomes of *Arabidopsis thaliana* and rice (*Oryza sativa*). These U.S. groups have coordinated with activities in Europe and Japan to decipher the entire genomes of these model plants, providing valuable resources to the international plant research community. The genome of *Arabidopsis* was finished in December 2000; progress on the rice genome has accelerated with an anticipated completion in 2004.

Recent Accomplishments

Plants capture light energy through an array of pigments and proteins that are associated with highly specialized lipid membranes found in chloroplasts. Recently, a novel protein was discovered that is involved in the biogenesis and assembly of photosynthetic membranes. This protein acts in the biosynthetic pathway for galactolipids, which make up the bulk of the chloroplast photosynthetic membranes. A reduction in the amount of galactolipids also results in impaired growth, chloroplast structure, and photosynthetic activity. The isolation and characterization of this novel protein may lead to improved strategies for manipulating membrane formation and energy capture efficiency.

Bacteriorhodopsin is a protein found in special membranes of salt marsh bacteria. Sunlight causes bacteriorhodopsin molecules to change shape and in the process transport protons across the membrane that can be harnessed to provide chemical energy for the bacterium. A high-resolution x-ray crystallographic structure of this light-driven proton pump has been obtained showing the molecule frozen mid-stroke in the proton transport cycle. This novel view of the intermediate conformation confirms spectral data on the relatively small conformational changes in bacteriorhodopsin that allow the unidirectional release of protons across the membrane. This fundamental research lends insight into the mechanisms by which biological systems capture and transform energy.

Plants employ a number of specialized regulatory proteins to control key developmental events such as the formation of flowers and leaves. These regulatory proteins, grouped into a diverse family based on structural similarities, can exercise control of both the timing and quantity of gene expression. A recent discovery shows the critical link between the molecular action of one of these regulators and the timing of senescence (cell death). The research demonstrates that constitutive and elevated expression of the regulator protein results in a significant increase in the longevity of flowers and a delay in the normal onset of tissue death. The understanding of this phenomenon has broad implications for the manipulation of biological regulatory mechanisms in plant growth and development for the enhanced production of chemical feedstocks and biobased products.

All living organisms use a common mechanism for translating genetic instructions from DNA blueprints into the proteins that carry out the biochemical functions within a cell. These proteins are synthesized from 20 different amino acid building blocks, with each having a different aminoacyl-tRNA synthetase to catalyze its addition to the growing protein chain. Therefore, when the entire genome sequences of two methane-producing bacteria were deduced, scientists were puzzled to find no genes encoding the tRNA synthetase responsible for adding the amino acid cysteine. An answer to this biological puzzle was provided by research showing that a single tRNA synthetase protein catalyzes the addition of two different amino acids, proline and cysteine. The ability of one enzyme to provide two different building blocks in protein synthesis raises interesting questions about the concept of substrate specificity and may lead to future applications in the directed functional design of proteins.

The chlorophyll pigments in plants capture light as an energy source for photosynthesis, while other pigments serve as light sensors for the regulation of growth and development. Phytochrome is the best-studied sensor pigment in plants, having the interesting property of reversing between an active or inactive shape according to the wavelength of light detected. Phytochromes and phytochrome-like proteins have been found in all photosynthetic organisms, including plants, algae, cyanobacteria, and purple bacteria. Two nonphotosynthetic bacteria have been added to the list. Examination of these two bacterial phytochromes showed that they are quite distinct in terms of structure in comparison to the phytochromes from photosynthetic organisms. They still undergo the interconversion between two light-absorbing shapes, which is the hallmark of phytochrome action. It is believed that comparative studies between these two types of phytochromes will result in a better understanding of the mechanisms of light regulation. These studies should have broad implications for the evolution of general mechanisms in biological signal transduction and energy capture.

Cellulose is the major constituent of plant cell walls and represents the most abundant biopolymer on earth. Cellulose consists of multiple chains of sugars tightly packed together to form a fiber. New research is providing the first experimental confirmation of an important structure involved in cellulose biosynthesis. The

research showed that the synthesis of cellulose occurs on structures that parallel the number of chains seen within the cellulose fiber. This research provides an exciting springboard for future applications in the efficient design of specific complex carbohydrates and other renewable carbon resources.

The program continues to participate in the Life Sciences Research Foundation post-doctoral fellowship program, with three-year fellowship support for the following individuals in Fiscal Year 2000:

Dr. Joann A. Conner (University of Georgia)
Dr. Kenneth C. Keiler (Stanford University)
Dr. Elizabeth E. Rogers (Dartmouth College)
Dr. David Mackey (University of North Carolina)
Dr. Gregory M. York (Massachusetts Institute of Technology)
Dr. Erik Vollbrecht (Cold Spring Harbor Laboratory)
Dr. Christina W. Bowers (Princeton University)
Dr. Elizabeth S. Haswell (California Institute of Technology)
Dr. Seth Jon Davis (University of Warwick, UK)
Dr. Franklin G. Harmon (The Scripps Research Institute)
Dr. Christine Pfund (University of Wisconsin)
Dr. Xuelin Wu (The Salk Institute for Biological Studies)

Each of the individuals is working in an area related to the Energy Biosciences program scope.

During Fiscal Year 2000 the program provided partial support for the following conferences, workshops or training activities:

1. University of Florida, Gainesville, FL 32611-0700, Interfacing Microbiology and Biotechnology Conference, May 17-19, 2000, Gainesville, Florida

2. University of Georgia, Athens, GA 30602-7229, The Power of Anaerobes Conference, May 17-19, 2000, Athens, Georgia

3. Dartmouth College, Hanover, NH 03755, 11th International Conference on Arabidopsis Research, June 24-28, 2000, Madison, Wisconsin

4. Gordon Research Conferences, Inc., Gordon Research Conference on Nitrogen Fixation, July 2-7, 2000, New London, New Hampshire

5. Brown University, Providence, RI 02912, International Conference on the Tetrapyrroles of Photosynthetic Organisms, July 25-30, 2001, Providence, Rhode Island

6. Gordon Research Conferences, Inc., 2000 Gordon Research Conference on Plant Cell Walls, August 20-25, 2000, Meriden, New Hampshire

7. Life Sciences Research Foundation, Baltimore, MD 21210, Developing Research Capabilities in Energy Biosciences

8. Marine Biological Laboratory, Woods Hole, MA 02543, Investigations into the Metabolic Diversity of Microorganisms as Part of Microbial Diversity, Summer 2000, Woods Hole, Massachusetts

9. Michigan State University, East Lansing, MI 48824, Minority Summer Research Program in the Plant Sciences, Summer 2000, East Lansing, Michigan

10. Washington State University, Pullman, WA 99164-6340, An Advanced Course in Plant Biochemistry, Pullman, Washington, Summer 2001

The Energy Biosciences program was provided approximately thirty-one million dollars in fiscal year 2000. The distribution of these resources is indicated in the following table:

	Number of Projects	FY 00 funding (in thousands)	Percent of total funds
University and Non-Profit Institutions	210	$ 22,868	74
Michigan State University Plant Research Laboratory	15	$ 3,200	10
National Laboratories Brookhaven National Lab Lawrence Berkeley Lab National Renewable Energy Lab.	11	$ 2,392	8
Small Business Innovation Research (SBIR) contribution, and Miscellaneous		$ 1,952	6
Conferences, Educational Activities	11	$ 588	2
	247	$31,000	

The Energy Biosciences Division staff wish to thank the hundreds of reviewers, both in this country and abroad, who have contributed their time and effort to the peer review process of the program through mail reviews, panel meetings and site visit reviews. Without your assistance the program would be unable to maintain the same quality.

The staff members of the Energy Biosciences program are:

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ABSTRACTS OF PROJECTS SUPPORTED IN FY 2000

(NOTE: Dollar amounts are for a twelve-month period using FY 2000 funds unless otherwise stated)

1. U.S. Department of Agriculture

Urbana, IL 61801
Biochemical and molecular analysis of a new control pathway in assimilate partitioning
Daniel R. Bush, Photosynthesis Research Unit
$127,444 (FY 99 funds – two years)

Plant leaves capture light energy from the sun and transform that energy into a useful form in the process called photosynthesis. The primary product of photosynthesis is sucrose. Generally, 50 to 80% of the sucrose synthesized is transported from the leaf to supply organic nutrients to many of the edible parts of the plant such as fruits, grains, and tubers. This resource allocation process is called assimilate partitioning and alterations in this system are known to significantly affect crop productivity. We recently discovered that sucrose plays a second vital role in assimilate partitioning by acting as a signal molecule that regulates the activity and gene expression of the transporter that mediates long-distance sucrose transport. Research progress this year showed that the sucrose-sensitive signaling system controls transport activity by regulating the abundance of the transport protein in the plasma membrane. We also showed that the responsive sucrose transporter is exclusively located in the companion cells of the plant's vascular system.

2. U. S. Department of Agriculture

Raleigh, NC 27695-7631
Role and Regulation of SNF1-like Protein Kinases in Plant Carbohydrate Metabolism
Steven C. Huber, USDA/ARS and Departments of Crop Science and Botany, NCSU
$99,000

Spinach (*Spinacia oleracea* L.) leaf sucrose-phosphate synthase (SPS) can be inactivated by phosphorylation of Ser-158 by calmodulin-like domain protein kinases (CDPKs) or SNF1-related protein kinases (SnRK1) in vitro. While the phosphorylation site sequence is relatively conserved, most of the deduced sequences of SPS from dicot species surrounding the Ser-158 regulatory phosphorylation site contain a proline residue at P-4 (where P is the phosphorylated Ser); spinach is the exception and contains an arginine residue at P-4. We found that a proline residue at P-4 selectively inhibits phosphorylation of the peptide by a CDPK relative to a SnRK1. The presence of a proline residue at P-4, by allowing a tight turn in the peptide substrate, may interfere with proper binding of residues at P-5 and beyond. Both kinases had greater activity with peptides having basic residues at P-6 and P+5 (in addition to the known requirement for an arginine at P-3/P-4), and when the residue at P-6 was a histidine, the pH optimum for phosphorylation of the peptide was acid shifted. The results suggest that a proline residue at P-4 (and perhaps other positions) can be a strong negative recognition element for CDPKs and thereby render a phosphorylation site to be specific for SNF1-like protein kinases. The results predict a variety of proteins from the databases that may be selectively phosphorylated by SnRK1s (as opposed to CDPKs), such as SPS in dicot species, or may be phosphorylated in a pH-dependent manner.

3. U.S. Department of Agriculture

Urbana, IL 61801-3838
Consequences of Altering Rubisco Regulation
Archie R. Portis, Jr., USDA/ARS and Departments of Crop Sciences/Plant Biology, University of Illinois
$57,719

Rubisco initiates photosynthetic carbon acquisition and its activity is limiting under high light at atmospheric levels of carbon dioxide. However, under either limiting light or when adequate sinks for the products of photosynthesis are not available, the activity of Rubisco is reduced below its maximal capacity even though

a limitation by the availability of its other substrate, RuBP, would be sufficient. The reasons for this response are unclear.

The activation state of Rubisco is determined by the activity of its regulatory protein, Rubisco activase. Rubisco activase is usually present as two isoforms, differing at the carboxyl terminus and generated by alternative splicing of the pre-mRNA. Earlier work examining mutant forms of the protein *in vitro* indicated that reduction/oxidation of the larger isoform dramatically altered the activity of the protein and suggested that this regulation might account for the modulation of Rubisco activity by light intensity. By using transgenic Arabidopsis plants expressing only one of the two isoforms and mutant forms of the larger isoform, we found that reduction/oxidation of the larger isoform is required in order to down-regulate Rubisco activity in response to limiting light intensities. These plants and transgenic plants expressing mutant forms of the activase that have an altered response to the ATP/ADP ratio, which may account for the down-regulation when adequate sinks are not available, are currently being investigated further in order to determine the consequences of altering Rubisco regulation on photosynthesis, growth, and the response of plants to their environment.

4. U.S. Department of Agriculture

Madison, WI 53706-1108

What is the Extent of Metabolic Plasticity in the Lignification Process, and Can it be Exploited?

John Ralph and Ronald Hatfield, USDA Agricultural Research Service; US Dairy Forage Research Center

$94,000

Lignin is a polymer that plants use to bind the fibers together and confer structural rigidity to stems as well as provide other functions for the well-being of the plant. However, utilization of plant resources is often limited by the difficulty of dealing with lignin. It is the polymer that must be removed to make fine paper, for example. Recently, natural mutant and transgenic plants in which the lignin biosynthetic pathway has been affected have become available. Such plants provide a rich source of insights into the chemistry of lignin formation that will allow more efficient uses of our plant resources in the future.

Recent work on a few mutant plants revealed that deprivation of the ability to produce lignin precursors results in increased incorporation of other plant phenols into the lignin. Some of the incorporated monomers were unexpected and not normally associated with the biosynthetic pathway. The large compositional shifts that are possible indicate considerable plasticity in the lignification process, suggesting new approaches to plant modification for improved utilization in processes ranging from polysaccharide digestion in ruminants to industrial chemical pulping.

The project, which is only just beginning in this reporting period, will involve the detailed characterization of lignins produced by plants that have altered lignification, to determine the impact of lignin pathway gene mutations on the structure and function of lignin. Such information will lead to a better understanding of the mechanisms controlling lignification and how they can be altered to produce desirable products.

5. U.S. Department of Agriculture

Beltsville, MD 20705

Controls on production, incorporation and decomposition of glomalin -- a novel fungal soil protein important to soil carbon storage

Sara Wright, Soil Microbial Systems Laboratory (Note: see also University of Montana, M.C. Rillig)

$66,491 (FY 99 funds – two years)

A group of beneficial soil fungi live on carbon supplied directly to them by plant roots. The fungi are called arbuscular mycorrhizal fungi or AM fungi. These fungi have long hair-like projections called hyphae that extend several cm from the root into soil. Glomalin is a glycoprotein that is produced on AM hyphae in large amounts, is released from hyphae, and attaches to soil particles. Glomalin is important because

concentrations in soil are correlated with soil aggregate stability, and large amounts of labile soil carbon are sequestered in aggregates. Plants fix more carbon under elevated CO_2 than under ambient CO_2, and more carbon is transported from roots to these fungi. We continue to find larger amounts of glomalin in planted soils exposed long-term or short-term to increased atmospheric CO_2. Preliminary evidence indicates that warming, without increased CO_2, is detrimental to aggregate stability. We found that glomalin production is influenced by plant species in the field. Laboratory studies indicated that glomalin production differed among fungal species, but not between corn and crimson clover. Incubation studies indicated that glomalin levels decline more rapidly in soils from the Midwest that have been conventionally tilled compared with no-till soils. We have evidence that glomalin makes up a large part of soil organic matter in an organic soil from Hawaii. Our work shows that glomalin is in the fraction of soil organic matter called humin – a fraction that was previously thought to be composed of undefined insoluble organic matter.

6. University of Alabama

Tuscaloosa, AL 35487-0336

A Combined Genetic, Biochemical, and Biophysical Analysis of the A1 Phylloquinone Binding Site of Photosystem I from Green Plants
Kevin Redding, Department of Chemistry
$194,001 (two years)

We are attempting to understand how plants convert electromagnetic energy in the form of light into chemical energy by studying the light-driven transport of electrons through the photosystem 1 (PS1) protein. Two phylloquinone molecules are embedded within PS1 as part of two symmetric chains of electron transport cofactors that route electron flow from one side of the protein to the other. We have made mutations of amino acids in the close vicinity of the phylloquinones, to see what role the protein plays in tuning the properties of this cofactor. We targeted several amino acids that possessed criteria making them more likely to be involved, as it was not clear at the time which ones were nearby. Our results have now focussed our attention upon a set of tryptophans that affect the spectroscopic and kinetic properties of the quinones. This allows us to map the quinone site to the polypeptide sequence. Moreover, one effect of the mutations was interesting: a 3-5 fold decrease in the rate of electron transport from the nearby quinone to the next cofactor in the chain. Although it had been assumed that only one chain was active, our mutants have allowed us to make the intriguing discovery that both are active. Furthermore, the environments of the two phylloquinones are sufficiently different to produce an order of magnitude difference in the rate of electron transport from them. We are expanding our project to pinpoint the origin of this difference.

7. Arizona State University

Tempe, AZ 85287-1604

Structure, Function and Regulation of Antenna Complexes of Green Photosynthetic Bacteria
Robert E. Blankenship, Department of Chemistry and Biochemistry
$118,000 (FY 99 funds – 18 months)

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, which are well adapted to energy collection in extremely dim light environments. The overall objective of this project is to determine the molecular organization of pigments in chlorosome antennas, as well as the mechanisms of excitation transfer and regulation of this unique antenna system. 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. The chlorosome pigments are organized in vivo into pigment oligomers in which direct pigment-pigment interactions are of dominant importance. A pigment-protein baseplate complex couples the chlorosome to the membrane. Green sulfur bacteria contain a redox-activated quenching mechanism for control of energy transfer efficiency mediated through quinone molecules found in the chlorosome. The quenching effect is a control mechanism that protects the cell from damage during conditions where light and oxygen are present simultaneously. Recent work has included

the biochemical isolation and characterization of the chlorosome baseplate complex. It contains a chlorosome-associated protein, CsmA, bacteriochlorophyll *a* and beta carotene.

8. Arizona State University

Tempe, AZ 85287-1601
Chlorophyll-Binding Proteins in the Cyanobacterium Synechocystis sp. PCC 6803
Willem F.J. Vermaas, Department of Plant Biology
$104,000

This year our studies focused on regulation of chlorophyll biosynthesis. If not properly bound near carotenoids, chlorophyll is toxic in the presence of light and oxygen due to formation of singlet oxygen and superoxide. Therefore, chlorophyll synthesis needs to be tightly regulated. In *Synechocystis*, we have found *scp* genes (coding for **s**mall **C**ab-**l**ike proteins) for small (5-8 kDa) membrane proteins with chlorophyll-binding motifs. Upon deletion of two single *scp* genes, regulation of chlorophyll and heme synthesis has been altered (the first part of the chlorophyll and heme biosynthesis pathways are identical). Our current working hypothesis is that chlorophyll binding to SCP proteins reports on the status of chlorophyll availability for the cell, and thus provides feedback regulation. Another important question regarding chlorophyll biosynthesis is how the relative abundance of chlorophyll *a* and *b* is regulated in plants. Cyanobacteria synthesize only chlorophyll *a*, but a plant gene for chlorophyll *a* oxidase, the enzyme converting chlorophyll *a* to chlorophyll *b*, can be expressed in *Synechocystis*. We observe that chlorophyll *b* is a major pigment in this system only when pea *lhcb*, the gene for a major chlorophyll *b*-binding light-harvesting protein, is present as well. Surprisingly, chlorophyll *b* in this system is associated with the photosystem II reaction center complex. Association of chlorophyll *b* with a reaction center complex is unprecedented in other systems, and yet chlorophyll *b*-containing photosystem II reaction centers are functioning rather normally. Functional and structural studies on these novel chlorophyll *b*-containing photosystem II complexes is continuing.

9. Arizona State University

Tempe, AZ 85287
Excitation energy transfer in the photosystem I core antenna: function of the clustered and connecting chlorophylls
Andrew N. Webber, co P.I. Neal W. Woodbury, Department of Plant Biology
$200,000 (FY 99 funds – two years)

Our goal is to understand how light energy, captured by the light harvesting chlorophyll pigments, is effectively transferred to specialized chlorophyll molecules that initiate photosynthetic electron transfer. In photosystem I, the approximately 86 antenna chlorophylls form a connected ring around the chlorophylls of the electron transfer chain. Between the antenna and electron transfer cofactors are two additional chlorophylls ("connecting chlorophylls") that we hypothesize form a functional connection between the two groups of chlorophylls. We are using various spectroscopic techniques to examine excitation energy in photosystem I mutants lacking the "connecting chlorophylls". Interestingly, these mutants show impaired quantum yields of photosystem I electron transfer. We are now investigating ultrafast excitation energy dynamics to determine how the mutants effect coupling between specific groups of chlorophylls. These results indicate that the unique arrangement of chlorophylls in photosystem I play a crucial role in harvesting light energy for photosynthesis.

10. University of Arizona

Tucson, AZ 85721-0088

Restructuring Metabolism for Photosynthesis Protection

Hans J. Bohnert and R.G. Jensen, Department of Biochemistry

$103,000

The modification of plant function is the focus of our project with the long-term goal of modifying biochemical pathways that confer increased whole-plant tolerance to drought and high salinity. We concentrated on altered polyol production, based on the realization that polyols are one of the water deficit stress responses in many species. Polyols, it seems, have three functions: (1) in osmotic adjustments through mass action, (2) as low molecular weight chaperones which in part also involves binding of transition state metal ions by which action the production of hydroxyl radicals is reduced, and (3) in redox control through their synthesis and accumulation. In an extension of the transgenic approaches multi-gene transformation vectors have been constructed and transferred into tobacco and rice. These vectors were designed to increase radical scavenging capacity, and to lead to the production of several different polyols in different compartments. In total, seven genes controlled by seven different promoters have been transferred and several 100 lines have been regenerated. Expression of the seven genes has been observed in the To and T1 generations but in later generations and increasing number of genes, apparently randomly, appears to be silenced. Analyses are ongoing.

11. University of Arizona

Tucson, AZ 85721-0036

Role of Cell Wall Degrading Enzymes in the Programmed Separation of Cells from Root Caps

Martha C. Hawes; co P.I. Ho-Hyung Woo, Department of Plant Pathology

$100,000

The longtime goal is to determine the mechanism by which plants control the production and delivery of border cells into the rhizosphere by controlling cell wall degradation. Each day, thousands of metabolically active root 'border' cells with unique patterns of gene expression are delivered from the root tip to the external environment where they function to protect the root from pathogen invasion and to facilitate development of beneficial relationships. The development of methods to precisely synchronize cell cycle leading to border cell production has made it possible to establish a molecular framework for root cap function and turnover. Cloning and manipulation of genes controlling the process at its inception (mitosis) and at its culmination (border cell separation) has been achieved, and the progress is described in seven research papers and two reviews published in the past year. We focused on two carbohydrate processing genes to establish that root cap development can be blocked genetically at its beginning or end, to yield distinctive phenotypes in pea, alfalfa, and *Arabidopsis thaliana*. In the process, we identified a product which may constitute a previously unknown class of plant hormones which acts on the regulation of cell cycle, and have for the first time confirmed the long-standing hypothesis that plant pectinmethylesterase plays a key role in cell wall degradation. Initial characterization of two additional cell wall processing enzymes--a polygalacturonase and a galactosidase--has been accomplished, and both genes have been cloned in preparation for detailed functional analysis of their roles in border cell production.

12. University of Arizona

Tucson, AZ 85721-0036

Transgene silencing, paramutation, and promoter-homology-based control of gene expression

Richard A. Jorgensen, Department of Plant Sciences

$94,000

The goal of this project is to explore how related genes interact and produce novel patterns of gene expression. We are using a convenient and efficient model system in which plant gene expression is monitored by changes in flower color. Chalcone synthase (Chs) is an enzyme necessary for flower color and we create duplications of the Chs gene by introducing additional copies of this gene into petunia plants. One

copy of an introduced Chs gene produces a simple pattern due to RNA silencing of the normal petunia Chs gene. Two copies of an introduced Chs gene interact with each other to produce new patterns of RNA silencing that are heritable. These new patterns have white flower veins. We have now shown that duplication of transcribed *Chs* sequences is sufficient for efficient induction of these vein-based patterns of *Chs* RNA silencing. Furthermore, we found that these duplication-induced changes are heritable even after segregation of the second transgene copy away from the first in sexual progeny, i.e., these events meet the broad definition of "paramutations." Paramutations are a special class of genetic changes that are usually temporary and reversible and are caused by interactions between related gene copies. Our observation that duplication of transcribed sequences is sufficient to produce paramutations that create vein-based patterns supports the hypothesis that the paramutation produces an RNA silencing signal that moves systemically throughout the plant, resulting in RNA silencing of Chs genes in flower veins, and therefore white vein patterns of Chs gene expression.

13. University of Arizona

Tucson, AZ 85721-0036
Dissection of Molecular Mechanisms Regulating Protein Body Formation in Maize Endosperm
Brian Larkins, Department of Plant Sciences
$104,000

Endosperm texture is an important quality trait in maize, as it influences the shipping characteristics of the grain, its susceptibility to insects, the yield of grits from dry milling, energy costs during wet milling, and the baking and digestibility properties of the flour. There appears to be a causal relationship between kernel hardness and the formation of protein bodies, as mutations affecting protein body number and structure are associated with a soft, starchy kernel. To better understand this relationship, we characterized the nature of storage proteins synthesized in the endosperm using a genomics analysis of endospserm ESTs. This study identified several new storage proteins and demonstrated the existence of novel protein storage vacuoles. Using the yeast two-hybrid system, we dissected protein interactions between zeins that are responsible for their association into a protein body. By deletion mutagenesis, we identified domains with an α-zein that cause it to interact with other zein proteins, particularly γ-zeins. This allowed us to develop a minimal α-zein gene construct that can be used as a vector to target heterologous proteins, such as green fluorescent protein, into protein bodies. In an effort to understand the mechanisms influencing endosperm texture, we used transcript profiling of eight different "opaque" mutants (*o1, o2, o5, o9, o11, Mucronate, Defective endospsperm B30,* and *floury2*) to identify patterns of gene expression that are consistently altered in all of them, or that are unique to each one of them. These mutants fall into two subgroups: one systematically manifests an "unfolded protein" response (*fl2, Mc, DeB30*) and the other (*o1, o2, o5, o9, o11*) does not. Genes encoding cytoskeletal proteins are generally up-regulated in all the mutants, and this may be associated with a higher lysine content in several of them.

14. University of Arizona

Tucson, AZ 85721-0036
Regulation of DNA Endoreduplication in Maize Endosperm
Brian Larkins, Department of Plant Sciences
$101,000

Endoreduplication occurs widely in metabolically active tissues of plants and animals, but its function is poorly understood. During this process, cells amplify their genome without chromatin condensation, segregation or cytokinesis, resulting in what appear to be multiple, uniform copies of the nuclear DNA. Endoreduplication could provide a mechanism to increase the availability of DNA templates and thus increase gene expression. It is also possible that endoreduplication maintains an optimum ratio between cell and nuclear size. The goals of this project are to determine the function of endoreduplication in maize endosperm and understand the molecular mechanisms that create this cell cycle. To address these questions, we examined the variability of endoreduplication among maize genotypes and characterized the genetic regulation of this trait. Based on the phenotypic variation we observed, we were able to begin

developing nearly isogenic lines that differ in their degree of endoreduplication. We also genetically engineered maize plants to alter cell cycle regulation and influence the process of endoreduplication. This led to the discovery that wheat dwarf virus RepA protein can induce endoreduplication in mitotically active cells, but it does not affect the cell cycle in maize endosperm cells undergoing endoreduplication. Interestingly, RepA expression increases the transformation efficiency of maize embryos, acting through a pocket protein-dependent mechanism. By expressing a mutant Cdc2 in maize endosperm, we reduced the level of endoreduplication by nearly half. Experiments are in progress to identify the genes regulating the activity of the S-phase kinase in maize endosperm and determine the mechanism that oscillates its activity during endoreduplication.

15. University of Arizona

Tucson, AZ 85721-0036

Molecular Characterization of the Role of a Calcium Channel in Plant Development
Karen S. Schumaker, Department of Plant Sciences
$93,000

A stimulus-induced change in cellular calcium levels is a critical component of energy transduction in plant and animal development. Demonstrating calcium's involvement in any developmental process requires identification of mechanisms that regulate these calcium changes. In plants, biochemical and electrophysiological studies have shown that the activity of calcium channels leads to increases in cellular calcium levels; however, molecular evidence for these transporters is lacking. We are using moss to establish a role for calcium in hormone-induced morphogenesis and to identify transporters responsible for increasing cytosolic calcium levels during this process. We have shown that calcium is important in the early events of cytokinin-induced bud assembly using 1,4-dihydropyridines (DHPs), molecules that block calcium movement through voltage-dependent channels in animal cells. In addition to inhibiting moss growth, these calcium channel blockers prevent calcium transport into moss cells and bind specifically to two proteins in the moss plasma membrane. We are purifying the DHP-binding proteins (putative calcium channel) with the ultimate goal of understanding channel expression, regulation, structure, and function during development. Experiments are in progress to identify components that respond to changes in cellular calcium levels during bud assembly. Growth studies with the calmodulin antagonist Ophiobolin A suggest that calcium changes may be transduced by calmodulin activation. To determine the role of the actin cytoskeleton, we have transformed the moss *Physcomitrella patens* with: (1) a GFP-talin construct which enables visualization of actin *in vivo* and (2) a moss Rho (a putative regulator of the actin cytoskeleton) construct to generate moss mutants with altered actin organization.

16. University of Arizona

Tucson, AZ 85721-0036

Manipulation of Phytoalexin Biosynthesis: Effects on Plant-Microbe Interactions
Hans D. VanEtten, Department of Plant Pathology
$100,000

Plants have the capability to produce large amounts of diverse chemicals commonly referred to as secondary metabolites. Contemporary research is beginning to elucidate the biological roles for many of these secondary metabolites. The objective of our research is to further determine the function of a class of secondary metabolites called phytoalexins. In particular, we have proposed to determine the role these chemicals play in plant-microbe interactions. The model system being used involves (+)pisatin, the isoflavonoid phytoalexin produced by pea (Pisum sativum), for which several of the biosynthetic genes are known. We have proposed to make pisatin-deficient transgenic lines of pea by blocking pisatin biosynthesis at three different steps in the pisatin pathway and to examine the effects of those mutations on plant-microbe interactions. The preliminary experiments are utilizing hairy root cultures because of the relative ease and speed of producing transgenic pea tissue compared to current methods for producing transgenic pea plants. Three plasmid vectors in which the two halves of the gene for the terminal step in pisatin biosynthesis are present in reverse orientation have been constructed. These vectors have been used to

transform hairy roots of pea in an attempt to silence the wild type gene. If pisatin-deficient hairy roots are obtained using one of these constructs that construct will be used to produce transgenic pea plants.

17. University of Arizona

Tucson, AZ 85721
Cytosolic HSP100 Proteins and Stress Tolerance in Plants
Elizabeth Vierling, Department of Biochemistry
$100,000

High temperature stress can severely limit crop productivity. During such stress plants express heat shock proteins (Hsps) which are hypothesized to protect them from heat damage. Using mutants of *Arabidopsis thaliana*, a model plant system for which the entire genome sequence has been determined, we have shown that one Hsp, Hsp101, is essential for the adaptation of plants to high temperature stress. Hsp101 protects germinating seeds as well as young seedlings from irreversible high temperature damage. Hsp101 is also expressed in developing seeds and may have an important role in survival of other stresses during germination. Regulated expression of Hsp101 in transgenic plants may provide enhanced heat tolerance. We are now testing whether Hsp101 also has a protective role against other stresses, including drought, salt and heavy metal stress. Hsp101 is proposed to help reactivate proteins damaged by heat. We can now identify these heat sensitive components by determining which proteins fail to be reactivated in Hsp101 mutants. These experiments will pinpoint heat sensitive targets for genetic engineering, as well as provide insight into the mechanism of Hsp101 action. The complete genome sequence of Arabidopsis has revealed two other genes that are related to Hsp101. While these genes cannot substitute for Hsp101 function in heat stress tolerance, they may provide tolerance to other stresses. We have isolated mutants in these two genes to test this hypothesis.

18. Brookhaven National Laboratory

Upton, NY 11973
Plant Molecular Genetics
Benjamin Burr and Frances Burr, Biology Department
$352,000

Molecular genetics is likely to have the greatest impact on plant improvement in two interrelated areas: the development of analytical techniques to increase the efficiency of plant breeding, and the elucidation of biological phenomena that affect plant traits. An example of the development of analytical tools is the use of genetic polymorphisms in DNA as genetic markers. Recently, we have focused on simple sequence repeats as genetic markers because of their ease of use and because they are relatively polymorphic. Genes that regulate the expression of other genes can have a profound effect on plant characters. We are focusing on the regulation of the biosynthesis of anthocyanin and carotenoid pigments in maize and the development and expression of cotton fibers.

19. Brookhaven National Laboratory

Upton, NY 11973
Regulation of Energy Conversion in Photosynthesis
Geoffrey Hind, Biology Department
$325,000

This project addresses the DOE strategic goal of securing the nation’s future energy supply. Energy from cultivated plant biomass is a benign option suited to utilization of marginal lands, where environmental stress is inherent. Our objective is to elucidate molecular mechanisms that protect photosynthetic tissues from stress-related damage in these environments.

Thylakoid protein kinases regulate excitation energy distribution between the photosystems by phosphorylating photosystem II light-harvesting and reaction center proteins. In so doing, they protect against photoinhibition. We described two serine/threonine protein kinases in thylakoid membranes of spinach; however, recent availability of the complete genome sequence for *Arabidopsis thaliana* has made this plant preferable for protein isolation and functional studies. Tools for protein sequence analysis reveal that many kinase precursors in *Arabidopsis* have putative targeting toward the thylakoid membrane. Fewer than ten of these will mature into polypeptides with masses consistent with the polypeptides we previously described as autophosphorylating protein kinases. We are investigating the most probable candidates among these by cloning, expression and purification. Future work will use improved screening tools and immunological approaches to localize and functionally identify chloroplast protein kinases generally, and those of the thylakoid membrane in particular, for which a key characteristic is activation by a reducing redox environment.

These accomplishments are important because they set the stage for identifying enzymes responsible for adjusting the efficiency of photochemical and photosynthetic processes. With this information, transgenic biomass plants could eventually be designed with improved resistance to environmental stress.

20. Brookhaven National Laboratory

Upton, NY 11973
Modification of Plant Lipids
John Shanklin, Biology Department
$350,000

Lipids and oils are a vital class of compounds found in all living systems that occur in a wide variety of forms, particularly the storage lipids of higher plants. In eukaryotes, saturated fatty acids are modified post-synthetically by the introduction of double bonds or by the addition of a variety of functional groups.

The focus of this research is to understand the molecular basis for these lipid-modification reactions by using the process of fatty acid desaturation as a model. We are using an integrated approach combining x-ray crystallography, spectroscopy, molecular genetics, and biochemistry to probe structure-function relationships within these enzymes. Understanding the factors that control the selectivity and specificity of these processes will allow us to redesign lipid-modification enzymes with improved function or for novel uses. While our major focus is to understand the biochemical details of the reactions, our long-term goal is to introduce these re-engineered enzymes into plants that will result in renewable sources of industrial starting materials currently derived from petrochemicals.

21. Boston College

Chestnut Hill, MA 02467
Osmoregulation in Methanogens
Mary F. Roberts, Department of Chemistry
$99,000

We are investigating different aspects of how methanogens respond to osmotic stress. In the cell, small molecules (e.g., glutamate and glutamine isomers, di-*myo*-inositol-1,1'-phosphate (DIP)) and K^+ are used to balance external NaCl concentrations. NMR and HPLC techniques are used to identify these solutes (termed osmolytes because their intracellular concentrations depend on extracellular NaCl) and to quantify changes in their intracellular concentration as a function of time after the external NaCl is varied. Amounts of intracellular water can also fluctuate when external NaCl is changed (in fact this is likely to be the immediate response of cells to NaCl stress). We are trying to monitor such changes using NMR field gradient techniques that exploit diffusion properties of molecules to separate intracellular from external water molecules. Cells also synthesize specific proteins in response to NaCl stress. Some may be enzymes involved in synthesis or turnover of the osmolytes, others may be proteins that aid in refolding of macromolecules (e.g., chaperonins). By introducing ^{35}S-methionine at the time of the NaCl increase or

decrease, we can label newly synthesized proteins. Those that are synthesized at early time points after NaCl stress will be identified by gel electrophoresis and mass spectrometry methods. Lastly, specific enzymes involved in osmolyte biosynthesis will be biochemically and structurally characterized. The goal from these diverse studies is to understand all the different facets of the osmotic response in methanogens and use this information to improve the osmotic tolerance of other organisms.

22. Boyce Thompson Institute for Plant Research

Ithaca, NY 14853
Post-transcriptional Gene Regulation in Chloroplasts
David B. Stern, Plant Molecular Biology Program
$92,000

This project has focused on two aspects of chloroplast gene regulation during plant development: coordinate accumulation of components of the cytochrome b_6/f complex, and the role of a soluble RNA binding protein/ribonuclease.

Cytochrome *f* is essential for photosynthesis, and its synthesis is under exquisite regulation in chloroplasts of *Chlamydomonas reinhardtii*, a eukaryotic green alga. To find out if this regulation also occurred in vascular plants, we manipulated tobacco chloroplasts to create mutant plants either unable to synthesize cytochrome *f* or one of its assembly partners. We found that tobacco cytochrome b_6/f complex mutants did not entirely resemble the analogous mutants in *Chlamydomonas*, another important model system for understanding photosynthesis. For example, they were chlorophyll deficient, and unlike in *Chlamydomonas*, cytochrome *f* synthesis was not strongly impaired. Since in *Chlamydomonas* this regulation is mediated by cytochrome *f* itself, and the tobacco protein has a very similar sequence, this points to additional unknown factors that permit continued synthesis in the plant but not the alga. Data such as these are also important indicators of the conservation of regulatory mechanisms in different photosynthetic model systems.

The second emphasis was on CSP41, a chloroplast ribonuclease. Ribonucleases in general are critical regulators of gene expression since they influence the level of messenger RNA accumulation. We have created CSP41 mutant plants and are studying their phenotypes. Our very preliminary indication is that they might have retarded senescence, suggesting that CSP41 might have a role in cell death or chloroplast longevity.

23. Brown University

Providence, RI 02912
The Magnesium Branch of the Chlorophyll Biosynthetic Pathway
Samuel I. Beale, Division of Biology and Medicine
$114,000

Chlorophylls are the essential pigments of biological energy acquisition, necessary for the conversion of sunlight into food by plants and other photosynthetic organisms. This project is focused on how photosynthesis organisms synthesize chlorophylls. The immediate objectives are to characterize enzymes that catalyze key steps of chlorophyll formation, and on identifying, cloning, and expressing the genes that encode these enzymes. The goals are to achieve an understanding of the physical properties of the enzyme proteins, catalytic mechanisms of the enzymes, regulation of the enzyme catalysis, and control of the expression of their genes. As experimental organisms, we are using the three microorganisms with which we have made the most progress in the recent past, the unicellular green alga Chlamydomonas reinhardtii, the cyanobacterium Synechocystis sp. PCC 6803, and the photosynthetic bacterium Rhodobacter capsulatus.

24. California Institute of Technology

Pasadena, CA 91125-0001
Molecular and Genetic Analysis of LEAFY, a Gene Controlling Floral Induction and Flower Development in Arabidopsis thaliana
Elliot Meyerowitz, Division of Biology
$145,000

In the past year we obtained new mutant lines of the plant Arabidopsis in which the ability of the plant to flower properly in response to environmental stimuli (such as day length) was altered in specific ways. The mutated genes are therefore suspected of coding for proteins with critical roles in assessing environmental conditions and promoting flowering. One of the genes was cloned and sequenced, and found to encode a protein that regulates the state of other genes (a chromatin remodeling ATPase) - and thus to act in the pathway from environmental stimulus to the gene responses that start the flowering processes.
We also developed a new set of methods for the culture of Arabidopsis cells in sterile conditions, in which the culture responds to activation of flowering promotion genes by producing numerous flowers on its surface. This gives us an abundant source of developing flowers for further experimentation, and should allow the cloning of genes activated when the decision to flower is made.

25. University of California

Berkeley, CA 94720-3102
Cellular Integration of MVA Synthesis and Protein Prenylation
Wilhelm Gruissem, Department of Plant & Microbial Biology
$26,794 (four months)

Carbon allocation to isoprenoid biosynthesis in plants is of considerable importance because isoprene intermediates and end products are required in cell growth processes, hormone biosynthesis, photosynthesis, plant defense mechanisms, most other metabolic pathways, as well as for the synthesis of pharmaceutically and commercially valuable compounds. Isoprenoids in plants are derived from two principal pathways, the cytoplasmic pathway from mevalonic acid (MVA) and the recently identified alternative pathway from 1-deoxy-D-xylulose-phosphate in the chloroplast. Regulatory interactions between the two pathways are currently not well understood, although cytoplasmic isoprene synthesis appears to be most closely associated with the regulation of plant growth. Inhibition of the cytoplasmic isoprenoid pathway results in arrest of growth and cell division, but a mutation in the alternative pathway allows growth to continue in the presence of sucrose. Modification of regulatory proteins by conserved prenyl transferases with the cytoplasmic isoprene intermediates farnesyl and geranylgeranyl could provide cells with an important link between a key biosynthetic pathway and control of cell growth. We are using genetic, biochemical and molecular approaches to dissect the cellular integration of MVA synthesis and protein prenylation. As part of this effort we are analyzing the enzymatic mechanism, function and regulation of farnesyl transferase (FTase) in more detail. Targeted inactivation of geranylgeranyl transferase-1 (GGTase-1) activity will also provide insights into the function of this enzyme and expose cellular functions that require proteins modified by GGTase-1. Together, these efforts will make significant new contributions to understanding the cellular network by which isoprenoid pathways are integrated with plant growth control.

26. University of California

Berkeley, CA 94720-3102
Protein and RNA Interactions Involved in the Pathogenesis of Tomato Bushy Stunt Virus
Andrew O. Jackson, Department of Plant and Microbial Biology
$81,000

The goal of our research has been to gain a better understanding of mechanisms and interactions involved in the replication and pathogenesis of Tomato Bushy Stunt Virus (TBSV). Our studies on replication have recently identified a cis acting element that is a potent inhibitor of the trans-replication (ITR) of defective interfering TBSV RNAs (DI-RNAs). Relocation of this 150 nucleotide element in an inverse orientation into

DI-RNAs whose replication in trans is supported very efficiently by wild type (wt) TBSV drastically inhibited the replication of the DI-RNAs. Insertion of the element in the sense orientation generated DI-RNAs that could replicate but were unable to interfere with replication or pathogenicity of the wtTBSV. Thus the properties of the ITR element are strongly influenced by the overall context of the element.

Additional studies are underway to understand how TBSV mediates movement from cell to cell. For this purpose, we have constructed GFP fusions in the P-19 and p22 nested gene products known to be required for movement and are evaluating the cytoplasmic interactions of these proteins. GFPp19 produces a diffuse cytoplasmic signal whereas fluorescence of GFPp22 is associated with membranes. P22 accumulates as small intense punctate foci around the cell membrane and as intense punctate bodies in the nucleus/nucleous and the endoplasmic reticulum. A mutational analysis is underway to determine the specific amino acids of p22 that are required for subcellular targeting and movement. A model to explain the nuclear accumulation of p22 is also being tested.

27. University of California

Berkeley, CA 94720-3102

Determinants of Environmental Stress Tolerance by Bacteria on Leaves

Steven E. Lindow, Department of Plant and Microbial Biology

$83,586 (FY 99 funds – two years)

Bacteria that live on the surface of plants are important as plant pathogens, in causing plant frost injury, and in altering plant productivity in other ways. The objectives of this study are to determine those genes in the plant-associated bacterium *Pseudomonas syringae* that are expressed on leaves but not in culture and to determine how these traits enable epiphytic bacteria to survive the stresses encountered on leaf surfaces. We have optimized an in vivo selection assay for plant inducing genes based upon restoration of ability of a methionine auxotroph of *P. syringae* to survive on dry leaf surfaces when harboring a plasmid library consisting of DNA fragments containing a plant inducible promoter fused to a promoterless *met*XW locus. The selection scheme was very efficient, enriching for promoter-containing fragments by over 80-fold in each round of selection. Over 50 putative plant-inducible promoters have been found to date, representing about 1.5% of the promoters in this species. Sequence analysis of proximal DNA regions in clones revealed putative functions to the plant-inducible genes in about 40% of the cases, while the function of many genes remain cryptic. The extent of plant-inducibility was assessed by producing fusions of promoters to an ice nucleation reporter gene; most genes exhibited from 8 to 20-fold inducibility. This selection scheme was very effective in isolating weakly expressed genes and preferentially selected such genes; the majority of genes isolated were expressed at low levels even though they exhibited substantial plant inducibility.

28. University of California

Berkeley, CA 94720

Phytochrome from Green Plants: Properties and Biological Function

Peter H. Quail, Department of Plant and Microbial Biology

$108,000

Informational light signals perceived by the phytochrome (phy) family of plant sensory photoreceptors are transduced to photoresponsive nuclear genes by poorly defined mechanisms. This project is aimed at investigating these mechanisms for phyB and phy C, using Arabidopsis as a model system. In genetic screens for mutants defective in phyB signaling, we identified two loci: srl1 and gi-100. We have cloned the wild-type GI-100 gene and shown that it encodes a novel nuclear protein called GIGANTEA, also involved in controlling flowering. The data suggest a potential role in gene regulation. In addition, we have shown that light-activated phyB binds reversibly to a basic-helix-loop-helix (bHLH) transcription factor, we call PIF3, that binds in turn to a G-box DNA-sequence motif present in the regulatory regions of various light-induced genes. From these and other results, we suggest that the phytochromes may function as integral, light-switchable components of transcriptional regulator complexes, permitting continuous and immediate sensing of changes in this environmental signal directly at target genes. This proposed direct targeting of light

signals to responsive genes represents a paradigm shift in concepts regarding potential intracellular signaling pathways for the phytochromes.

29. University of California

Berkeley, CA 94720-3102

Molecular Analysis of Pathogen Recognition and Signal Transduction Events Specifying Plant Disease Resistance

Brian J. Staskawicz, Department of Plant and Microbial Biology

$114,000

Our research has focused on the molecular mechanisms of how plants recognize and defend themselves against bacterial pathogen attack. We have employed *Arabidopsis thaliana* as the model plant to identify and characterize genes from the host plant that control the recognition of bacterial pathogens and expression of plant disease resistance. Our DOE-supported research has allowed us to clone the first NBS/LRR disease resistance gene, *RPS2,* from *Arabidopsis thaliana.* This class now represents the major class of disease resistance genes in all the major crop species. Our research has specifically addressed the molecular mechanisms by which pathogens deliver avirulence effector proteins directly to the plant cell host. This work has focused on the secretion and translocation of effector proteins to plant cells. Furthermore, we are in the process of identifying the cellular targets for these proteins in both resistant and susceptible interactions. Finally, we have demonstrated that the NDR1 protein is a glycosylated protein and has a putative GPI-anchor. Preliminary results suggest the NDR1 protein is localized in the extracelluar matrix of the plant. Interestingly, overexpression of this gene leads to enhanced disease resistance to several phytopathogenic bacteria . Our current research is focused on characterizing the early molecular events that specify resistance at both the biochemical and cellular level.

30. University of California

Davis, CA 95616

The Phosphate Starvation Response Pathway in Arabidopsis thaliana

Steffen Abel, Department of Vegetable Crops

$105,000

Plants have evolved elaborate metabolic and developmental adaptations to low phosphorus availability. Biochemical responses to phosphate (Pi) limitation include increased production of Pi-acquisition proteins such as nucleases, acid phosphatases, and high-affinity Pi-transporters. However, the signal transduction pathways that sense Pi-availability and integrate the Pi-starvation response in plants are unknown. We have isolated 22 mutant lines that show reduced growth on medium containing DNA as the only source of phosphorus, but which recover on Pi-medium. Characterization of 9 lines demonstrates inability to utilize either DNA or RNA. One line, *psr1* (*phosphate starvation response*), has reduced activities of Pi-starvation-inducible isoforms of ribonuclease and acid phosphatase under Pi-limiting conditions. The data suggest that a subset of the mutations impair the expression of more than one Pi-starvation-inducible enzyme required for utilization of exogenous nucleic acids, and may thus affect regulatory components of a Pi-starvation response pathway in higher plants. Current research focuses on a detailed characterization of the most interesting *psr* mutants such as *psr1*. In a complementary approach, we have evaluated the suitability of the Pi analog, phosphite, to be used for selecting mutant plants with a constitutive response to low Pi availability. We showed that phosphite inhibits plant growth and represses Pi-starvation-inducible enzymes. However, growth inhibition by phosphite is relieved by addition of exogenous Pi. Thus, mutant plants that constitutively express Pi-starvation-inducible enzymes survive on media containing nucleic acids and phosphite. We are currently using these conditions to select for constitutive *psr* mutants.

31. University of California

Davis, CA 95616
Developmental Genetics of Nectaries in Arabidopsis and Gossypium
John L. Bowman, Section of Plant Biology
$100,000

Nectaries are secretory organs involved in offering rewards for pollinators in flowering plants. In the Brassicaceae, nectaries are positioned at the stamen bases. We have shown that while nectaries are associated with stamens in wild-type *Arabidopsis* flowers, their development does not depend on the presence of the stamens since nectaries develop in mutant strains that lack stamens. Genetic evidence suggests nectary development is independent of the ABC genes that specify the identity of the other floral organs. Nectaries form an integral component of the third whorl of the *Arabidopsis* flower. Loss-of-function alleles of *CRC* result in the loss of all morphological and biochemical signs of nectary development. However, gain-of-function alleles of *CRC* are unable to induce ectopic nectaries suggesting that *CRC* must act with a partner(s) to promote nectary development.

The aims of this proposal are three-fold. First, we wish to extend our characterization of the role of *CRABS CLAW* in nectary development in *Arabidopsis* by functionally dissecting the *CRC* promoter to identify elements that are critical for expression in nectaries. In addition, we will search for the putative partner of *CRC* required for nectary development. Second, we will extend our molecular genetic analyses of nectary development to another genera, *Gossypium*, which has nectaries of a different structure and disposition than those of *Arabidopsis*. From this phylogenetic perspective we hope to gain insight into the mechanisms of morphological evolution. And third, we will examine whether we can alter nectar composition by ectopically expressing catabolic enzymes specifically in the nectary.

32. University of California

Davis, CA 95616-8535
The Role of Rub (Related to Ubiquitin) Family of Proteins in the Auxin Response
Judy Callis, Section of Molecular and Cellular Biology
$98,000

The plant growth hormone auxin mediates multiple aspects of plant growth and development. This ability of auxin has been exploited commercially; several auxin-like compounds are herbicides, while other formulations function as root enhancing agents in horticulture. Despite their wide-spread use, we do not understand how auxin works. The goal of our DOE supported research is to understand the role of a protein called RUB in how plant cells respond to auxin. Toward this goal, we have generated plants designed to have reduced levels of the RUB protein in the model system plant, Arabidopsis. In our initial studies, we have looked at multiple independent plant lines and a significant fraction of these lines have marked changes in their growth habit. Plants are stunted with marked leaf curling. These changes are consistent with previously observed altered auxin responses, indicating that RUB is important for the auxin response. Because this protein is highly conserved, we are also studying RUB in yeast as a model for our plant studies. We have identified new proteins in yeast to which RUB attaches. This result gives us related proteins to identify in Arabidopsis. There are multiple RUB-like proteins. We are performing experiments to determine whether these proteins have identical or unique functions. The overall goal of these studies is to understand how auxin affects plant growth and responses to environmental cues. Information obtained from our studies could be useful for enhancing the growth and yield of crop plants.

DOE supported research is providing a rich environment for students. Currently assisting in experiments are two special students. One is a High School Senior, who is participating in a school sponsored internship program. Another is a Hispanic student participating in a UCD program, Biology Undergraduate Scholars Program, designed to enrich the research experience of students that might not otherwise participate in undergraduate research. In addition, partial support of two Ph.D. students has been possible.

33. University of California

Davis, CA 95616

The Mechanism and Regulation of Cellulose Syntheses in Plants
Deborah P. Delmer, Section of Plant Biology
$200,000 (20 months)

This project seeks to contribute basic knowledge concerning the mechanism and regulation of cellulose biosynthesis in plants. Because of the great abundance and commercial importance of cellulose, this pathway is of special importance for understanding the regulation of biomass production on earth. Research concentrates on studies with the developing cotton fiber—a single cell of commercial importance that deposits massive amounts of cellulose. Using this system, we have identified two genes, GhCesA-1 and GhCesA-2 that are highly-expressed in fibers during the massive phase of cellulose deposition and are believed to catalyze the polymerization of the β-1,4-glucan chains of cellulose. We have recently shed new light on the mode of action of CGA 325'615, an herbicide that specifically inhibits the synthesis of crystalline cellulose. We find that this herbicide also causes accumulation of a novel β-1,4-glucan that appears to be a unique intermediate in cellulose synthesis. The glucan is tightly-associated with CesA protein and also has the sterol sitosterol associated with it. Further work now indicates that sterol-cellodextrins may serve as primers for glucan chain polymerization, a process inhibited by another herbicide, DCB. Thus, these studies have shed new light on the both the mechanism of glucan chain initiation and elongation and show that crystallization of cellulose can be uncoupled from glucan chain polymerization. In other studies, we are also identifying unique domains within CesA proteins that may serve as interaction sites for assembly of the cellulose synthase complexes and also may regulate stability and turnover of these complexes.

34. University of California

Davis, CA 95616-8535

Structure, Function and Assembly of the Clostridium cellulovorans Cellulosome
Roy H. Doi, Section of Molecular & Cellular Biology
$120,000

The properties of the Clostridium cellulovorans cellulosome (cellulase complex) are being investigated, since the rate limiting step in cellulose degradation is the conversion of cellulose to cellobiose. If the cellulase enzyme can be engineered to be a more efficient enzyme complex and the host cell can be engineered to produce more enzyme, this will reduce the cost of converting cellulolytic biomass to ethanol and make this technology cost competitive. To this end we have studied the structure of the cellulosome and found that it is a very versatile enzyme complex capable of digesting not only cellulose, but also xylan, mannan, and pectin. Furthermore, sequencing of the genes coding for subunits of the cellulosome has revealed a gene cluster containing genes for the scaffolding protein as well as seven other enzymatic subunits and one small cell membrane associate protein that can bind enzymes. Experiments have also shown that the four hydrophilic (surface layer homology or SLH domains) domains in the scaffolding protein CbpA are capable of binding the enzyme complex to the cell surface. This allows the enzyme complex to be tightly bound to the cell surface and increases the efficiency of the cell to utilize the hydrolytic products produced by the cellulosome.

35. University of California

Davis, CA 95616-8537

Regulation of Embryonic Development in Higher Plants
John J. Harada, Section of Plant Biology
$105,000

The Arabidopsis *LEAFY COTYLEDON1* (*LEC1*) gene plays a central role in the control of embryogenesis. *LEC1* is required to suppress the embryonic potential of the suspensor, to specify cotyledon identity, to maintain the maturation phase, and to prevent developing seed from germinating prematurely. Ectopic expression of *LEC1* which encodes a HAP3 subunit of the CCAAT binding transcription factor induces

somatic embryo formation from vegetative cells, suggesting that *LEC1* is sufficient to establish a cellular environment that permits embryo development to occur. Insight into the mechanisms by which LEC1 regulates the transcription of genes that confer embryogenic competence came from the identification of another Arabidopsis gene with *LEC1* function. We showed that the Arabidopsis HAP3 family member *LEC1-LIKE* (*L1L*) that shares highest sequence similarity with LEC1 can complement the *lec1* mutation when expressed under the control of the *LEC1* promoter. Other Arabidopsis HAP3 subunits cannot complement the mutation, suggesting that a structural feature(s) common to LEC1 and L1L differentiate them from other HAP3 subunits. LEC1 and L1L share sequence similarity only in a central region of the protein, designated the B domain. This result suggests that the B domain underlies LEC1 function. Preliminary "domain swap" experiments in which the amino-terminal, carboxyl-terminal, and central B domains of LEC1 are exchanged with a HAP3 subunit provide independent support for this hypothesis. Work is currently in progress to define the specific amino acid residues in the B domain that account for the specific role of LEC1.

36. University of California

Davis, CA 95616-8537
Cellular and Molecular Characterization of Vascular Plasmodesmata
William J. Lucas, Section of Plant Biology
$126,000

The cell-to-cell trafficking of proteins and ribonucleoprotein complexes, via plasmodesmata, plays an important role in orchestrating physiological and developmental processes within the plant. Although recent studies have implicated the phloem in the long-distance transport of information macromolecules, only a limited knowledge exists as to how such molecules enter and exit the phloem translocation pathway. Our recent finding that pumpkin phloem sap contains a unique population of RNA molecules supports the hypothesis that the phloem functions as an information superhighway. To expand our understanding of the processes that underlie the operation of the phloem, both as a nutrient delivery system and as a potential information superhighway, we have continued our studies on the identification and characterization of proteins and RNPs that enter and exit the translocation stream. These proteins are being used to test the hypothesis that trafficking of proteins, between the CC and the phloem translocation stream, is a regulated process. Ongoing experiments have revealed that the phloem sap contains a unique set of RNA-binding proteins that likely play a central role in mediating the entry, translocation and controlled exit of long-distance signaling macromolecules. Finally, we have now established a model heterograft system to test the hypothesis that delivery of specific transcripts to the apex, via the phloem, can influence developmental processes. These studies will provide a solid foundation for the elucidation of the role played by plasmodesmal-mediated trafficking of long-distance information macromolecules in the integration of physiological and developmental processes that take place in distantly located organs of the plant.

37. University of California

Davis, CA 95616-8665
Physiology and Genetics of Energy Conservation in Chemoautotrophic Sulfur-oxidizing Bacteria
Douglas C. Nelson, Section of Microbiology
$88,000

Worldwide, roughly one-tenth of all organic carbon produced by photosynthetic plants, algae and cyanobacteria is degraded by microbes termed dissimilatory sulfate-reducing bacteria. These bacteria dominate organic matter degradation in marine sediments due the high concentration of their required substrate [sulfate, 28mM] in natural seawater where their anaerobic respiration is what drives biological production of hydrogen sulfide. When this compound comes into contact with oxygen, potential energy stored in the hydrogen sulfide molecules can, in turn, be harnessed by chemoautotrophic sulfur-oxidizing bacteria -- a globally important but poorly characterized group of microbes. The biochemical pathways by which sulfur-oxidizing bacteria consume hydrogen sulfide and other reduced-sulfur compounds have been deduced based almost entirely by enzyme studies. The current proposal will begin a genetic analysis of the

sulfur oxidation pathways in *Thiobacillus denitrificans* and certain marine *Beggiato*a species. The initial approach will be to focus on transformations of sulfur compounds for which these bacteria appear to have redundant but biochemically different enzyme systems. Mutations will be generated separately in the different paths to allow an evaluation of the relative importance of each. Hydrogen sulfide is a poison to most animals; hence the bacteria that remove it by biological oxidation play a protective, but poorly understood, role in ecosystems. Problems associated with hydrogen sulfide may increase if sulfur compounds derived from coal, high-sulfur petroleum and natural gas increasingly impact the biosphere.

38. University of California

Davis, CA 95616-8665
Genetic Control of Nitrate Assimilation in Klebsiella oxytoca
Valley Stewart, Section of Microbiology
$93,999

Nitrate is a significant nitrogen source for plants, most fungi, and many bacteria. Nitrate is formed in soil and water through bacterial nitrification, and is also applied as fertilizer. Our work focuses on *Klebsiella oxytoca*, a genetically-amenable enterobacterium that inhabits soil and water environments. We previously identified and characterized the *nasFEDCBA* operon encoding enzymes for nitrate uptake and assimilation. Expression of the *nasF* operon is regulated by both general nitrogen control (NtrC activation) and pathway-specific transcription antitermination. We identified and characterized the nitrate- and nitrite-responsive NasR protein as an RNA-binding transcription antitermination factor, and also characterized its RNA target in the *nasF* operon leader region. Our current focus is to further characterize general nitrogen control of *nas* gene expression. We found that *nasR* gene expression is subject to NtrC-dependent transcription activation. However, the *nasR* control region contains no apparent upstream activation sequence for NtrC binding, unlike almost all other NtrC-dependent control regions characterized to date. Most recently, we have constructed a strain that expresses decreased levels of NtrC protein. In this strain, *nasR* expression was no longer induced by nitrogen limitation. This result argues against one simple hypothesis, that the *nasR* control region has no need for an upstream activator sequence because it is unusually sensitive to NtrC activation. Rather, as with conventional NtrC-dependent promoters, that for *nasR* is active only with the normal high level of NtrC protein that accumulates during nitrogen-limited growth. We are currently considering alternative hypotheses to explain NtrC-dependent *nasR* gene expression.

39. University of California

Davis, CA 95616-8537
Protein Import and Assembly in Chloroplasts
Steven M. Theg, Section of Plant Biology
$100,000

This project seeks to understand the mechanism of trans-membrane protein transport via the thylakoid Tat pathway. This pathway is unique in its reliance on the trans-membrane pH gradient to supply the energy necessary for protein transport. We are making simultaneous measurements of protein transport and thylakoid energetic parameters, such as the magnitude of the pH gradient and the number of protons pumped during the transport reaction. Our studies will provide a quantitation of the absolute energy input per protein transported, and ultimately, should lead us to an assessment of the total energy cost to eukaryotic cells of their protein trafficking activities.

40. University of California

Irvine, CA 92697
Membrane Bioenergetics of Salt Tolerant Organisms
Janos K. Lanyi, Department of Physiology and Biophysics
$200,001

The energy costs of salt tolerance in the extremely halophilic bacteria include prominently those needed for the generating ion gradients across the cell membrane. Sodium extrusion from the bacteria is driven by exchange of protons for sodium ions, but protons are extruded and chloride ion is accumulated with active pumps. We study the molecular mechanisms of the proton and chloride transporting bacterial rhodopsins in these organisms. These are small membrane proteins in which photoisomerization of a retinal (the chromophore) initiates a sequence of reactions, the “photocycle” and either a proton or a chloride is moved across the membrane. Our studies of bacteriorhodopsin and halorhodopsin describe the atomic structure of these proteins. They explore the thermodynamics of the transport, the chromophore and protein changes that determine the change in the connection of the active site to the two membrane surfaces during the transport cycle, and the means by which the transported ions are conducted to and from the buried retinal Schiff base. The principal methods we use in this work are site-specific mutagenesis, time-resolved optical multichannel and infrared spectroscopy, and high-resolution x-ray diffraction of 3-dimensional crystals.

41. University of California

La Jolla, CA 92093-0116
The Signal Transduction Pathway of the Unfolded Protein Response
Maarten J. Chrispeels, Department of Biology
$105,000

Cells contain thousands of different proteins, each encoded by a different gene. They perform multiple functions and may be enzymatic catalysts, nutrient transporters, structural components of chromosomes or storage products in seeds, for example. Each protein molecule is a long strings of amino acids that needs to be folded in the correct 3-dimensional configuration to perform its function. Strangely enough, the process of folding is helped by yet other proteins called chaperones. Proper folding occurs when chaperones are present and the cells are growing and functioning optimally. However, when there is stress (physical or chemical), folding may go awry and the cells then need to make more chaperones. How do the cells know that they are under stress? When malfolded or unfolded proteins first accumulate in cells, a signal goes to the cell nucleus that activates genes that encode chaperones and directs them to make more chaperones, which then restore proper folding.

This signal is transmitted by a "sensor protein kinase", an enzyme located in the extensive internal membrane of the cell and binds an unfolded protein at one end. This triggers an enzymatic reaction at the other end of the protein, which begins to propagate the signal. *We have succeeded in isolating and identifying two genes that encode these sensor protein kinases. This is the first step in unraveling the pathway.*

Why is it important to know this? Stresses (drought, heat, unusual chemicals) are quite common and an understanding of these signals could help us maintain proper folding at a high level, even when cells experience stress. This process seems to be especially important during seed development, when cells make massive amounts of protein.

42. University of California

La Jolla, CA 92093-0116

Physiology and Regulation of Calcium Channels in Stomatal Guard Cells
Julian I. Schroeder, Division of Biology
$165,000

Stomatal pores in the epidermis of leaves regulate the diffusion of CO_2 into leaves for photosynthetic carbon fixation and control water loss of plants during drought periods. Guard cells sense CO_2, water status, light and other environmental conditions to regulate stomatal apertures for optimization of CO_2 intake and plant growth under drought stress. The cytosolic second messenger calcium plays central roles in guard cells by transducing signals including the hormone abscisic acid (ABA) and CO_2. Studies suggest that both plasma membrane Ca^{2+} influx channels and vacuolar/organellar Ca^{2+} release channels contribute to ABA-induced Ca^{2+} elevations in guard cells. Recent research in the P.I.'s laboratory has led to identification of a novel major cation-selective Ca^{2+}-permeable influx channel (I_{ca}) in the plasma membrane of *Arabidopsis* guard cells. These advances will allow detailed characterization of I_{ca} plasma membrane Ca^{2+} influx channels in guard cells. The long term goal of this research project is to gain a first detailed characterization of these novel plasma membrane Ca^{2+}-permeable channel currents in *Arabidopsis* guard cells. The proposed research will investigate the hypothesis that I_{ca} represents an important Ca^{2+} influx pathway for ABA and CO_2 signal transduction in *Arabidopsis* guard cells. These studies will lead to elucidation of key signal transduction mechanisms by which plants balance CO_2 influx into leaves and transpirational water loss and may contribute to future strategies for manipulating gas exchange for improved growth of crop plants and for biomass production.

43. University of California

Los Angeles, CA 90095-1606

Suspensor Differentiation During Early Plant Embryogenesis
Robert B. Goldberg, Department of Molecular, Cell and Developmental Biology
$107,000

What are the genes responsible for regulating embryo development in higher plants? Little is known about how cells within the plant embryo are specified to follow distinct developmental pathways. A novel plant with "giant" embryos, the Scarlet Runner Bean (*Phaseolus coccineus*), is being used as a system to dissect events controlling the earliest stages of embryo development. Genomics experiments are being carried out using micro-dissected regions (suspensor and embryo proper) of this embryo three to four days after fertilization (globular stage) in order to uncover genes and proteins required for the differentiation of unique parts of a plant embryo. EST sequencing, transcript profiling, and *in situ* hybridization studies have identified genes that are expressed asymmetrically in these regions shortly after fertilization. Analysis of one suspensor-region-specific gene, designated as *G564*, indicated that embryo-region-specific genes are controlled primarily at the transcriptional level by processes that are activated shortly after the zygote divides asymmetrically into two distinct-sized cells – the apical and basal cells of the two-celled embryo. Bioinformatics experiments have identified related genes in *Arabidopsis*, and "knock-out" mutants have been uncovered that lead to embryo-defective phenotypes. Finally, experiments using GeneChips that contain 8,300 *Arabidopsis* genes are being carried out in order to identify novel gene sets that regulated coordinately during plant embryo development, and, eventually, to identify regulatory circuits in the plant genome that are responsible for allowing a plant to "make a seed".

44. University of California

Los Angeles, CA 90095-1489
Molecular Biology and Genetics of the Acetate-Utilizing Methanogenic Bacteria
Robert P. Gunsalus, Department of Microbiology and Molecular Genetics
$113,212

Methane biosynthesis by the Methanosarcina species, in contrast to other methanogens, occurs from the full range of methanogenic substrates that include acetate, methanol, tri-methyl, di-methyl, and methyl-amine, and in most instances, H2/CO2. The Methanosarcina are also versatile in their ability to adapt and grow in habitats of varying osmolarity ranging from fresh water environments, marine environments, and to hyper saline environments (ca to 1.2 M NaCl). To facilitate studies that address the biochemistry, molecular biology and physiology of these organisms, we are constructing a whole-genome microarray chip to identify and characterize different classes of differentially expressed genes in M. mazei Gö1. Our goal is to identify genes involved in the synthesis and/or transport of osmolytes in the cell, and to study how they are regulated. Osmolytes include N?-acetyl-?-lysine, ?-glutamate, betaine, and potassium whose levels are modulated within the cell in order to provide appropriate osmotic balance. We will also search for and characterize gene families for use of the different carbon substrates for methane formation. In continuing studies we have examined the cellular uptake of the osmolyte betaine in halophilic methanogens. They contain high affinity transporters similar to those found in the Methanosarcina sp. These genetic and physiological studies will enhance our understanding of how methanogens respond to their environment, and adapt by adjusting their physiology to thrive in changing anaerobic habitats.

45. University of California

Los Angeles, CA 90095-1606
Sensory Transduction of the CO2 Response of Guard Cells
Eduardo Zeiger, Department of Biology
$103,000

Carbon dioxide is a key environmental signal for stomatal movements. The sensory transducing cascade coupling CO_2 perception in the guard cells with the regulation of stomatal apertures is not well understood. DOE-sponsored research in our laboratory has shown that the guard cell content of the chloroplastic carotenoid, zeaxanthin, a blue light photoreceptor of guard cells, changes in response to changes in ambient CO_2 concentrations at constant light and temperature. In *Vicia faba* leaves, ambient CO_2 concentration, zeaxanthin content, and stomatal apertures were linearly related under a broad range of ambient CO_2 concentrations. In the presence of dithiothreitol, an inhibitor of zeaxanthin formation, a decrease in ambient CO_2 concentration failed to elicit changes in zeaxanthin content and in stomatal apertures. These data suggest that illuminated guard cells can sense ambient CO_2 concentration *via* a sensory transducing cascade that couples changes in zeaxanthin content with changes in stomatal apertures. This hypothesis also links photosynthetic carbon fixation in guard cells with the regulation of zeaxanthin content and of stomatal aperture. A recent study in our laboratory has shown that guard cells from *npq1*, a zeaxanthin-less Arabidopsis mutant, lack a specific blue light response. This mutant provides a valuable genetic tool to study stomatal responses to CO_2 in zeaxanthin-less guard cells. The stomatal responses to CO_2 in *npq1* are currently under investigation.

46. University of California

Riverside, CA 92521-0124
Growth and Development Regulation by Rop GTPase Signaling in Arabidopsis: A Genome-Wide Study
Zhenbiao Yang, Department of Botany and Plant Sciences
$85,000

The Rop GTPase acts as a molecular switch that turns on or off many signaling pathways that control plant growth and development. The goal of this project is to elucidate the function of various Rop GTPases in

Arabidopsis, particularly the one involved in the control of shoot apical meristem maintenance. The most direct means of studying the function of Rops is to knockout Rop genes. Thus, the major objective within this funding period (July 2000 through September 2000) was to continue isolating knockout mutants for various Rop genes. Within this period, we isolated knockout mutants for two Rop genes in Arabidopsis and are in the process of analyzing the changes in plant growth and development induced by these mutations and several rop knockouts previously isolated. Although we have not yet identified the Rop that controls meristem maintenance, we have evidence that one Rop is involved in the negative regulation of responses to the plant hormone abscisic acid (ABA). ABA responses are important for plant growth and development and regulate plant biomass. This study will provide basic knowledge useful for improving the production of plant biomass under suboptimal growth conditions.

47. University of California

Santa Cruz, CA 95064

Regulation of Vacuolar pH in Citrus limon

Lincoln Taiz, Department of Molecular, Cellular and Developmental Biology

$106,000

Plant cells are characterized by large central vacuoles which occupy up to 90% of the cell volume. The central vacuole performs a number of vital functions for the cell, including water uptake to provide the turgor pressure needed for cell expansion, the storage of organic compounds required for metabolism, the storage of toxic ions and organic molecules needed for plant defense, and pigmentation. The uptake of many ions and organic solutes into the vacuole is driven by the vacuolar H+-ATPase. The vacuolar H+-ATPase pumps protons into the vacuole, lowering its pH and generating a membrane electrical potential. Although much has been learned about the structure of V-ATPases in recent years, little is known about their regulation. Although most vacuoles are maintained at a mildly acidif pH (pH 5), some plant vacuoles are maintained at much lower pHs. For example, the pH of lemon fruit vacuoles can be as low as 2.2. Our research has been aimed at studying the mechanism of regulation of the V-ATPase in lemon. An understanding of the mechanism of vacuolar regulation would open the door to using genetic engineering to modify the acidity, and therefore the taste, of important crop plants, such as fruits and vegetables. Using lemon, we have been able to compare the properties of the fruit V-ATPases, which hyperacidify the fruit vacuoles, with epicotyl V-ATPases, which normally acidify to about pH 5.5. We have shown that the V-ATPase of lemon fruits is specialized for hyperacidification. However, membrane lipids also contribute to hyperacidification.

48. Carnegie Institution of Washington

Stanford, CA 94305-4101

Genetic Engineering of Biomaterials

Chris Somerville, Department of Plant Biology

$526,535

The overall goal of this project is to expand the range of biomaterials produced from higher plants and increase the efficiency of biomass production. Toward this end we are pursuing several objectives. One project is focused on basic problems associated with production of industrially useful fatty acids by genetic engineering. We have discovered that several of the key enzymes involved in plant lipid metabolism are regulated posttranscriptionally. The mechanism appears to involve a region of the proteins near the C-terminus that causes the proteins to be rapidly degraded. We have found that by masking this sequence, the activity of the enzymes can be increased. A second project is focused on the characterization of a family of genes that exhibit homology to cellulose synthase and may, therefore, play a role in cell wall synthesis. We have isolated mutations in Arabidopsis that inactive most of these genes. We are using the mutants to understand what the functions of the cellulose synthase-like genes are and to discover their roles in growth and development. Knowledge of the properties of these enzymes may permit the use of genetic engineering techniques to modify the amount or composition of cell walls in economically important species. In related studies of cellulose synthase we have demonstrated that cellulose synthase is the target for several classes of herbicides and have obtained evidence indicating that each cell may require more than

one type of cellulose synthase polypeptide. We have also found that protein glycosylation is required for cellulose synthesis.

49. Carnegie Institution of Washington

Stanford, CA 94305-4150
Powdery Mildew Disease Resistance
Shauna C. Somerville, Department of Plant Biology
$100,000

The genetic basis for powdery mildew resistance in two highly resistant accessions of Arabidopsis thaliana was determined via quantitative trait loci analysis (QTL) of recombinant inbred lines. In one accession, Kas-1, three resistance loci were identified while in Wa-1 two loci were found. These results suggest that Arabidopsis is a rich source of powdery mildew resistance genes. Furthermore, the kinds of resistance mechanisms identified in Arabidopsis appeared to differ from classic resistance genes identified to date. Thus, the cloning and characterization of the Arabidopsis genes promises to provide new insights and sources of powdery mildew resistance. An alternate source of disease resistance genes is artificially-induced mutants. In a screen for powdery mildew resistant mutants, 18 mutants that develop exaggerated chlorotic and necrotic patches late in the infection cycle were recovered. The necrotic response does not develop spontaneously nor does it appear to develop in response to non-biotic stresses, distinguishing this mutant class from lesion-mimic mutants. The gene responsible for one of these mutations has been cloned and was found to encode a novel protein with two distinct regulatory motifs. Future studies of this group of mutants will contribute to our understanding of the steps leading to the necrotic defense response. DNA microarrays, which permit the measurement of transcript levels of thousands of genes simultaneously, is a powerful new tool for characterizing host-pathogen interactions. Our preliminary studies confirm the utility of this technology for implicating additional genes in plant defenses and for bringing new insight into the interactions between known plant defense pathways.

50. University of Chicago

Chicago, IL 60637
Cell-cell Interactions pollen tube growth in Arabidopsis
Daphne Preuss, Department of Molecular Genetics and Cell Biology
$104,000

This project employs molecular and genetic experiments to characterize the genes necessary for the growth of pollen tubes to the ovules. During the past year, we have characterized a mutation (pop2-1) in the POP2 gene of Arabidopsis thaliana. This gene is essential for a late step in reproduction - the guidance of pollen tubes to ovules. The POP2 gene has been cloned and the gene sequence shares high degree of homology to the class III omega aminotransferases. In addition to pop2-1 allele, we have also identified three different T-DNA insertional alleles of POP2. Our current work focuses on utilizing these POP2 alleles to understand the function of POP2 in pollen tube guidance by (i) identifying and characterizing the substrate for POP2 aminotransferase and (ii) identifying the genes through microarray experiments that show altered expression in the pop2-1 mutant compared to the wild-type plants.

51. Cold Spring Harbor Laboratory

Cold Spring Harbor, NY 11724
The Iojap Gene in Maize
Robert A. Martienssen
$102,000

Chlorophyll variegation in higher plants is a well known but poorly understood phenomenon. Single recessive nuclear mutations are often responsible and, for a few mutants, defective plastids are transmitted through to the next generation. For more than 50 years the variegated *iojap* mutation in maize has been a

model system for cytoplasmic inheritance. *iojap*-affected plastids transmitted through the female egg cell remain defective independent of the parental nuclear genotype. The striping pattern of *iojap* plants indicates that the Ij protein acts early in leaf development. Mature leaf cells can have all normal chloroplasts, or all mutant ones, or both normal and mutant plastids (heteroplastidic cells). Their arrangement on the leaf defines the pattern of striping, and may reflect the cytoplasmic inheritance of defective plastids in certain meristematic lineages. We have shown that the protein encoded by *Iojap* (Ij) is a soluble, chloroplast localized protein that associates with chloroplast ribosomes. Consistent with the proposal that Ij plays an essential role in translation, hypothetical proteins with sequence similarities to Ij have been identified in all sequenced bacterial genomes with the exception of mycoplasmas and archaebacteria. They have also been found in animal genomes, as well as in other higher plants. The combined use of plant and bacterial molecular genetic systems will serve to answer fundamental questions pertaining to variegation and cytoplasmic inheritance.

52. University of Colorado

Boulder, CO 80309-0215

Microbial Production of Isoprene

R. Ray Fall, Department of Chemistry and Biochemistry

$93,000

As petroleum availability declines, there will be increasing interest in finding alternative sources of useful hydrocarbons, especially those larger than methane. We have discovered that certain bacteria produce and emit the hydrocarbon isoprene (2-methyl-1,3-butadiene). If isoprene-producing enzymes and their genes could be harnessed, useful hydrocarbon-producing systems might be constructed. We are focusing our efforts on a common soil bacterium, *Bacillus subtilis*, since it produces isoprene in relatively large amounts, it is a very common industrial microorganism, its genome has been sequenced, and its genetics and cell development are well understood. We have shown that in *B. subtilis* isoprene is released in three distinct phases, corresponding to utilization of different carbon sources and the early stages of sporulation. We have isolated the enzyme responsible for isoprene formation, and demonstrated that its activity rises and falls dramatically with the phases of isoprene release. These results point the way to obtaining the cloned isoprene synthase gene via a proteomics approach, which should allow us to a) determine if the isoprene synthase gene is in a known operon, b) overexpress and further characterize the enzyme, c) use gene silencing or enhancement methods to assess the physiological role of isoprene production, and d) establish if isoprene is a metabolite that can be overproduced.

53. University of Connecticut

Storrs, CT 06269-3125

Genetic Analysis of Sugar Nucleotide Interconversions in Arabidopsis

Wolf-Dieter Reiter, Department of Molecular and Cell Biology

$95,000

The synthesis of plant cell wall polysaccharides depends on the availability of nucleoside diphospho sugars representing activated monosaccharides which are generated via nucleotide sugar interconversion reactions. We have chosen the plant model system *Arabidopsis thaliana* to isolate and characterize genes involved in the *de novo* synthesis of the monosaccharides L-fucose, L-arabinose, D-xylose and D-galacturonate which represent important components of plant cell wall material. By utilizing *Arabidopsis* mutants with changes in their cell wall composition we were able to clone genes in the synthesis of L-fucose and L-arabinose. An evaluation of the data generated by the *Arabidopsis* Genome Initiative revealed that these coding regions are members of small gene families which enabled us to clone and characterize additional isoforms of these enzymes. Based on sequence information on capsule biosynthesis genes in bacteria, we identified putative proteins in *Arabidopsis* which we suspect to catalyze the conversion of UDP-D-glucuronate into UDP-D-galacturonate and/or UDP-D-xylose. These activated monosaccharides represent essential substrates for the synthesis of pectins and hemicelluloses. Experiments are currently underway to determine the functions of these proteins via genetic and biochemical approaches. In the long

term we wish to understand the regulation of these nucleotide sugar interconversion pathways to modify the composition and properties of cell wall material by changing the availability of monosaccharide precursors. Since plant cell wall material is a the most abundant sink for photosynthetically fixed carbon, we hope that our work will help to make more efficient use of a major source of renewable energy.

54. Cornell University

Ithaca, NY 14853-2703
Energy Transduction in Plant Mitochondria
Maureen R. Hanson, Department of Molecular Biology and Genetics
$108,000

For optimum energy flow during the operation of biosynthetic and metabolic pathways, close association of different organelles or subcellular structures could be advantageous, permitting efficient exchange of molecules. By labeling organelles and other components of plant cells with fluorescent dyes or proteins, we have been probing the dynamic nature of two energy-transducing organelles, plastids and mitochondria. We have observed close interaction of plastids and mitochondria in certain tissues, and have explored the role of tubules emanating from plastids in movement of materials within the cell. We have observed that plastid tubules are particular abundant in large, non-green cells. Measurements of the rate of movement of a fluorescent protein through plastid tubules indicate that the internal environment of plastids and tubules is highly viscous, so that movement by diffusion is much slower than in the cytosol. Furthermore, we have unexpectedly discovered that molecules can move within plastid tubules by active transport, suggesting that these tubules may play a role in long-distance distribution of molecules within the cell. We have also investigated whether mitochondria play a role in heat production under cold temperature stress through activity of uncoupling proteins. We also considered whether mitochondrial signals are involved in programmed cell death during senescence of flower petals. Unlike in apoptosis in animal cells, we did not detect cytochrome c release from mitochondria during early stages of petal senescence, although our observations indicate that senescence exhibits features of programmed cell death.

55. Cornell University

Ithaca, NY 14853-8101
Regulation of Denitrification in Rhodobacter sphaeroides
James P. Shapleigh, Department of Microbiology
$92,997

Like humans, bacteria respire oxygen to generate the energy required for growth. Unlike humans, however, bacteria can respire compounds other than oxygen. Nitrate is one of the compounds that bacteria can use as an alternative respiratory substrate. Nitrate respiration that produces nitrogen gas as an end product is referred to as denitrification. Denitrification is the part of the global nitrogen cycle that results in the transformation of more readily utilizable forms of nitrogen like nitrate to gaseous compounds. Our laboratory is interested in understanding how bacteria sense that conditions are favorable for denitrification in an effort to better understand what regulates the flow of nitrogen through this part of the nitrogen cycle.

We have focused our effort on understanding the processes controlling metabolism of nitric oxide, an essential intermediate in denitrification. Nitric oxide production is the defining step in denitrification and it is a biologically important molecule in higher organisms, including humans. Bacteria apparently use the concentration of nitric oxide around the cell to determine if conditions are favorable for nitric oxide metabolism. We have found a single protein that senses nitric oxide and activates production of proteins used to metabolize this compound. We have also found a second set of proteins that the cell uses to ensure that nitric oxide is only produced when oxygen is absent. The interaction of the oxygen and nitric oxide respiration systems is a critical factor in determining if a cell will reduce nitric oxide and we are currently investigating this in more detail.

56. Cornell University

Ithaca, NY 14853
Cold Acclimation of Herbaceous Species: Effect of Sugars on Membrane Cryostability
Peter L. Steponkus, Department of Crop and Soil Sciences
$116,000

The ultimate goal of this project is to provide a mechanistic understanding of the cellular and molecular aspects of freezing injury and cold acclimation to provide for rational strategies for the improvement of freezing tolerance of crop species. Currently, the focus is on the role of sugars in the cold acclimation process and their interactive effects with membrane lipid alterations, the *COR* genes and the transcriptional regulators that control their expression (*DREB1A/CBF3*). In studies of *Arabidopsis thaliana* Columbia and transgenic lines that constitutively express *DREB1A* we observed a prodigious increase (10 C) in the freezing tolerance of the transgenic lines that was equal to or greater than that of the maximally cold-acclimated wild type. The increased freezing tolerance was a consequence of preclusion of freeze-induced formation of the hexagonal II phase—the primary lesion that limits the freezing tolerance of non-acclimated herbaceous plants. The increased freezing tolerance was not only associated with expression of the *COR* genes, but there were greatly increased levels of sugars—especially sucrose and raffinose—and proline and glutamine. The freezing tolerance of non-acclimated transgenic plants was increased to that of cold-acclimated transgenic lines (18 C) by incubation in a 100 mM sucrose solution for 48 hours in the dark at 23 C. This suggests that alterations in membrane lipid composition that normally occur during exposure to low temperatures also occur in the transgenic lines without exposure to low temperatures. Preliminary studies of the lipid composition of the plasma membrane are consistent with this interpretation.

57. Cornell University

Ithaca, NY 14853
Studies of the Genetic Regulation of the Thermomonospora fusca Cellulase Complex
David B. Wilson, Section of Biochemistry, Molecular and Cell Biology
$96,000

A major plus for our *T. fusca* cellulase research was the determination of the sequence of the *T. fusca* genome by the DOE Joint Genome Institute in November 2000. *T. fusca* contains three cellulase genes, besides the six we had previously cloned. However, none of these genes has an adjacent upstream copy of the 14-base operator sequence we have found upstream of each of our cloned genes. Thus, the additional cellulase genes are not induced by cellobiose and probably do not function in cellulose degradation. The operon that we had cloned and sequenced last year, containing three genes that code for a potential cellobiose transport system and a β-glucosidase gene, does have an adjacent upstream operator sequence and probably functions in both cellobiose metabolism and cellulose digestion. There are two other β-glucosidase genes in the genome, but neither is adjacent to an operator sequence. There are fifteen copies of the operator sequence in the genome. Nine are adjacent to the six cellulase genes and the cellobiose operon, one is adjacent to a xylanase gene, one is adjacent to a mannanase gene, while the other four are inside unrelated genes and probably do not function in regulation. Thus, it appears that there are only twelve *T. fusca* genes that are induced by cellobiose.

We completed our research on the cloning and sequencing of the reducing-end attacking exocellulase gene Cel48A, as well as the characterization of this enzyme. By itself, it has very low activity on any tested substrate, but it does increase the activity of mixtures of other cellulases and can synergize with endocellulases, non-reducing end attacking exocellulases and the progressive endoglucanase, Cel9A. Our studies of cellulase regulation have shown that there is a mutation in the CelR gene in one of our cellulase constitutive mutants. The mutant CelR was purified and shown to bind more weakly to the operator site than wild-type CelR. In addition, cellobiose reduced its affinity more than it did wild-type CelR. These results provide conclusive evidence that CelR is involved in cellulase regulation.

58. University of Delaware

Lewes, DE 19958
Plant Growth with Limited Water
John S. Boyer, College of Marine Studies
$110,000

In land 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 decreased growth when water is limited. Studies so far showed that 1) single cells require turgor pressure for elongation, 2) despite high turgor, elongation ceases in stems of seedlings when water is in short supply, 3) water potential gradients are significant in stems but not single cells, 4) the gradients are locally disrupted when water is in short supply, and 5) the gradient disruption prevents water from flowing into the elongating cells and thus blocks growth. These events are followed by a loss in the extensibility of the cell walls, an increase in certain wall proteins, a decrease in wall biosynthesis but increases in wall mass. Xyloglucan endotransglycosylase (XET) activity was related to the lower extensibility and increased wall mass. These metabolic effects are being further tested and the gradient concepts were explored in fully established maize plants where the growth inhibition was found to rely on similar mechanisms. We also explored the origin of the gradients that are so susceptible to disruption. It was proposed that although turgor is high, wall yielding prevents turgor from being as high as it otherwise would be and thereby creates a growth-induced water potential gradient. The hypothesis was tested by pressurizing the shoots of germinating seedlings to decrease wall yielding. As yielding diminished, the gradient likewise became smaller, supporting the wall yielding hypothesis.

59. Donald Danforth Plant Science Center

St. Louis, MO 63105
Regulating Expression of Cell and Tissue-Specific Genes by Modifying Transcription
Roger N. Beachy, President, Donald Danforth Plant Science Center
$207,000 (FY 99 funds – two years)

The goal of this project is to develop a better understanding of the regulated expression of the promoter from rice tungro bacilliform badnavirus (RTBV) and to use the information to control gene expression in transgenic plants. The RTBV promoter was shown in this and previous work to be expressed only in primary cells of vascular tissues in transgenic rice and tobacco plants. Following isolation of the cDNAs encoding two b-zip transcription factors, RF2a (previously reported) and RF2b (current study), the proteins were produced in *E. coli*. The two proteins were isolated because they bind with high affinity as homodimers and as heterodimers to a DNA sequence *cis* element immediately upstream of the TATA box of the RTBV promoter. Affinity of binding of RF2a and RF2b to the *cis* element was increased by directed mutagenesis of the *cis* element: increased binding of the factors was correlated with reduced expression of the promoter in transfected protoplasts. We have also conducted studies to express RF2a and/or RF2b in BY-2 protoplasts and in transgenic plants that also contained a reporter gene comprising the RTBV promoter and the *uid A* coding sequence. In related studies we are studying the activity of each of the proteins in *in vitro* transcription assays using extracts derived from rice cell cultures. We also developed a series of mutants RF2a and RF2b to identify the regions of the proteins that confer regulation of transcription *in vitro* and *in vivo*: one of our goals is to identify activation domains as well as mutants that can repress gene expression. A goal of these studies is to develop tools that can be used to up-regulate or repress gene expression at will in transgenic plants.

60. East Tennessee State University

Johnson City, TN 37614-0703

Functional Analysis of Chloroplast Early Light Inducible Proteins (ELIPs)
Carolyn M. Wetzel, Department of Biological Sciences
$84,959

Plants are faced with the paradoxical problem of needing to maximize absorption of light energy for photosynthesis while minimizing damage from excess light absorption. Because they are fixed in location and cannot escape from adverse environmental conditions, plants have evolved a suite of biochemical and biophysical mechanisms for photoprotection. One potential photoprotective mechanism that has recently been discovered involves a class of proteins called "early light inducible proteins" (ELIPs). The presence of ELIPs in plant cells correlates with other plant responses to high-light stress. No one, however, has yet experimentally tested the role of these proteins in the stress response. The purpose of this research project is to establish and use an experimental system in which to test the function of ELIPs in the high-light stress response. The system consists of transgenic plants that either lack or contain lower-than-normal levels of ELIP, with protein levels manipulated by molecular genetic techniques. The low-ELIP plants are being compared with normal plants to assess the role of ELIPs in plant photoprotection. In addition to the functional analyses, the project addresses the expression of the protein; i.e., what signals associated with high light stress trigger production of the protein. Together, the expression information and the functional analyses will provide us with a better picture of what causes these proteins to accumulate in the plant cells and what they are doing there.

61. Florida State University

Tallahassee, FL 32306-4370

Role of Sucrose in Modulating Stomatal Aperture
William H. Outlaw, Jr., Department of Biological Science
$84,000

Gas exchange between a leaf and the atmosphere occurs through adjustable stomata, each of which is surrounded by a pair of guard cells. It is well known that certain conditions stimulate guard cells to accumulate potassium salts. The special cell-wall architecture forces these cells to bow outward upon the consequent osmotic-water influx; this alteration enlarges the pore. Stomatal closure occurs when the guard-cell pair loses solutes. The aperture size is generally a compromise between the opposing priorities of permitting CO_2 uptake and avoiding H_2O-vapor loss. As CO_2 is required for photosynthesis and water is usually the most limiting resource for a terrestrial plant, regulation of stomatal aperture size is one of the most crucial aspects of the physiology of a plant.

Recently, an important osmotic role for internal sucrose in stomatal regulation has emerged. Our work indicates that external sucrose concentration fluctuations are also important, providing feedback if transpiration is high. This conclusion results from studies of the accumulation of a model compound outside guard cells, the movement to and accumulation of photosynthetically produced sucrose in the guard-cell wall, and the dissipation of that sucrose when transpiration rate is lowered. In the past year, this project has also supported publications regarding movement of ABA (a water-stress hormone that causes stomatal closure) from other parts of the leaf to guard cells and identification of an ABA-upregulated gene in guard cells.

62. University of Florida

Gainesville, FL 32611
Ethanol-Tolerant Biocatalysts for Fuel Ethanol Production
Lonnie O. Ingram, Department of Microbiology and Cell Science
$100,000

Over half of the petroleum used in the United States each year is imported, an amount roughly equivalent to total imported oil. This year, approximately 2 billion gallons of ethanol will be produced from corn starch and used to replace 1% of domestic automotive fuel. Benefits from the use of ethanol as a component of automotive fuel include a reduction in greenhouse gases, an increase in rural employment, improvement of the environment, and a reduction in our strategic dependence on imported oil. Ethanol and MTBE are used as oxygenates in reformulated gasoline and as fuel extenders. With the phasing out of MTBE due to toxicity in ground water, there is an immediate need to increase fuel ethanol production as well as a longer term need to increase domestic fuel production and reduce strategic dependence on imported oil. While ethanol production from corn starch can expand to a maximum of around 5 billion gallons per year, production of additional fuel ethanol must be developed from other renewable feedstocks. Our work focuses on the development and improvement of biocatalysts for the production of fuel ethanol from lignocellulosic materials such as crop residues, wood waste, and energy crops. Recombinant biocatalysts have been developed under this grant which are able to produce ethanol efficiently from all sugars found in the polymers of lignocellulose. During the past year, we have continued our investigations of physiological and genetic methods to improve the tolerance of these biocatalysts to ethanol and other compounds derived from crop residues. Improvements in tolerance to these compounds will offer an opportunity to reduce the number of steps in a lignocellulose-to-ethanol process, reduce the costs of fuel ethanol production, and improve efficiencies.

63. University of Florida

Gainesville, FL 32611-0690
Genetic Control of Abscisic Acid Biosynthesis in Plants
Donald R. McCarty, Horticultural Sciences Department
$105,000

Our genetic studies of ABA biosynthesis in maize and arabidopsis led to the discovery of a new class of enzymes that are responsible for synthesis of carotenoid derived hormones in plants and animals. In man, for example, Vitamin A and retinoid signalling molecules are derived from beta-carotene derived from food vegetables. In plants, the hormone abscisic acid (ABA) is synthesized by a related biochemical process. ABA is an important regulator of seed development and processes that enable plants to adapt to severe drought and other environmental stresses. However, the regulation of ABA synthesis and transport within the plant remains poorly understood. A key goal of our current research is to understand how synthesis of ABA is regulated in the plant during normal development and in response to drought. Through an analysis of the complete genome sequence of the arabidopsis plant we have identified a family of nine related genes that potentially control synthesis of ABA and related compounds. By making mutations in each of these genes we can determine its specific role in developmental control of ABA synthesis. Detailed analysis of the expression of each gene in the arabidopsis plant will reveal which cells and tissues synthesize ABA in normal and stressed environments.

64. University of Georgia

Athens, GA 30602-7229
The Metabolism of Hydrogen by Hyperthermophilic Microorganisms
Michael W. W. Adams, Department of Biochemistry & Molecular Biology
$112,000

Hyperthermophiles are a recently discovered group of microorganisms that have the remarkable property of growing optimally near and even above 100°C. Our understanding of the metabolism and biochemistry of

these organisms is at an early stage, in spite of their enormous biotechnological potential. The goals of this research are to elucidate the pathways that lead to hydrogen production by hyperthermophiles and to characterize the key enzymes involved. The model organism is the anaerobe *Pyrococcus furiosus* (Pf) which grows optimally at 100°C. It ferments carbohydrates and peptides to organic acids and hydrogen gas. Several of the key enzymes in the fermentation pathways are unusual oxidoreductases that contain the tungsten, a metal rarely used in biological systems, as part of their catalytic sites. These oxidoreductases provide reductant to a cytoplasmic hydrogenase for hydrogen evolution. In the prior funding period two new hydrogenases were purified from Pf. One is cytoplasmic and one membrane bound, but their functions remain unclear. A third tungstoenzyme (FOR) that appears to be involved in amino acid catabolism was also characterized. Pf contains two more members of this family according to genome analyses. A novel iron-containing enzyme termed superoxide reductase (SOR) was also discovered in Pf. It was proposed to be part of a new pathway for oxygen detoxification by anaerobic microbes in general. The crystal structures of FOR and SOR were determined in collaborative studies. A long term objective of this research is to assess the utility of hyperthermophilic enzymes in industrial energy conversions involving hydrogen.

65. University of Georgia

Athens, GA 30602-4712
Structures and Functions of Oligosaccharins
Peter Albersheim, Complex Carbohydrate Research Center
$170,000

The goals of this project are to determine the functions in plants of oligosaccharide signal molecules (oligosaccharins) and the factors that generate and destroy oligosaccharins in plants and in plants and microbes when they are interacting. The multiple mechanisms in plants that result in accumulation of bioactive levels of oligosaccharins and the corresponding mechanisms in pathogens that prevent this accumulation point to the importance of oligosaccharins in plants and their pathogens. Many of the biochemical battles that determine whether the plant overcomes or succumbs to the pathogen take place in the extracellular matrices of plants and their microbial pathogens. This research project is currently targeting three important host-pathogen interactions and the role of oligosaccharins of cell wall origin in these interactions. The three subprojects investigate the mechanisms of action of (i) plant cell wall-localized "polygalacturonase-inhibitor proteins" (PGIPs) and the fungal extracellular enzymes they inhibit or activate; (ii) a family of fungi-secreted *endo*-β-1,3-glucanase-inhibitor proteins (GIPs) and the family of *endo*–β-1,3-glucanases present in the extracellular matrix or vacuoles of plants that the GIPs inhibit; and (iii) six different *endo*-β-1,4-xylanases secreted by the fungus that causes rice blast disease and the roles of the xylanases in activating and defeating the defense responses of rice plants.

66. University of Georgia

Athens, GA 30602-4712
Center for Plant and Microbial Complex Carbohydrates
Peter Albersheim and Alan Darvill, Complex Carbohydrate Research Center
$615,000

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. This grant provides support for personnel, equipment, and consumables for a portion of this resource that is devoted to plant and microbial research. The CCRC faculty currently has 13 tenured or tenure-track members. The grant supports collaborative service research, analytical services, and training in the study of plant and microbial complex carbohydrates. Six of the CCRC's faculty members 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. Eighteen (18) undergraduate and 30 graduate Ph.D. students are currently conducting research projects or pursuing graduate degrees in the CCRC; a total of 11 undergraduate and four (4) graduate students are working in plant or microbial carbohydrate science. Week-long, hands-on laboratory training courses are held annually

for scientists from academic institutions and industries located throughout the United States. Course participants learn how to isolate, purify, and structurally characterize a broad range of complex carbohydrates. The plant and microbial carbohydrate program has provided service to 470 individuals by analyzing 1656 samples in the time that the service program has been active. Over the past year, CCRC personnel performed 260 analyses. These analyses variously include determination of glycosyl-residue and glycosyl-linkage compositions, and the acquisition and interpretation of one-dimensional NMR and mass spectra. In addition, collaborative service research provides in-depth analyses and consultations on specific carbohydrate problems requested of the CCRC's service personnel. The faculty and staff of the CCRC are currently involved in more than 158 internal or external collaborative research projects of which 81 are part of the plant and microbial carbohydrate program. The CCRC has assisted more than 33 corporations during the past year and a total of 139 different corporations since the plant and microbial program was initiated.

67. University of Georgia

Athens, GA 30602-4712
Structural Studies of Complex Carbohydrates of Plant Cell Walls
Alan Darvill, Complex Carbohydrate Research Center
$385,000

Plant primary cell walls control many important properties of plant cells (including the rate and direction of cell growth) that ultimately determine the shapes of cells, tissues, and organs. Primary cell walls form a barrier to pathogens, are the source of oligosaccharins that elicit plant defense responses in host-pathogen interactions, and appear to participate in regulating growth and development in healthy plants. Primary cell wall components are also a major source of biomass and are used in a variety of industrial and medical applications, thus constituting an important natural resource. The long-term goal of this research project is to determine the structures, functions, and locations of the non-cellulosic matrix polysaccharides of primary cell walls that, with cellulose, account for more than 90% of the dry weight of the walls. Our structural studies emphasize development of analytical methods and using them to elucidate the detailed structures of hemicellulosic (e.g., xyloglucan) and pectic (e.g., rhamnogalacturonan II) wall polysaccharides. We have recently shown that a highly specific borate di-ester cross-link of rhamnogalacturonan II is essential for normal growth and development of *Arabidopsis*. We are also examining the cell-, tissue-, and species-dependent expression of cell wall epitopes using monoclonal antibodies. This research has identified developmentally and spatially regulated variations in the fine structure of non-cellulosic polysaccharides of primary cell walls. The objectives of this research are to elucidate additional functions of primary cell wall polysaccharides and to identify how and to what extent the structures of wall polysaccharides are modified *in muro*.

68. University of Georgia

Athens, GA 30602-2152
Jeffrey F.D. Dean, School of Forest Resources
Structure-Function Relationships in Plant Laccases
$92,000

This project seeks to develop a better understanding of structure-function relationships in the laccase-type multicopper oxidase (MCO) gene family in plants. Results from this work are expected to help uncover the physiological functions of the various plant laccase isozymes. Phylogenetic analyses completed in the past year have demonstrated that plant MCO gene families are more complex than previously appreciated, falling into at least four major classes. The emergence of at least three of these classes predates the evolutionary split between the angiosperms and gymnosperms suggesting an ancient functional divergence. Our phylogenetic analyses have facilitated the development of robust computational models of plant MCO structure, and these models have allowed us to identify new structural motifs that may play important roles in regulating the functions of these enzymes. One of these motifs suggests that certain plant MCOs may be targeted to the plastids and, at least in Arabidopsis, one of these genes is expressed specifically in roots. We have cloned this particular gene and are focusing on its characterization and localization. A completely

unexpected outcome of our work on this particular MCO has been the identification of a new MCO with a similar motif in E. coli. With the expectation that improved understanding of the expression, structure and physiological function of this bacterial MCO would provide novel insights into the plant MCOs, we cloned and characterized the E. coli enzyme. Results from our studies of the bacterial enzyme have recently been submitted for publication in a pair of research papers.

69. University of Georgia

Athens, GA 30602-7229
Fermentation of Cellulose and Hemicelluloses by Clostridia and Anaerobic Fungi
Lars G. Ljungdahl, Center for Biological Research Recovery
$172,000

Worldwide photosynthetic fixation in carbon dioxide per year amounts to 150 x 10^9 tons of dry plant material consisting of cellulose (28-50%), hemicelluloses (20-30%) and lignin (18-30%). This biomass can be converted to fuel and industrial feedstock by enzymes from anaerobic microorganisms. We are studying the anaerobic fungus *Orpinomyces* strain PC-2 and the anaerobic bacteria *Clostridium thermocellum* and *C. thermoaceticum* (*Moorella thermoacetica*). *C. thermocellum* and *Orpinomyces* effectively degrade lignocellulose. This is attributed to their production of multiprotein cellulase/hemicellulose complexes called cellulosomes consisting of more than 25 different polypeptides. Most of these are enzymes with endoglucanase, cellobiohydrolase, xylanase, chitinase, lichenase, acetyl xylan esterase, and feruloyl esterase activities. Combined, they hydrolyze cellulose and hemicellulose to sugars, which are fermented to ethanol, acetate, lactate CO_2 and H_2. The enzymes are modular, each having a catalytic site and a dockerin domain. The latter binds to cohesin domains of a scaffolding polypeptide. Other domains of the enzymes include cellulose binding (CBD) immunoglobin-like (IgD), fibronectin-3-like (Fn3D), additional catalytic, and unknown domains. The ultimate goal of our work is to find how enzymes of the cellulosomes efficiently hydrolyze cellulose and hemicelluloses, and how they can work in industrial processes. This involves studies of interactions between the different subunits, dockerins and cohesins and the role of CBDs, IgD, and Fn3D. The acetogenic *C. thermoaceticum* fixes CO_2 and H_2 via the autotrophic acetyl-CoA pathway yielding acetate. This fixation generates energy and how this occurs is the subject of work with *C. thermoaceticum*. It involves electron transport and its coupling to energy generation.

70. University of Georgia

Athens, GA 30602-2605
Bacterial Nickel Metabolism and Storage
Robert J. Maier, Department of Microbiology
$108,000

Nickel is required for the growth of many microorganisms, as the metal functions as a key component of a metal center for several enzymes. Such enzymes play roles in conserving energy by harvesting of H2, utilizing carbon monoxide, or breaking down urea into molecules that can be beneficially used by organisms. However, after it is transported into cells, nickel must undergo specific protein-dependent reactions. My work has focused on the role of one such nickel-binding protein, HypB (also know as Nickelin) in two different bacteria. The nickel dependence of synthesis of the Ni-containing hydrogenase in the nitrogen-fixing bacterium Bradyrhizobium japonicum is dependent on a functional HypB. The role of HypB in storage of nickel is associated with its high Ni-sequestering ability, whereas its complementary role of Ni-mobilization into hydrogenase is associated with the energy utilizing function of HypB. The two roles can be assigned to different areas within the protein. Both roles are important in the synthesis of active nickel enzymes. The nickel-containing enzyme urease breaks down urea, enabling organisms to use the carbon and nitrogen in urea for cell maintenance and growth. A unique role for HypB in urease synthesis was discovered. Its role is related to inserting nickel into the urease enzyme. This was uncovered by use of an organism that contains both hydrogenase and urease, namely the peptic-ulcer causing bacterium Helicobacter pylori.

71. University of Georgia

Athens, GA 30602-7271

Genetic Analysis of Polyamine Synthesis in Arabidopsis

Russell L. Malmberg, Department of Botany

$108,000

The polyamines are small, positively charged, compounds that play a variety of roles in metabolism, in stress response, and in cell and developmental biology in all kingdoms. In plants there are strong correlations of polyamines with stress response, with floral development, and senescence. This correlative evidence is not definitive, and leaves open the questions of the function of polyamines in plant physiology. Research in animals and plants has also shown that the regulation of polyamine synthesis has surprising complexities and details. Our experimental goals are to use genetic tools, primarily mutants, to understand the functions of polyamines in plants, and the complex regulation of their pathway that exists. In the research leading up to this proposal, we have isolated both regulatory and structural gene mutations for loci encoding enzymes in the pathway in the model plant Arabidopsis thaliana. We are using these mutants to study the function of polyamines, and the regulation of polyamine synthesis. Additional genetic experiments are being performed performed to analyze the interactions of polyamine synthesis with other developmentally important pathways. The proposed experiments are weighted towards understanding the function and regulation of the genes encoding arginine decarboxylase, the first enzyme of the pathway. However, the long-term goal is to analyze the entire pathway, taking advantage of the powerful resources that are becoming available in Arabidopsis thaliana.

72. University of Georgia

Athens, GA 30602-7223

Mechanisms and Determinants of RNA Turnover: The Role of PAB2 in the Post-Transcriptional Apparatus

Richard B. Meagher, Department of Genetics

$106,000

The Arabidopsis genome contains only 25,000 genes, but the encoded proteome is much more complex. This grant is concerned with how post-transcriptional events control gene expression. The poly(A) tails of eukaryotic mRNAs are complexed with poly(A) binding protein (PABP). Within *Arabidopsis thaliana* the several characterized PABP genes examined so far exhibit an extreme degree of sequence divergence and are differentially expressed. While *PAB2* RNA is expressed in roots, stems, leaves, flowers, pollen, and siliques of *Arabidopsis* promoter/reporter fusions revealed spatial and temporal regulation in each organ. *PAB2* protein was also strongly expressed in the transmittal tissues of both *Arabidopsis* and tobacco, raising a possibility of its involvement in the pollination dependent poly(A) tail shortening of transmittal tissue specific mRNAs. Yeast strains defective in PABP function and complemented by PAB2 alleles were used to explore PAB2's molecular functions. PAB2 can participate in poly(A) tail shortening, demonstrating it interacts with the poly(A) nuclease complex. *PAB2* is required for translation and maintains intact polysome structures. Consistent with its role in translation initiation, poly(A) was found to enhance PAB2 binding to *Arabidopsis* eIF-iso4G *in vitro*. In addition, PAB2 can restore the linkage between deadenylation, decapping, and mRNA decay in yeast. Our results suggest that *Arabidopsis* PABPs participate in numerous complex post-transcriptional processes and do this in a tissue specific manner

73. University of Georgia

Athens, GA 30602-7271
Identification of Novel Cell Wall Components
Michelle Momany, Department of Botany
$172,000 (two years)

Filamentous fungi are among nature's best degraders. They secrete enzymes that break down bulky organic matter in the environment and absorb the resulting small products for food. The cell wall is the interface between a fungus and its environment. Information about food sources, the enzymes that break down organic molecules, and digested nutrients all travel through the wall. Fungi find food by growing long tubes, or hyphae, into new areas. If their walls are removed, fungi lose their characteristic hyphal shapes. They also lose the ability to forage for nutrients by growing into new areas.

Despite its importance, many questions about the fungal cell wall remain. We do not know all of the components that make up the wall or how these components get to the right part of the cell. We do not know how parts of the wall fit together or what happens as the wall gets older. My lab has identified nine *Aspergillus nidulans* mutants with abnormal shapes that may have defects in making the wall. We are cloning the genes represented by these mutants and studying the roles of the proteins they encode.

74. University of Georgia

Athens, GA 30602-7271
Molecular and Physiological Studies of Photosynthetic Adaptation in Nitrogen Deficiency
Gregory W. Schmidt and Brigitte U. Bruns, Department of Botany
$200,000 (FY 99 funds – two years)

It is becoming increasingly apparent that gene expression in photosynthetic organisms is intricately integrated with their metabolic activities which become optimized through poorly understood responses to light and nutrient availability. Because nitrogen availability most commonly restricts terrestrial plant growth and productivity, we focus on the means by which its availability affects photosynthetic and downstream pathways and, in particular, how it impacts on the synthesis of chlorophyll and chlorophyll-binding proteins. When nitrogen levels are insufficient, the overwhelming result is the pale-green coloration of photosynthetic tissues and diminished capacity for light-energy utilization. Using the unicellular green alga *Chlamydomonas reinhardtii* as a model system for studies of the nitrogen control pathway, we have found that the major pigment binding proteins of light-harvesting complexes and many of the key enzymes in the chlorophyll synthesis pathway are precisely regulated at the level of gene transcription by signaling pathways that are nitrogen-dependent. Reinforced by studies with mutants blocked at various steps in pigment synthesis, it is evident that chlorophyll precursor molecules somehow modulate the nitrogen signalling response and altering their levels by provision of optimal nitrogen levels somehow leads to prolific biosynthesis of a large group of both nuclear- and chloroplast-encoded photosynthetic proteins. On the other hand, sustained nitrogen-deficiency is marked by important metabolic and protective adaptations that enable survival despite greatly diminished growth rates. The nature of the mechanisms for photoprotection and metabolic cycling pathways that alter consumption of products of carbon dioxide fixation that are engaged during nitrogen deficiency are additional subjects of investigation.

75. University of Georgia

Athens, GA 30602-7223
Structure, Regulation and Evolution of the R transcriptional activators from maize and rice
Susan Wessler, Department of Botany
$112,000

The availability of increasing amounts of rice (*O. sativa*) genomic sequence has furnished a bonanza of transposable elements (TEs) which account for up to 25% of the genome. Two projects are utilizing this database in very different ways to study rice TEs. Miniature inverted repeat transposable elements (MITE)

account for over 5% of the genome. In maize, we determined that MITEs frequently insert into other MITEs, creating multimers. To determine whether multimers were a common feature of MITEs, over 25Mb of rice sequence was analyzed and MITE multimers identified and characterized. Over 10% of the 6000 MITEs were part of multimers. Furthermore, some MITE families were more likely to form multimers than others. For these families, self-insertions predominated. Multimers also provided a window on the order of amplification during genome evolution. The finding that MITEs were almost 100 times more likely to insert into MITE DNA than into retrotransposons suggested that the bulk of MITE amplification occurred prior to retrotransposition. In a second project, a search of the rice BAC end database (CUGI, Clemson) led to the discovery of a non-autonomous LTR-retrotransposon called *Dasheng*. With 1000 to 2000 copies displaying over 90% sequence identity, *Dasheng* may be the highest copy number retroelement in the *O. sativa* genome. Mapping of over 200 elements revealed their pericentromeric localization, a finding confirmed by FISH. It appears that *Dasheng* elements have succeeded in the small rice genome by targeting gene poor areas for insertion.

76. University of Georgia

Athens, GA 30602-2605
Biochemistry and Genetics of Autotrophy in Methanococcus
William B. Whitman, Department of Microbiology
$211,000 (two years)

In nature, the methanogenic fermentation of organic matter to CH4 and CO2 requires a complex consortium of microorganisms. The methanogenic archaebacteria, which catalyze the terminal step in this process, have a very narrow substrate specificity and can not directly oxidize organic compounds. Instead, they utilize H2 and formate, which are produced by other organisms in the anaerobic environment. Developing the appropriate microbial consortium is a major constraint on the practical application of the methanogenic fermentation, and the development of methanogens with the capacity to oxidize organic compounds might have a great utility. One goal of our studies is to understand the physiological basis for this narrow substrate specificity of the methanogens in order to learn how it might be broadened. For instance, we have shown that although the methanococci contain the enzymatic machinery to oxidize organic substrates such as sugars and pyruvate, this activity is not utilized in the presence of H2. Current studies are underway to examine the molecular basis for this effect. In addition, genetic methods have been developed to identify genes necessary for the autotrophic fixation of CO2 to acetate, and mutations at several loci are currently being characterized. In these environment, CO2 fixation is an anomaly and the reverse of the fermentation of organic matter to CH4 and CO2. Lastly, a shuttle vector for expression of foreign genes in methanococci has been developed.

77. University of Georgia

Tifton, GA 31793-0748
Development of Innovative Techniques and Principles That May Be Used as Models to Improve Plant Performance
Wayne W. Hanna and G. W. Burton, USDA-ARS and Department of Crop and Soil Sciences
$61,183

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. Germplasm was identified in the primary gene pool that will be useful for manipulating maturity to extend the growth period under favorable growing conditions and to shorten the growing period under adverse growing conditions. Striga resistance was identified in all three

gene pools. A system was developed to identify striga resistant germplasm in Africa, incorporate resistance into cultivated germplasm in the USA, and evaluate progress and stability.

New cytoplasms that induce improved male sterility stability were identified in the primary gene pool and have been incorporated into cultivated germplasm.

78. University of Hawaii

Honolulu, HI 96822

Mechanisms regulating blue light-activated psbD transcription in plant chloroplasts
David A. Christopher, Department of Molecular Biosciences and Biosystems Engineering
$98,578

The broad goal of this project is to understand the mechanisms underlying the efficient conversion of light into cellular energy such that rationale strategies can be developed for improving crop growth and productivity in a changing light environment. This project focuses on the genetic mechanisms and signaling pathways controlling the biogenesis of the photosystem II reaction (PSII) center in higher plants. The chloroplast psbD gene encodes the D2 subunit of PSII. In higher plants, high-fluence blue light, but not red nor far-red light, activates psbD transcription from a phylogenetically conserved blue light-responsive promoter (BLRP). Blue light-activated psbD transcription assists with maintaining adequate levels of D2, which is photodamaged and turned over in plants exposed to high light. We have isolated several proteins from Arabidopsis chloroplasts that interact with cis-elements in the psbD BLRP. We hypothesize that the interacting proteins are downstream components of a blue light signaling pathway that is modulated by phytochrome A and that regulates psbD transcription in chloroplasts. The immediate goals are to clone the cDNAs for these proteins and characterize their function and isolate new genes in the signaling pathway. Among organelle genomes in multicellular organisms, blue light-activated gene transcription is unique to higher plant chloroplasts. Thus, this work has the high potential to broaden our understanding of signal sensing and transduction processes and to provide new insights into the processes coordinating of nuclear and organelle genomes.

79. University of Hawaii

Honolulu, HI 96822

Xanthophyll Cycle and Photoprotective Systems in Higher Plants
Harry Y. Yamamoto, Department of Molecular Biosciences and Biosystems Engineering
$107,000

Light intensity in the natural environment is often higher than plants need for maximum rates of photosynthesis, being about four times higher at full sunlight. Plants need systems to protect against the excess light since it is potentially damaging. The xanthophyll cycle, comprising of reversible changes in the carotenoids, violaxanthin, antheraxanthin and zeaxanthin, is such a system. Plants become sensitive to high light when the cycle deleted or suppressed by mutagenic or molecular methods. Surprisingly, such plants still tolerate high light, especially if given time to develop seemingly alternative or parallel protective systems. The objectives of this research are to better understand the relationships among the various protective systems using molecular, biochemical and physiological approaches. What are these alterative systems? What is the relationship between the cycle and these systems? Are these other systems induced or up-regulated in response to a limited xanthophyll-cycle protective activity or are they parallel protective systems? These studies will take advantage of transformed tobacco and Arabidopsis plants that we developed in which the xanthophyll cycle is suppressed or enhanced. Mutant plants with deletions in essential components of the protective system that have been developed by other laboratories will also be used in cooperative studies. The results of these studies are expected to contribute to a better understanding of the fundamental process of high-light tolerance by plants and possibly offer means for improving light tolerance and plant productivity.

80. University of Illinois

Chicago, IL 60612-7344
Bacterial resistance to silver cations: molecular genetics, physiology and biochemistry
Simon Silver, Department of Microbiology & Immunology
$113,000

We reported in *Nature Medicine* and GenBank in 1999 the first silver resistance determinant to have ever been characterized, sequenced, and transcriptionally analyzed. It consists of seven named genes with known or predicted functions, plus two additional small open reading frames that need characterization. The system appears to be regulated by the two component sensor kinase/responder proteins SilRS, products of two genes that are transcribed separately from other genes in this system. Next to these two genes, the *silE* gene encodes a small periplasmic protein that binds 5 Ag^+ cations using 10 histidine residues in the polypeptide (there are no cysteines). More than a gram of this protein has been purified and analyzed by CD, NMR and other physical chemical methods. Those results were submitted to *J. Biol. Chem.* and hopefully will be published shortly. The four remaining genes are transcribed together in the opposite orientation and appear to encode two cation efflux pumps, a three polypeptide "RND" family system and a one polypeptide P-type ATPase. In addition to our original IncH silver resistance plasmid, pMG101, we have subsequently identified more than 50 additional bacteria with related *sil* genes by dot blotting and Southern blotting DNA/DNA analysis. These have inevitably been located on large plasmids, IncH type when identified, and not on the chromosome. When gene-specific PCR products from new isolates were sequenced, the typical difference in sequences was about 50 nucleotides per kilobase, or 5%, which suggests considerable sequence diversity, and relatively "ancient" origin for the silver resistance system. The new arsenite oxidase genetic determinant is for this molybdopterin protein with two identified Fe-S clusters that was purified from the periplasmic space of *Alcaligenes faecalis* by G. Anderson and R. Hille (1992). When we started this project even the subunit structure (previously thought to be monomeric and now thought to consist of a large and a small subunit) were not clear and some N-terminal direct amino acid sequence data was available from Anderson and Hille (but uncertain). Using degenerate oligonucleotide primers based on unpublished information from these authors, we obtained a 2 kb PCR product that clearly represents the middle 80% of the large subunit. We are currently walking in both directions by inverse PCR and seeking genomic clones from a library that has been produced, using the initial PCR product in Southern blotting.

81. University of Illinois

Urbana, IL 61801-3364
Studies on Cytochrome bo_3 from *Escherichia coli*
Robert B. Gennis, Department of Chemistry
$132,000

The long-term goal of this grant is to understand how the respiratory oxidase, cytochrome bo_3 from *E. coli*, generates a proton motive force. The enzyme catalyzes the two-electron oxidation of ubiquinol and reduces dioxygen to water. In the past year we have made significant progress to define the binding site for ubiquinol by using a combination of site-directed mutagenesis and spectroscopy. The crucial development was the publication by the group of Iwata of the X-ray structure of the enzyme. Although no quinone was present in this structure, a binding site was tentatively identified. Using the information in this publication, we designed a set of mutants to test whether this was the correct location. Several mutations were made and evaluated by steady state quinol oxidase activity, EPR spectroscopy and FTIR spectroscopy. Several mutants largely eliminated enzyme activity and, importantly, eliminated the semiquinone radical which is stabilized in the wild type enzyme. This semiquinone radical defines a "high affinity" quinone binding site. The lack of tightly bound ubiquinone was also verified by FTIR redox difference spectroscopy. In addition to this, we completed an ENDOR measurement of the semiquinone radical in a collaboration with Dr. Charles Scholes at SUNY, Albany. This work defined the nature of the protein residues hydrogen bonding to the radical.

82. University of Illinois

Urbana, IL 61801
Studies on the Microbial Formation of Methane
Ralph S. Wolfe, Department of Microbiology
$84,626

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 or in hydrothermal vents. Because the pathways of methanogenesis are becoming generally understood, we have been studying enzymes of CO_2 fixation, pyruvate carboxylase and phosphoenolpyruvate carboxykinase, that are involved in the synthesis of oxaloacetate. Although the genome of *Methanococcus jannaschii* was the first archaeal genome to be sequenced, this information has not been exploited by the scientific community due to the difficulty in mass culture of this organism. So we developed a procedure for reliable culture, which yields 8 g wet weight of cells per liter of medium. To initiate a study of proteomics this organism was grown at two levels of hydrogen partial pressure, very low (650 Pa) and high (178 kPa). When cells were exposed to hydrogen excess conditions, they possessed very low or undetectable levels of four flagella-related polypeptides, whereas, when hydrogen became limiting, these proteins were synthesized. Thus, use of proteomics showed for the first time that this methanogen can regulate expression of proteins and these experiments open the door for general studies of regulation in this hyperthermophile.

83. Iowa State University

Ames, IA 50011-2010
Analysis of a signal transduction pathway involved in maize epidermis and aleurone differentiation
Philip W. Becraft, Zoology and Genetics and Agronomy Department
$94,000

Becraft's project seeks to understand the signal transduction pathway mediated by the CRINKLY4 (CR4) receptor kinase, which is important for the differentiation of the leaf epidermis and for the aleurone layer in the endosperm of the kernel. Both tissues are important; the epidermis is the protective layer against pathogens and abiotic stresses while the aleurone is an oil-rich tissue that has potential for modifying seed composition. The two major goals are to identify other proteins that function with CR4 in this pathway and to identify genes that are regulated by this pathway. One protein that appears to function in this pathway is Thioredoxin h (TRXH). CR4 phosphorylates TRXH, increasing TRXH activity. TRXH also increases the kinase activity of CR4 leading to our hypothesis that it functions as a positive feedback mechanism to amplify CR4 signal transduction. We expect these results to be published soon in *The Plant Cell*. A gene that is positively regulated by CR4 signal transduction is the alanine amino transferase gene, which is expressed highly in the growing regions of normal maize but only weakly in *cr4* mutants.

84. Iowa State University

Ames, IA 50011-3211
Mechanism of Methane Oxidation in Cells Expressing the Membrane-Associated Methane Monooxygenase
Alan DiSpirito, Department of Microbiology
$94,444

One focus of this project still involves attempts to improve to existing purification procedures, and we have been successful in making a number of improvements in the consistency as well as the activity levels in our preparations. The second focus involves determining whether the loss of enzymatic activity by the purified enzyme is the result of (a) inactivation of a percentage of the enzyme population, (b) inactivation or alteration of the hydroxylase component of the enzyme, or (c) due to a decrease in the reduction rate of the hydroxylase component. One experiment used to address this question involved the use of the irreversible

inhibitor, ^{14}C-acetylene. In this study we examined the level of labeling by the pMMO polypeptides in whole cells, in the washed membrane fraction, and in purified pMMO preparations. The results showed the intensity of polypeptide labeling by ^{14}C-acetylene was essentially identical in all three fractions, indicating the general population of the hydroxylase component was functional. Examination of the mechanism of catalysis of trichloroethylene by whole cells and by the purified pMMO demonstrated the mechanism of catalysis by the isolated enzyme was not altered during purification (Lontoh et al., 2000a). These results as well as previous studies indicate enzyme reduction is the rate-limiting step in our purified preparations. We have substantial evidence that the enzyme is coupled to the cytochrome bc_1 complex and are attempting to reconstruct the system using purified components.

85. Iowa State University

Ames, IA 50011
Function of the Maize Starch Synthase zSSIII/DU1 in Amylopectin Biosynthesis
Alan M. Myers, co P.I. Martha G. James, Department of Biochemistry, Biophysics and Molecular Biology
$171,000 (two years)

This project investigates the mechanisms that operate within plants to assemble glucose into the storage polymers in starch. Of particular interest is the way that the architecture of starch is attained, because this determines the physical properties of starch and how this renewable resource can be used as an energy source, food, or industrial raw material. So far in the project we have constructed a series of corn mutants that vary by the activity of zSSIII/DU1, one component of the system. Starches were isolated from each mutant, and these are being analyzed for structural differences from normal corn plants. We are looking for altered starch structures with potential novel utilities. Also we are trying to correlate changes in zSSIII/DU1 activity in the different mutant lines with changes in starch structure, thus trying to understand the specific function of this enzyme in starch biosynthesis.

A second aspect of the project is to alter starch biosynthesis by completely novel means, again with the aim of generating new starches with potential industrial utilities. ZSSIII/DU1 contains a long extension in addition to the portion of the protein that is responsible for enzyme activity. We suggest that disconnecting this extension from the enzyme may subtly change the organization of the biosynthetic system, resulting in unusual changes in the product. We are now in the process of constructing transgenic plants in which zSSIII/DU1 has been clipped so that the extension and the enzyme parts are separated. These plants will be characterized in the next project period.

86. Iowa State University

Ames, IA 50011-1020
Regulation of Carotenoid Biosynthesis: The *immutans* Mutant of *Arabidopsis*
Steven R. Rodermel, Department of Botany
$90,000

The *immutans* (*im*) variegation mutant of *Arabidopsis* has green and white leaf sectors due to the action of a nuclear recessive gene. IM is a chloroplast homolog of alternative oxidase of the inner mitochondrial membrane. Because the white sectors of *im* accumulate the noncolored carotenoid, phytoene, IM likely serves as a redox component in phytoene desaturation. Analyses of *im* plants have revealed that IM has a global impact on plant growth and development, and that it is required for the differentiation of multiple plastid types, including chloroplasts, amyloplasts and etioplasts. Consistent with these observations, *IM* promoter activity and *IM* mRNAs are expressed ubiquitously in *Arabidopsis*. *IM* transcript levels do not necessarily correlate with carotenoid pool sizes, raising the possibility that IM function is not limited to carotenogenesis. Leaf anatomy is radically altered in the green and white sectors of *im*. In particular, mesophyll cell sizes are dramatically enlarged in the green sectors and palisade cells fail to expand in the white sectors. These findings suggest that *im* interrupts plastid-to-nucleus signaling pathways that control *Arabidopsis* leaf developmental programming. The green *im* sectors have significantly higher than normal

rates of O_2 evolution and significantly elevated chlorophyll *a/b* ratios, typical of those found in “sun” leaves. We conclude that the changes in structure and photosynthetic function of the green leaf sectors are part of an adaptive mechanism that attempts to compensate for a lack of photosynthesis in the white leaf sectors, while maximizing the ability of the plant to avoid photodamage.

87. University of Iowa

Iowa City, IA 52242-1109
Molecular Biology of Anaerobic Aromatic Biodegradation
Caroline Harwood, Department of Microbiology
$93,000

The soil bacterium *Rhodpseudomonas palustris* can degrade aromatic compounds that are resistant to biodegradation by humans and other organisms. Aromatic compounds include pollutants such as benzene and toluene. These compounds also occur naturally in trees and other green plants where they have been polymerized to form lignin, a major constituent of wood. We have been working to identify *R. palustris* genes that are required for the degradation of aromatic compounds in oxygen poor environments. To do this we have started to take advantage of the recently completed genome sequence of this bacterium. *R. palustris* can degrade 15 different kinds of aromatic compounds. Over the years we have identified 45 genes required for the degradation of two different lignin-derived aromatic compounds. With the genome sequence of *R. palustris* in hand we now have the ability to combine bioinformatics with traditional laboratory approaches of mutant analysis and microbial physiology. By using this combination of approaches we expect to be able to rapidly identify genes for the degradation of the remaining 13 aromatic compounds that *R. palustris* can degrade. Such information should be of eventual practical value as the anaerobic degradation of aromatic compounds is critical to the recycling of plant biomass on a global scale. It is also important for environmental bioremediation because substantial amounts of toxic aromatic compounds are produced industrially and make their way into anaerobic groundwaters and sediments.

88. Johns Hopkins University

Baltimore, MD 21218-2685
Transport of Ions Across the Inner Envelope Membrane of Chloroplasts
Richard E. McCarty, Department of Biology
$105,000

The study of ion transport into plant chloroplasts is important for understanding the global process of photosynthesis. Our lab has been involved in the determination of physiological activities and development of new strategies for identifying proteins associated with ion transport.

Calcium transport is being studied using intact chloroplasts isolated from *plumbaginafolia* plants expressing aequorin, a calcium-sensitive luminescent protein. This method has allowed us to measure free calcium levels within the chloroplast and determine conditions which affect those levels. This study is being performed by Dr. Richard Shingles in collaboration with Dr. Carl Johnson of Vanderbilt University and is part of an overall study to determine how calcium levels may be regulated during photosynthesis.

Dr. Shingles has also been using two-dimensional gel electrophoresis to generate a “map” of the chloroplast membrane proteins. Using immunoblotting and proteomic techniques several proteins have been identified including; members of the protein import apparatus, a solute channel, an iron storage protein and a putative calcium ATPase. Steve Millward, an undergraduate student, worked on a related project to isolate protein complexes associated with the chloroplast membrane. The complexes he was able to identify include the protein import apparatus and the carbon-fixing complex Rubisco.

Marisa North, an undergraduate student, has been working with Dr. Shingles to develop an assay to measure iron transport across the chloroplast inner membrane utilizing fluorescent iron indicators. This work

has identified a zinc-sensitive iron transporter. Both iron and zinc are essential for components of photosynthesis to function.

89. KAIROS Scientific Inc.

Santa Clara, CA 95054
Macromolecular Scaffolds for Energy Transfer
Douglas C. Youvan
$137,000

In order to demonstrate the feasibility of finding novel sequences from a complex mixture of microorganisms, genomic DNA was isolated and purified from hydrogen sulfide rich 'black mud'. Plasmid DNA was isolated from each culture and the clones were sequenced using fluorescently labeled primers directed to the flanking vector sequence. Partial or complete ORFs from each clone were analyzed by the BLASTX program to find sequences in the nr database that produce significant (high-scoring) alignments. Alignments were analyzed for percent similarity, which includes both identical amino acid matches and conservative replacements. The highest-scoring alignments include matches to known genes and to conserved hypothetical genes. When the highest-scoring matches are grouped by functional category, the list indicates that the distribution of putative genes *by functional category* is similar to the distribution found within the sequenced genomes of single species of bacteria. This result indicates that plethogenomic sequencing is capable of finding gene sequences that are reasonably representative of all the individual genomes contained within the microbial population. Since the microorganisms in the 'black mud' marine sediment sample are uncultivated, these results also indicate that it is possible to retrieve identifiable gene sequences from such organisms.

90. Keck Graduate Institute of Applied Life Sciences

Claremount, CA 91711
Regulation of Gene Expression by Methanol in the Yeast *Pichia pastoris*
James M. Cregg
$84,000

The ability to utilize methanol as the sole carbon and energy source is limited to prokaryotes and a few species of yeasts. In yeasts, growth on methanol requires a specific set of pathway enzymes whose synthesis is highly regulated at the transcriptional level. The long-term goal of this project is to understand the molecular mechanisms by which yeasts coordinately regulate the expression of genes in response to methanol. As a model system for these studies, we have selected *Pichia pastoris*, primarily because the glucose repression and methanol induction mechanisms are distinct and readily separable in this yeast. We have succeeded in cloning a gene, *MXR1*, whose product, Mxr1p, is a transcription factor required for gene expression in response to methanol. Currently, we are defining the specific sequences to which Mxr1p binds. We have also identified a region of approximately 250 base pairs located upstream of the highly methanol-regulated alcohol oxidase 1 (*AOX1*) gene that is necessary and sufficient to confer methanol regulation upon reporter genes.

In addition to providing important insights into the basic mechanisms that regulate expression of methanol pathway genes, these studies will contribute to the use of *P. pastoris* as a system for the production of recombinant proteins. This system is utilized by hundreds of academic and commercial labs worldwide. Results of this research are expected to result in the development of stronger methanol-regulated promoters and strains that overexpress transcription factors controlling the *AOX1* promoter.

91. University of Kentucky

Lexington, KY 40506-0055

Acetyl-CoA cleavage and synthesis in methanogens: Mechanistic, enzymological, and metabolic studies

Edward DeMoll, Department of Chemistry

$119,000 (two years)

The long term goals of this research are to understand how a multienzyme complex, designated the acetyl-CoA decarbonylase (ACDS) synthase complex functions in acetyl-CoA synthesis and cleavage in methanogens. Approximately two-thirds of the global methane produced is derived from acetate, and all of this acetate is cleaved by the ACDS complex for further reduction to methane. The ACDS complex has a molecular mass of approximately two million and is composed of five different subunits, each being present in equimolar ratios. Research approaches include enzyme kinetic studies, isothermal microcalorimetry measurements, and mutagenic experiments to better elucidate the catalytic mechanism of this important enzyme.

92. University of Kentucky

Lexington, KY 40546-0091

Molecular Regulatory Mechanisms of Two Senescence-Specific Genes in Arabidopsis

Susheng Gan, Department of Agronomy

$94,000

Our long goal is to unveil the molecular regulatory mechanisms underlying leaf senescence so that the senescence process can be manipulated for increased biomass accumulation and CO2 consumption by plants. Our specific aims are to identify, characterize, and clone genetic loci that control the expression of subsets of genes that share regulatory mechanisms with two previously identified senescence-specific genes. The overall strategy is to use a reporter gene (GUS or GFP) fused to the promoters of the two senescence-specific genes as a genetic screening marker for Arabidopsis mutants that display altered expression patterns of these two genes. The mutations will be mapped and the corresponding genes will be cloned using positional cloning method. For the first year of funding we have focused on (1) the analysis of putative mutants that exhibit elimination of the expression of one of the genes, (2) generation of new reporter gene lines in a different genetic background so that the mutation loci can be mapped, (3) generation of promoter-GFP transgenic lines for the isolation of mutants that display altered expression of the other gene. In addition, during the analysis of the mechanisms involved in regulating the second senescence-specific gene, we have discovered a genetic insulator that is the first in plants. Further analysis of the potential mutants and the insulator will provide insights into the regulatory mechanisms underlying leaf senescence.

93. University of Kentucky

Lexington, KY 40546-0091

Mechanism and Significance of Post-Translational Modifications in the Large and Small Subunits of Ribulose Bisphosphate Carboxylase/Oxygenase

Robert L. Houtz, Department of Horticulture

$87,252

In all plants atmospheric carbon dioxide is ultimately fixed during photosynthesis through the activity of one enzyme, Rubisco. This enzyme is strictly localized to plant chloroplasts, where it undergoes several significant modifications, which cannot be predicted by gene sequence alone. This project is focused on determining the mechanism and significance of these modifications to Rubisco, and exploring the potential genetic manipulation of the enzymes responsible for these modifications, in hopes of creating a more active or stable Rubisco. Such improvements in the stability or activity of Rubisco could lead to significant increases in plant biomass and greater assimilation of carbon dioxide from the Earth’s atmosphere. This project has successfully characterized several of the unique and potentially useful genes responsible for the

modifications found in Rubisco. These genes and the associated gene products are being explored for their ability to introduce site-specific modifications in Rubisco in several different plant species utilizing molecular genetic engineering techniques. Additionally, some structural features of the enzymes which modify Rubisco suggest that parts of these enzymes can be used as molecular vehicles to deliver other useful proteins or factors directly to Rubisco in vivo.

94. Lawrence Berkeley National Laboratory

Berkeley, CA 94720
CAM Biomolecular Materials Program
M.D. Alper; A.P.A. Alivisatos, C.R. Bertozzi, J. Clarke, J.M.J. Fréchet, J.T. Groves, P.G. Schultz, R.C. Stevens, Materials Sciences Division
$150,000

The program goal is the study and use of biological concepts, processes, structures and molecules as the basis for the synthesis of new materials and the enhancement of biological processes for energy applications. One component focuses on the use of natural, engineered and artificial enzymes to catalyze synthetic reactions. Enzymes exert a unique level of control over structure and can catalyze reactions at low temperature. This allows their use in the synthesis of materials with structures and therefore properties that cannot be achieved using conventional synthesis routes. Another project involves the study of the surface interactions and interfaces between inorganic materials and organisms or materials with surfaces composed of biological molecules. As biology, chemistry, physics, and materials science continue to converge, and structures and devices involving both biological and non-biological features are developed, our understanding of the nature of the surface interactions between the two types of materials must be expanded. Cell and materials surfaces are being engineered to control these interactions. Another project involves the development of metabolic engineering techniques involving the “restructuring” of groups of biochemical reactions carried out by cells to enhance their energy related functions. Since natural evolution has led to processes that are simply “good enough”, it is expected that many can be engineered and further optimized. A fourth project involves the use of short stretches of DNA of defined sequence as scaffolds for aligning nanocrystals, tubes, dendrimers and other active structures in precise two and three dimensional arrays. Preliminary structures have allowed precise control of optical and electronic interactions. Finally, collaborative work with Clarke and Alivisatos is also progressing on the use of the SQUID microscope and magnetic nanoparticles as extremely sensitive sensors.

95. Lawrence Berkeley National Laboratory

Berkeley, CA 94720
Structural Basis of Plant Signal Transduction
Sung-Hou Kim, Physical Biosciences Division
$100,000

Signal transduction pathways in higher plants are beginning to be identified. A few putative receptors and factors controlling gene expression in response to radiation, environmental chemicals, and plant hormones are being identified. Although most molecules in the pathways between signal perception and response are still unknown, some of the genes and gene products have been studied at biochemical level, but none at three-dimensional structural level. The current program is to exploit the expertise in structural biology of scientists from Structural Biology Department of LBNL to do structural studies on plant signal transduction proteins. The on-going project is the structural studies of ethylene receptors and their components. We have so far cloned the cytoplasmic domain of an ethylene receptor and subcloned kinase subdomain and response regulator subdomain of the receptor. The latter has been crystallized and the crystal structure of the protein is being determined.

96. Lawrence Berkeley National Laboratory

Berkeley, CA 94720

Energy Conversion in Photosynthesis - Mechanism of Water Oxidation and Oxygen Evolution
Kenneth Sauer, Melvin P. Klein, and Vittal K. Yachandra, Physical Biosciences Division (Note: Dr. Klein died during this past year)

$220,000

Green plants and algae generate almost all of the oxygen present in the atmosphere by the oxidation of water; a reaction on which all aerobic life depends. This light-induced oxygen-evolution is catalyzed by a tetranuclear Mn cluster present in the photosynthetic apparatus of plants and algae. The structure and oxidation states of the Mn complex, as the system is advanced through its five intermediate states (S_0 through S_4 states) by the sequential absorption of four photons, and the role of the necessary cofactors Ca and Cl, are important for understanding the mechanism of oxygen-evolution. We have shown earlier that the Mn complex contains at least two di-μ-oxo and one mono μ-oxo bridged Mn motifs in the dark state (designated the S_1 state). We have now shown that the structure of the Mn complex does not change appreciably after the absorption of one photon (S_1 to S_2 transition) but changes dramatically after the absorption of two photons (S_2 to S_3 transition). We suggest that this is the onset of water oxidation. We have used a new spectroscopic method, that measures the energy-resolved Mn X-ray fluorescence, to show that Mn is oxidized during the S_0 to S_1 and S_1 to S_2 transitions, but not during the S_2 to S_3 transition. These results also implicate water oxidation during the S_2 to S_3 transition. We have replaced Ca with Sr and by using these samples for X-ray spectroscopy studies we have demonstrated that Ca/Sr is proximal to the Mn cluster; raising the possibility that the cluster is a Mn/Ca heteronuclear cluster. These results have important implications to how plants oxidize water to oxygen using visible light in nature.

97. Lawrence Berkeley National Laboratory

Berkeley, CA 94720

Kenneth Sauer, Melvin P. Klein and Vittal K. Yachandra, Physical Biosciences Division
Energy Conversion in Photosynthesis - Photosynthetic Light Reactions
$216,000

Light absorption by the chlorophyll pigments in photosynthetic organisms determines their efficiency of growth and ability to adapt to different environments. The red (Q_y) absorption band maximum of bacteriochlorophyll *a* (BChl) in photosynthetic bacteria occurs over a range from 770 to 890 nm, depending on the immediate pigment environment and local interactions with other pigment molecules *in vitro* and *in vivo*. Studies of the solvent- and temperature-dependence of BChl spectra show that particular features can be assigned to (1) hydrogen-bonding interactions with a ring acetyl substituent that effects its orientation, (2) solvent matrix interactions and (3) excitonic coupling among adjacent molecules. [Bellacchio and Sauer, 1999]. The Kennard-Stepanov relation applied to the fluorescence and absorption spectra of these pigments provides a clear indication of spectral heterogeneity that can be applied as well to BChl-proteins in reaction centers or antenna complexes in photosynthetic membranes. We have also investigated chlorophyll *a* (Chl) in complexes in Photosystem I of higher plants. Spectroscopic heterogeneity is readily identified using PARAFAC analysis of fluorescence quenching and through extended Kennard-Stepanov theory. [Talbot, 2000] The examination of the Kennard-Stepanov spectral temperature as a function of excitation/emission wavelength is found to be an excellent indicator of heterogeneity; i.e., incomplete equilibration of excitation among molecules heterogeneously arranged in an ensemble. The K-S spectral temperature can also be used to indicate the participation of triplet states in the relaxation of excitation. [Knox, et al. 1999] By the application of this new set of experimental tools that make use of conventional steady-state spectroscopic measurements, we are able to extract information about the variety of environments and roles that chlorophyll pigment molecules play in transferring and trapping energy in the light reactions of photosynthesis.

98. Lawrence Berkeley National Laboratory

Berkeley, CA 94720
Valerie Vreeland, Division of Materials Science
Vanadium Haloperoxidase: Functional organization and regulation of catalysis in Fucus zygote adhesion
$135,000

Vanadium peroxidase in the cell wall of zygotes of the marine brown alga *Fucus* catalyzes the assembly of an adhesive coating. Understanding the process of initial adhesion in a single-cell plant system can contribute to understanding extracellular oxidative crosslinking processes in higher plants. It can also lead to new biomaterials and efficient oxidations such as lignin degradation and waste decolorization utilizing this unusually stable redox enzyme. Our goals are to identify the functional parts of vanadium peroxidase and to understand how cell wall crosslinking and bioadhesive formation are controlled. Full length and shortened recombinant forms of *Fucus* embryo vanadium peroxidase were produced in bacteria. The catalytic half of vanadium peroxidase had enzyme activity in the absence of the other half of the enzyme. This result was unexpected from the known structure of a related enzyme. Further shortening at both ends of the catalytic part provided evidence that the minimal catalytic unit consists of a bundle of four helices, located in the core of the enzyme. The helical bundle forms a rigid frame for binding vanadate in the catalytic site at one end of the bundle. The catalytic helices are arranged next to each other along the protein chain, with two intervening loops. These loops are located on the enzyme surface, and may bind the enzyme to the cell wall. Recombinant catalytic fragments lacking these loops were prepared for activity studies, and fragments representing all noncatalytic parts were made to identify which part of the enzyme binds to the cell wall.

99. Louisiana State University

Baton Rouge, LA 70803-1715
Identification of Chloride-Binding Domains in Photosystem II
Terry M. Bricker, co P.I. Laurie K. Frankel, Department of Biological Sciences
$94,000

Elucidation of the functional properties and structural organization of membrane protein complexes is one of the central objectives of current biochemical investigation. Biological membranes are involved in virtually every aspect of cellular organization and activity. One of the most intriguing aspects of membrane function is its role in the mediation of energy transduction in photosynthetic organisms. Light energy, which is the product of a most violent physical process, fusion, is transformed into biological energy equivalents utilized by the photosynthetic cell. The photosynthetic process provides both the carbohydrate which lies at the base of virtually all food chains and, as a byproduct, all of the atmospheric oxygen utilized by heterotrophic organisms. In our laboratory, we are interested in identifying which proteins bind the chloride ions essential for the oxygen evolution activity of Photosystem II. We have identified amino acid residues (Arg448, Lys321) on the CP 47 protein which, when modified by site-directed mutagenesis, drastically affect the binding of chloride to Photosystem II . Alteration of these residues leads to a dramatic stabilization of the S2 and S3 states of the oxygen-evolving complex. We hypothesize that Arg448 is involved in the formation of the low affinity, rapidly exchanging site previously identified (Lindberg, K. and Andreasson, L.-E. (1996) Biochemistry 35, 14259). We have also isolated a number of intergenic suppressors of these site-directed mutations and are in the process of mapping their location in the cyanobacterial genome.

100. Marquette University

Milwaukee, WI 53201-1881
K. Dale Noel, Department of Biology
Bacterial lipopolysaccharide structures required for root nodule development
$94,000

Understanding the basic mechanisms of legume root nodule development is of great importance agriculturally and environmentally. The bacteria in these nodules make nitrogen in the atmosphere available

to the plant so that energy-costly and highly-polluting nitrogen fertilizer is not required. In previous work we found that the bacterial O antigen, the outermost segment of the abundant lipopolysaccharide on the bacterial surface, is crucial for the entry of bacteria into developing root nodules. During the past year we have demonstrated that the plant will not tolerate even a 50% decrease in these structures, which normally may be abundant enough to cover the bacterial surface almost completely. We have identified two specific chemical modifications of the O antigen that the bacteria make in the presence of the plant. Bacterial mutants unable to carry out one of the modifications were deficient in the development and invasion of nodules. Other bacterial mutants, which lacked one component sugar (quinovosamine) of the polysaccharide, were greatly impaired in infection as well. From these and previous results, we hypothesize that the O-antigen polysaccharide plays two roles. It nonspecifically protects the bacteria from toxic compounds that are part of plant defenses, and it acts as a signal in which specific portions of its structure interact with recognition molecules on the plant cell surfaces. The O antigen, therefore, appears to be an important member of a cadre of bacterial carbohydrates that are specifically tailored by chemical modifications to foster intimate association with the plant.

101. University of Maryland

Baltimore, MD 21202
Physiology and Genetics of Catabolic Metabolism in the Aceticlastic Methanogens
Kevin R. Sowers, Center of Marine Biotechnology
$95,000

Biomass conversion catalyzed by methanogenic consortia is a widely available, renewable resource for both energy production and waste treatment. The efficiency of this process is directly dependent upon the dismutation of acetate to methane, which is the rate-limiting step in the degradation of soluble organic matter. Acetate utilization is highly regulated, and we have shown that acetate catabolism is mediated in part by regulation of gene transcription in the archaeon *Methanosarcina*. A combination of physiological and genetic approaches are being utilized to investigate the cellular mechanisms that control expression of the catabolic gene encoding CO dehydrogenase (c*dh*), which catalyzes the dismutation of acetate in *Methanosarcina* spp. We have tested the *in vivo* expression of several fusion constructs, developed a functional archaeal *cdh*A::*lac*Z reporter vector and confirmed that *cdh* expression is controlled at the level of transcription. Methanosarcinal recombinant cells containing the reporter fusion are being grown in a chemostat to assess the effects of acetate and alternative substrate thresholds on the expression of CO dehydrogenase, which will enable us to determine the kinetics of *cdh*A expression. Concurrently, we are identifying regions of DNA that are critical for regulation of gene expression with deletion constructs in *Methanosarcina acetivorans.* Genes that encode signal proteins and transcription factors will be identified subsequently by disruption of the regulatory genes and screening on different substrates. Functional interactions will be confirmed *in vivo* by assaying the effects of gene dosage and site-directed mutagenesis of the regulatory gene. Results of this study will reveal factors that limit the rate of acetate catabolism at the molecular level and enable us to determine which portions of the degradative process would be potentially amenable to biotechnological enhancement.

102. University of Maryland

College Park, MD 20742-5815
Suppressors and enhancers of an Arabidopsis ethylene receptor mutant
Caren Chang, Department of Cell Biology and Molecular Genetics
$77,000

Ethylene gas has profound effects on numerous aspects of plant growth and development. The mechanisms of ethylene perception and signal transduction are starting to be revealed, largely based on research in *Arabidopsis*. Numerous ethylene-response mutants have been isolated, leading to the identification of a number of genes acting at different steps of the pathway, including five ethylene receptor genes. In this project, we are dissecting the ethylene-response pathway further by isolating new mutants, with the eventual goal of cloning the corresponding genes. We have isolated several mutants that suppress

or enhance a particular ethylene receptor mutation, which alone, confers ethylene insensitivity in *Arabidopsis*. Two of the new mutants are defective in the same gene (designated *RTE1*), causing the plant to have a normal ethylene response even though it carries the defective ethylene receptor. We are close to isolating the *RTE1* gene on the basis of its chromosomal location; we localized *RTE1* to a small chromosomal region containing eight genes, and we are in the process of determining which of these is *RTE1*. Future analyses of *RTE1* and its gene product will enhance our understanding of the ethylene-response pathway. Using a different approach, we have isolated a novel enhanced ethylene-response mutant, called *eer1*, which has defects specific to the hypocotyl and stem in *Arabidopsis*. Analysis of the *eer1* mutant revealed that stem thickening in *Arabidopsis* occurs as an ethylene response. An improved understanding of ethylene signaling will allow us to better manipulate plant processes in support of novel energy-related biotechnologies.

103. University of Maryland

College Park, MD 20742

A New Pathway for Isopentenyl Pyrophosphate Synthesis in Bacteria and Plants

Elisabeth Gantt and Francis X. Cunningham, Jr., Department of Cell Biology and Molecular Genetics

$99,004

In plants isoprenoid biosynthesis is required for many essential compounds. Among these are vitamin precursors, carotenoids for photoprotection of photosynthesis and for flower and fruit coloration, and quinones for electron transport. Isoprenoid biosynthesis by the non-mevalonate pathway, beginning with pyruvate and glyceraldehyde-3-phosphate and leading to isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), has been identified as a major pathway in plant chloroplasts and in some bacteria. The cyanobacterium *Synechocystis* PCC6803 is being used as a model for plant chloroplasts. A conversion of IPP to DMAPP, catalyzed by IPP isomerase, is an essential step in the mevalonate pathway. However, we found that isoprenoid synthesis in two cyanobacteria (*Synechocystis* PCC6803 and *Synechococcus* PCC7942) may not involve an interconversion of IPP and DMAPP in the non-mevalonate pathway. IPP isomerase activity, as reported for plants and *E. coli* , is lacking in these cyanobacteria consistent with the absence of an obvious homologue for the IPP isomerase enzyme in the genome of *Synechocystis* PCC6803. A separate origin of IPP isomerases in plants and green algae is suggested from the analysis of plant and algal cDNAs. Also, a new gene (*lytB*) essential for the non-mevalonate pathway was identified and its function is being characterized in mutants and by biochemical studies with the recombinant enzyme. The involvement of two additional novel genes for this pathway is being explored in *Synechocystis*. Elucidation of the non-mevalonate pathway will give new targets for development of antibiotics and is relevant to food production, plant and animal pathogens, and amelioration of environmental pollutants.

104. University of Maryland

College Park, MD 20742-5815

Investigating the molecular mechanism of TSO1 function in Arabidopsis cell division and meristem development

Zhongchi Liu, Department of Cell Biology and Molecular Genetics

$96,000

Shoot apical meristem (SAM), the small number of cells at the tip of a shoot, is the source of all aerial parts of plants. Our long-term goals are to understand the molecular mechanism underlying cell proliferation control in SAM and the critical roles of cell division in plant SAM organization and development. With the support from DOE, we have been focused on the molecular isolation and analyses of *TSO1*, an important regulator of *Arabidopsis* SAM. In *tso1* mutants, the SAM bifurcates to give rise to two or more SAM, resulting in a multiply branched and cauliflower-like inflorescence. In addition, cell division in *tso1* mutant flowers is abnormal; these mutant cells exhibit increased DNA contents and partially formed cell walls. Using a map-based approach, we isolated the *TSO1* gene and showed that *TSO1* encodes a putative nuclear protein with two conserved cysteine-rich repeats. Each repeat bears similarity to *Drosophila*

Enhancer of zeste family of chromatin repressors. This cysteine-rich repeat in *CPP1*, a soybean protein with sequence similarity to TSO1, was shown to directly bind DNA. We propose that *TSO1* encodes a novel plant regulatory protein involved in transcriptional repression of several target genes for cell division and SAM organization. We are in the process of examining the tissue distribution and subcellular localization of *TSO1* protein using newly raised anti-TSO1 antibody and Green Fluorescent Protein reporters. By illuminating the molecular mechanism underlying *TSO1* function, we will contribute to the general understanding of cell division control and its relation to meristem development in higher plants, an area largely unexplored until recently.

105. University of Maryland

College Park, MD 20742-5815
Structure and Regulation of Calcium-Pumping ATPases and Plants
Heven Sze, Department of Cell Biology and Molecular Genetics
$99,000

The spatial and temporal changes in intracellular [Ca^{2+}] during growth and during responses to hormonal and environmental stimuli indicate that Ca^{2+} influx and efflux transporters are diverse and tightly regulated in plants. Yet we are just beginning to identify and characterize calcium transporters at the molecular level. Ten or more Ca^{2+} pumps in Arabidopsis function not only to extrude Ca^{2+} from the cell, and to fill internal stores, but also are proposed to shape Ca^{2+} spikes or waves that are important determinants of cellular functions and responses. Of the >10 Ca^{2+}-ATPases, only a few have been functionally characterized, though the activity modulation, and the physiological roles of each protein are not understood. We identified the first active Ca^{2+} pumps from plants after functional expression in a yeast mutant. This expression system has opened the way to a genetic and biochemical characterization of the regulatory and catalytic features of diverse Ca^{2+} pumps. At**E**CA1 and At**A**CA2, now serve as models for '**E**R-type' and '**A**utoinhibitor-type' pumps, respectively. AtECA1 has been localized to endomembranes, including the ER, and is not regulated by calmodulin. AtACA2 also found on the ER is subject to regulation by calcium. A novel N terminal regulatory domain serves multiple functions, including autoinhibition, calmodulin-binding that leads to activation, and a site for phosphorylation by calcium-dependent protein kinase. Phosphorylation blocks calmodulin activation. These findings would support the idea that the shape of Ca^{2+} transients can potentially be altered by the activity of Ca^{2+} pumps. Thus studying the regulation of Ca pumps is a step towards understanding their roles in plant growth and responses to the environment.

106. University of Massachusetts

Amherst, MA 01003-5720
Microbial Fermentation of Abundant Biopolymers: Cellulose and Chitin
Susan B. Leschine, Department of Microbiology
$100,000

Cellulose is the most abundant biopolymer and renewable energy source on Earth. The decomposition of cellulose, which is carried out almost exclusively by microorganisms, is a key step in the cycling of carbon in the biosphere. Vast quantities of cellulose are degraded in environments devoid of oxygen where cellulose decomposition is effected by communities of physiologically diverse microorganisms, including strict and facultative anaerobes. Long-term goals of our research program are to advance understanding of the physiology, ecology, and diversity of cellulolytic members of anaerobic cellulose-decomposing microbial communities, and to discern the nature of key interactions among community members contributing to the efficient degradation of cellulosic materials. Chitin also is produced in enormous quantity in the biosphere as a structural biopolymer in cell walls of fungi, shells of crustaceans, and exoskeletons of insects. As the most abundant nitrogen-bearing organic compound found in nature, chitin is widely distributed in soils and in freshwater and marine sediments. In these environments, cellulose is present in proximity to chitin, and some organisms have evolved the ability to degrade both insoluble biopolymers. Our research program is directed toward expanding understanding of processes involved in the anaerobic decomposition of cellulose and chitin, the biology of the cellulolytic and chitinolytic microbes involved, and the cellulase and chitinase

enzyme systems produced by these microbes. Results of this research will provide fundamental information on the physiology and ecology of cellulose- and chitin-fermenting bacteria that will be invaluable in the development of practical applications, such as the bioconversion of cellulose- and chitin-containing wastes to fuels such as ethanol.

107. University of Massachusetts

Amherst, MA 01003
Mechanisms for Microbial Reduction of Humics and Structurally Related Compounds
Derek R. Lovley, Department of Microbiology
$96,000

Microbial reduction of humic substances (humics) under anaerobic conditions can have an important role in: 1) the natural cycling of organic compounds, nutrients, and metals; 2) plant-microbe interactions; and 3) the remediation of organic and metal contaminants. The only microorganisms known to use humics as an electron acceptor are dissimilatory metal-reducing microorganisms. The purpose of this research is to determine the mechanisms for electron transfer to humics in these microbes. These studies have been carried out with *Geobacter sulfurreducens* because microorganisms in the *Geobacteraceae* family are the most abundant humics-reducing microorganisms in a variety of sedimentary environments. Biochemical studies demonstrated that many redox-active molecules in *G. sulfurreducens* and other microorganisms could reduce humics, making it difficult to determine the physiologically relevant humics reductase with just a biochemical approach. Therefore, a genetic system for *G. sulfurreducens* was developed, which permitted the generation of knock-out mutations to determine which proteins were important for the reduction of humics. This is a major accomplishment, considering that not even effective mechanisms to plate this strict anaerobe were available prior to these studies. To date, mutations have been made in several genes expected to code for cytochromes and other redox proteins involved in electron transport to humics. In addition to providing insights into the mechanisms for the reduction of humics, these genetic tools will greatly aid in understanding other environmentally significant processes in *G. sulfurreducens* such as the reduction of radioactive and toxic metals and the degradation of organic contaminants.

108. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824
Molecular Basis of Symbiotic Plant-Microbe Interactions
F.J. de Bruijn
$205,769

The generation of nitrogen-fixing root and stem nodules on leguminous plants by soil bacteria belonging to the Rhizobiaceae is a highly evolved and 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 the colonization and the early infection of legume roots. To increase our understanding of these early stages, we are examining how microbes respond at the molecular level to environmental stresses, and the importance of these responses for microbial persistence and root colonization. Once the infection process has been initiated, distinct sets of plant and bacterial genes are induced, which are involved in nodules development and in symbiotic nitrogen fixation. We are using the model legume Lotus japonicus and its symbiont Mesorhizobium loti to identify genes that are involved in this process. In L. japonicus, we are studying novel symbiotic mutants generated via chemical mutagenesis in addition to symbiosis-specific genes identified via differential display of messenger RNA's. In M. loti, we helped sequence the 500-kb “symbiosis island” which has been proposed to carry most if not all of the microbial genes required for symbiotic nitrogen fixation. This information will aid us in the generation of M. loti symbiotic mutants. They systems and projects described above provide an excellent opportunity to increase our understanding of the molecular basis of symbiotic plant-microbe interactions.

109. Michigan State University-DOE Plant Research Laboratory
East Lansing, MI 48824
Molecular Mechanisms That Regulate the Expression of Genes in Plants
Pamela J. Green
$260,769

The control of mRNA stability is an important component of eukaryotic gene expression. A major goal of our research is to elucidate mechanisms that target highly unstable mRNAs for degradation because these mechanisms provide plants with the means to make rapid changes in gene expression in response to a variety of stimuli. To this end we isolated mutants of Arabidopsis, designated *dst1* and *dst2* that are defective in an mRNA decay pathway mediated by the mRNA instability sequence DST. Current efforts focus on mapped-based cloning of the *DST1* and *DST2* genes, and microarray analysis of the mutants to determine which other genes are affected. Microarray analysis is also being developed to monitor mRNA stability directly. Other projects include the characterization of intra- and extracellular ribonucleases for their roles in mRNA decay and other processes.

110. Michigan State University-DOE Plant Research Laboratory
East Lansing, MI 48824
Molecular biology of plant-bacterial interactions
Sheng Yang He
$240,769

Plant disease is a leading cause of crop loss worldwide. Bacterial pathogens are responsible for many of the plant diseases and are quite diverse in their taxonomical properties and hosts, and in the disease symptoms they cause. Remarkably, many of them contain a conserved protein secretion system known as the type III protein secretion system, which is also present in several animal and human pathogens. This secretion system is believed to inject bacterial virulence proteins directly into host cells and plays a central role in bacterial pathogenesis. We study the type III secretion system of a model pathogen, *Pseudomonas syringae* pv. *tomato* DC3000, and its interaction with the host *Arabidopsis thaliana*. To this end, we have i) obtained evidence for type III injection of virulence proteins into *Arabidopsis* cells, ii) identified several type III virulence proteins using biochemical and genomic approaches, iii) discovered a novel pilus which guides type III secretion in this bacterium, and iv) determined the effects of the type III virulence system on *Arabidopsis* gene expression by microarray. Our research is beginning to gain insights into the mechanism by which a representative bacterial pathogen attacks a plant host, which should in turn provide information for the design of control measures aimed at disarming pathogens.

111. Michigan State University-DOE Plant Research Laboratory
East Lansing, MI 48824-1312
Molecular and Biochemical Basis of Induced Resistance
Gregg A. Howe
$240,769

The long-term goal of our research is to understand, at the molecular and biochemical level, mechanisms of plant defense against insects and pathogens. Towards this goal, we are investigating a class of bioactive compounds called oxylipins that regulate plant defensive processes. One project seeks to understand how the plant hormone jasmonic acid (JA), a potent oxylipin, regulates defenses triggered by insect attack or wounding. We used a genetic approach to identify mutant varieties of tomato that fail to perceive JA as a regulatory signal. Two such mutants were identified and shown to be deficient in the defense reactions that are normally switched on by JA. Identification of the gene(s) defined by these mutations will provide new insight into the mechanism of JA-mediated plant defense. A second project was to identify novel oxylipins, and to determine their function in plant growth and development. Our experimental approach was to search DNA databases for plant genes that we hypothesize to be involved in the biosynthesis of oxylipins. This work led to the discovery of a tomato gene encoding the enzyme divinyl ether synthase (DES). DES

produces divinyl ether compounds that are thought to protect plants against *Phytophthora infestans*. This fungus was responsible for the Irish potato famine, and continues to cause devastating crop loses worldwide. The discovery of the DES gene may facilitate the engineering of plants for increased resistance to *Phytophthora* and perhaps other pests as well.

112. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824
Plastid Biogenesis
Kenneth Keegstra
$270,769

Plastid biogenesis requires the coordinated accumulation of gene products from two different genomes, i.e. plastid and nuclear. Because the plastid genome has a limited coding capacity, most plastid proteins are encoded in the nuclear genome, synthesized in the cytosol as precursors and imported into plastids via a posttranslational transport process. Nuclear-encoded proteins need to be directed to six compartments within chloroplasts, the most complex member in the family of plastids. Although a basic outline of precursor transport into plastids is now available, many important details are still missing. Our laboratory is investigating some of the unsolved problems of protein targeting into plastids using both genetic and biochemical strategies. Mutants containing T-DNA inserts in genes encoding selected components of the transport apparatus have been isolated and are being characterized. Other biochemical efforts are aimed at investigating the molecular chaperones involved in providing the driving force for import into chloroplasts. Finally, we are also exploring the pathways for targeting proteins to the inner envelope membranes of chloroplasts.

113. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824
Studies on Hormone Action in Vegetative Growth
Hans Kende
$260,769

We are using deepwater rice as a model plant to study the action of plant hormones on stem elongation. Deepwater rice is a subsistence crop in areas of Southeast Asia that are flooded during the rainy season. Survival of this rice depends on its capacity to elongate rapidly when it becomes submerged and to keep part of its foliage above the rising waters. Three plant hormones ethylene, abscisic acid (ABA), and gibberellin (GA)participate in regulating growth. The immediate growth-promoting hormone is GA. We have investigated the effect of GA on gene expression in rice internodes. We found three categories of genes whose expression increases upon treatment with GA: genes encoding cell-cycle-related proteins, genes encoding the wall-loosening proteins expansin, and genes with unknown function. In the latter category, we became particularly interested in one growth-related gene, *Os-GRF1*, whose product has all the hallmarks of a novel transcription regulator. Screening the databases, we have found that -expansins are encoded by at least 27 genes in the rice genome. We have studied their expression pattern and found that 10 of them are expressed in the internode. We also identified 16 -expansins, six of which are expressed in the internode. GA induces the expression of both types of expansin genes. The role of expansins in the growth is being investigated in transgenic rice in which expansin gene expression is increased or decreased. In addition to examining the function of Os-GRF1 in rice, we are also investigating the role of similar proteins in Arabidopsis using knock-out mutants.

114. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824
Photoperiodic Induction and the Floral Stimulus
H. Kende, L. McIntosh, J.A.D. Zeevaart
$235,769

Differential display of mRNA was used to identify genes associated with floral induction in the cotyledons of the short-day plant *Pharbitis nil*, strain Violet. A *Pharbitis* ortholog of the Arabidopsis *CONSTANS* (*CO*) gene was identified, which will be referred to as *PnCO*. Expression of *PnCO* was high after a 14-h night, but low when the dark period was 12 h or less. This indicates that the level of the *PnCO* transcript is photoperiodically regulated. After transfer from continuous light to darkness, *PnCO* showed a circadian pattern of expression. A major portion of the *PnCO* transcripts contained an unspliced intron. Only the intron-free *PnCO* was able to complement the *co* mutant of Arabidopsis by shortening the time to flower under short-day conditions. Thus, the CO protein is functional in the induction of flowering in both short-day and long-day plants.

Translocation in the phloem involves not only sugars and other small molecules, but also peptides, proteins and nucleic acids. Thus, the floral stimulus, which moves in the phloem from induced leaves to the shoot apex, could be any or a combination of these components. To investigate the nature of the floral stimulus, phloem sap from flowering and vegetative *Perilla* is being analyzed by HPLC and MALDI-MS for possible differences in peptide composition. A further goal of this project is to employ cDNA-AFLP fingerprinting to identify potential nucleotide signals for flowering. The technique is being developed with phloem exudate collected from cucumber.

115. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824
Interaction of Nuclear and Organelle Genomes
Lee McIntosh
$250,769

We are interested in the genetic and biochemical mechanisms that allow plant organelles to mediate energy transduction, biosynthesis of carbon intermediates, and environmental sensing. Inherent in this is a basic understanding of organelle/nuclear communication, especially mechanisms that allow the plant to survive environmental stress. Plant mitochondria directly influence plant stress responses, carbon and nitrogen flux, plastid function and biogenesis and, ultimately, cell death. The mitochondrial mediating signals (and receptors) that influence nuclear gene expression are virtually unknown in plants. Learning how these capacities are coordinated and linked to cellular function, with emphasis upon biochemical/physiological consequences to the organism, is a primary goal of our group. To explore these signal systems we are employing two approaches: 1) characterization of mitochondrial signal transduction mutants in Arabidopsis and 2) genomic approaches to expression of nuclear genes regulated by mitochondrial signals.

Two photosynthetic reaction centers act in series to convert visible photons into chemical free energy: Photosystem II (PS II), capable of oxidizing water; and Photosystem I (PS I), generates a strong reductant capable of reducing NADP+. PS I, the 'least studied' photosynthetic complex, contains three [4Fe-4S] clusters FX, FA and FB. We know from other, bacterial, organisms possessing critical iron-sulfur clusters that there are essential genes involved in iron-sulfur cluster assembly: cysteine desulfurization, Fe and S mobilization, and assembly/proofreading of subunits containing Fe-S. We are working on a new question concerning Photosystem I: what genes regulate [4Fe-4S] cluster assembly and thus control PS I complex biogenesis?

116. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824
Molecular Mechanisms of Protein Trafficking Through the Secretory System
N. V. Raikhel
$260,769

Cargo proteins destined for plant vacuoles contain positive sorting signals that lead to their segregation from the default secretory pathway to the cell surface. Two of these sorting signals, an N-terminal propeptide (NTPP) and a C-terminal propeptide (CTPP) are directed to the vacuole by distinct pathways. Transport of protein cargo to the plant vacuole is accomplished by membrane-bound transport vesicles in a manner similar to trafficking steps throughout the secretory system. Fusion of a transport vesicle with its target organelle requires the presence of a v-SNARE isoform on the vesicle membrane and a t-SNARE isoform found on the target organelle. We have characterized several components of the machinery involved in the sorting of NTPP-type cargo, including a trans-Golgi network (TGN) localized cargo receptor, AtELP, and several SNARE components involved in vesicular traffic between the TGN and the prevacuolar compartment (PVC) in Arabidopsis. We have found that the t SNAREs of these organelles are encoded by small gene families, the members of which reside on the same target organelle. Our functional analyses, including gene knock-out data, indicate that the members of these gene families perform distinct essential functions and are not redundant. We believe these distinct functions result from interactions with unique factors that provide specificity. For this reason, we have started to analyze the interacting factors unique to each gene family member through genomic and proteomic methods. We believe that the CTPP pathway may be unique to plants. We have initiated a genetic screen for mutants defective in the vacuolar targeting of CTPP-bearing protein reporters. We are now working to characterize two mutants derived from this screen.

117. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824
Cell Wall Metabolism
N. V. Raikhel, Kenneth Keegstra, H. Kende, J. Walton
$230,769

Despite the importance of cell walls to the biology of plants, little is known about the biosynthesis of their major macromolecular components. >From the known complexity of the cell wall we can predict that its synthesis requires hundreds of enzymes, but biochemical approaches have been unsuccessful in characterizing more than a few of them. Comparative molecular genetic studies have not been useful because the walls of other organisms, such as bacteria and yeast, are fundamentally different in composition, structure, and function from those of plants. We are using a functional genomics approach to unravel the molecular genetics and biochemistry of hemicellulose biosynthesis. Advances in the past few years, including from our laboratories (Raikhel and Keegstra), have identified the first genes encoding wall biosynthetic enzymes. We are building on this knowledge to identify other genes involved in this fundamental process. We have identified several genes as being putatively involved in cell wall biosynthesis and are now using a variety of biochemical and genetic approaches to study the functions of these genes and of the proteins they encode. Our other objective is to study the function of a Golgi-associated 40-kD protein that is possibly have a role in xyloglucan biosynthesis. We have isolated several knock out mutants lacking the gene encoding this protein. These mutants will be used to investigate the role of the 40-kD protein, if any, in xyloglucan biosynthesis.

118. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824
Biochemical and Molecular Aspects of Plant Pathogenesis
J. D. Walton
$260,769

The objective of this project is to further our understanding of the biochemical events that are important in the interactions between fungal plant pathogens and their hosts. We are studying examples of pathogen factors that determine basic compatibility (the ability of a fungus to infect any plant) and those that determine specificity (the ability of a fungus to infect a particular plant, i.e, the basis of host-range). As examples of basic compatibility factors, we are studying the extracellular enzymes that degrade the polymers of the plant cell wall. Circumstantial evidence suggests that these enzymes are important for colonization of host tissue and also can act as triggers of plant defense responses. We have purified enzymes, including pectinases, xylanases, glucanases, cellulases, and proteases, from the ascomycetous maize pathogen Cochliobolus carbonum, cloned the corresponding genes, and tested their importance in pathogenesis with targeted gene disruption. The host-selective toxin, HC-toxin, is a specificity determinant because it affects only maize of certain genotypes. We established that the maize resistance gene Hm encodes a reductase that detoxifies HC-toxin, and have now shown that the site of action of HC- toxin is histone deacetylase, a nuclear enzyme that influences chromatin structure and gene expression by modifying core histones. We are studying why inhibition of this enzyme leads to the establishment of a compatible (susceptible) disease interaction. In the fungus, HC-toxin production is controlled by a single genetic locus, TOX2. TOX2 contains multiple copies of multiple genes necessary for HC-toxin synthesis, including a large cyclic peptide synthetase, a fatty acid synthase, and a toxin export pump. All of these genes are present only in isolates of C. carbonum that make HC-toxin and are distributed over 540 kb on the same dispensable chromosome.

119. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824
Developmental Biology of Nitrogen-Fixing Cyanobacteria
C. Peter Wolk
$240,769

We are analyzing cellular differentiation and pattern formation in *Anabaena* species. These filamentous cyanobacteria have three kinds of cells: vegetative cells, which photosynthesize and grow; heterocysts, which form at spaced intervals along the filaments and assimilate nitrogen gas, providing nitrogen to the other cells; and resistant spores called akinetes, about whose development remarkably little is known despite their importance in natural ecosystems. To identify a gene whose activity is indicative of akinete formation, an *Anabaena cylindrica* protein was isolated that appeared akinete-specific. From the sequences of tryptic peptides of the protein and DNA-sequence data for *Anabaena* sp. strain PCC 7120, corresponding genes were identified and cloned from PCC 7120 and also from *Anabaena variabilis*, in which all vegetative cells can become akinetes. In *A. cylindrica*, heterocysts induce adjacent cells to form akinetes. To analyze the induction process, we are developing a genetic system for *A. cylindrica*. A plasmid bearing methylase genes was constructed to protect against DNA being destroyed upon transfer to *A. cylindrica*. Analysis of DNA fragments that correct two mutants of PCC 7120 in which the pattern of spacing of heterocysts is altered implicated hitherto unidentified genes in spacing. An efficient system of transposon mutagenesis that we developed for *Anabaena variabilis* was used to isolate mutants of the unicellular cyanobacterium, *Synechococcus*, affected in cell division, and the mutated genes were identified. Mutations of corresponding *Anabaena* genes appear to affect cellular differentiation as well as division.

120. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824

Frankia Genetics

C.P. Wolk, T. Newman, S.Y. He, and F.J. de Bruijn

(No cost extension)

Frankia spp., bacteria in the Gram positive actinomycete group, form nitrogen-fixing nodules with a wide variety of non-leguminous woody plants. *Frankia*-nodulated plants have great, world-wide potential as sources of fuel, fiber, shade, and forage, as well as for reforestation. Their potential, and opportunities for understanding the interesting biology of *Frankia*, are strongly curtailed by the lack of methodology for genetic manipulation of these bacteria. Our short-term objective is to develop techniques for stable, reproducible genetic transformation of *Frankia*. Longer term objectives include the further development of techniques for genetic manipulation of *Frankia*, and analysis of the generation of N_2-fixing symbioses of *Frankia*. As a step toward the construction of potential shuttle vectors, we earlier determined the DNA sequence of plasmid pFQ11 from *Frankia alni* strain CpI1. Four significant open reading frames were identified in pFQ11. Quantitative RT-PCR reactions produced results consistent with expression of those ORFs. Transformation of *Frankia* requires appropriate selectable markers as well as a means of transferring DNA. We are attempting conjugal transfer of DNA to *Frankia*. Cassettes were constructed that bear five genes that confer resistance to different antibiotics, with and without a gene for green fluorescent protein (all, of codon usage suitable for *Frankia*), and (for selection in donor bacteria) a gene that confers resistance to streptomycin and spectinomycin. Conjugally transferable vectors bearing these cassettes have also been developed.

121. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824

Environmental Control of Plant Development and Its Relation to Plant Hormones

Jan A.D. Zeevaart

240,769

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. In long-day rosette plants, stem growth in long days (LD) is due to an increase in gibberellin (GA) levels, particularly in the shoot tips. This is mainly due to a strong up-regulation of the gene encoding GA 20-oxidase under LD. The subsequent step in the pathway, catalyzed by 3b-hydroxylase, is not affected by daylength. However, when GA 20-oxidase is over-expressed in transgenic plants, more bioactive GA is produced. This shows that in wild-type plants 3b-hydroxylase is limited by the substrate provided by GA 20-oxidase.

Abscisic acid (ABA) is a plant hormone that plays a role in seed maturation and stress responses. The objective is to determine how the ABA content in plants is regulated by biosynthesis and breakdown. The limiting step for ABA biosynthesis is the cleavage of 9-*cis*-epoxycarotenoids to give xanthoxin, the precursor of ABA, and C_{25}-apo-aldehydes. This is true for both environmentally (water-stressed leaves) and developmentally (fruit ripening) regulated ABA biosynthesis. In each case, up-regulation of the cleavage enzyme occurs at the transcriptional level. On the other hand, ABA 8'-hydroxylase, which oxidizes ABA to give phaseic acid (PA), is induced by its own substrate. Its activity is also strongly increased by rehydration of water-stressed leaves. It is suggested that both increased synthesis and reduced breakdown must be considered for genetic manipulation of ABA levels in transgenic plants.

122. Michigan State University

East Lansing, MI 48824-1319
Regulation of Thylakoid Lipid Head Group Biosynthesis in Arabidopsis thaliana
Christoph Benning, Department of Biochemistry
$92,000

The galactolipids mono- and digalactosyldiacylglycerol, and to a lesser extent the sulfolipid sulfoquinovosyldiacylgycerol, are the most common non-phosphorus membrane lipids in the biosphere. Galactolipids account for 80% of polar lipids in green plant tissues. These lipids are major constituents of photosynthetic membranes (thylakoids) in plant chloroplasts and are crucial to oxygenic photosynthesis. We recently discovered that one of the most potent environmental stimuli affecting the overall glycerolipid composition of plant membranes is phosphate deprivation. Our recent observations suggest that plants can substitute phospholipids with non-phosphorus glycolipids under phosphate-limiting growth conditions. Furthermore, we discovered a phosphate deprivation-induced pathway of galactolipid biosynthesis. This pathway revealed itself in the apparent restoration of galactolipid biosynthesis in phosphate-starved leaves of the normally galactolipid-deficient *dgd1* mutant of *Arabidopsis*. Multiple lines of independent evidence suggest that these newly formed galactolipids are not exclusively associated with plastid membranes, but with extraplastidic membranes. This finding represents a significant deviation from the past dogma that Aall galactolipids are exclusively located inside plastids@. Based on the analysis of newly isolated lipid mutants such as *mgd1* (monogalactosyldiacylglycerol-deficient), *phg1* (deficient in plastidic phosphatidylglycerol), and very recently, an *sqd2* T-DNA tagged mutant (sulfolipid-deficient), progress has been made in better understanding several aspects of plant lipid biosynthesis. Similarly, the construction of mutants with multiple metabolic defects, has aided in the deconvolution of lipid biosynthetic pathways. Overall, a more complete and more complex picture of plant lipid biosynthesis has emerged, which still poses many more questions.

123. Michigan State University

East Lansing, MI 48824-1312
Biosynthesis of Triacylglycerol in Developing Oilseeds
John B. Ohlrogge, Department of Botany and Plant Pathology
$103,194

Oilseed crops produce 100 million tons of vegetable oil (triacylglycerol) per year which is valued at approximately $50 billion. Two thirds of these biologically produced oils are used for food and one third for a wide range of industrial applications. A long-term goal of our research is to develop renewable and sustainable resources to replace limited petrochemical reserves. We have studied the biochemical and genetic factors which determine how much oil is produced in plant seeds. This research has lead to the understanding that fatty acid production is one limiting factor in how much triacylglycerol (oil) accumulates in the seed. Based on these results, we are developing new methods to genetically engineer plants to allow higher levels of fatty acid production. Some seeds produce unusual fatty acids which could be useful for industrial purposes if available in large quantity at low cost. Analysis of the enzymes and proteins involved in producing these seed fatty acids has revealed that plants use specific forms of cofactors (acyl carrier protein and ferredoxin) to produce specialized fatty acids found only in the seed. Genes for these cofactors have been isolated and transgenic plants produced which produce the higher-value fatty acids.

124. Michigan State University

East Lansing, MI 48824-1319
Structure-Function Relationships of ADP-Glucose Pyrophosphorylase: Manipulation of the Plant Gene for Increased Production of Starch in Plants
Jack Preiss, Department of Biochemistry and Molecular Biology
$92,000

The structure-function relationships of ADPglucose pyrophosphorylase (ADPGlc PPase) and of branching enzyme (BE) from plants (potato ADPGlc PPase and maize BEI and BE II) and from bacteria (*Escherichia*

coli ADPGlc PPase and BE) will be studied using various approaches. Biochemical and molecular biology studies have identified those amino acids important for binding of the regulatory effectors and substrates of the ADPGlc PPases. We have obtained crystals of the potato tuber and bacterial ADPGlc PPase that diffract well in X-Ray studies and will provide us with information of the crystal structure of these enzymes. A three-dimensional structure would allow us to determine the locations of regulatory sites and the catalytic regions of ADPGlc PPase and allow us to manipulate the enzyme activity to increase plant starch yields. Crystallization of branching enzyme is an important effort as no three-dimensional structure of any BE is known whether it be mammalian, bacterial or plant. The determination of the BE structure will provide information on those amino acids in BE that are involved in branching of amylose to synthesize glycogen or starch. The BE crystal structure data are complete and the structure analysis shows locations of all secondary structures and domains. Locations of the amino acids in the structure are being refined. There is no information of the structure of any ADPGlc PPase or BE and these enzymes are of great importance in manipulating the structure and levels of starch, an economically important product of nature.

125. Michigan State University

East Lansing, MI 48824-1101
Molecular Biology and Biochemistry of Basidiomycete Laccases
C. A. Reddy, Department of Microbiology
$91,997

Laccases, lignin peroxidases (LIPs), and manganese-dependent peroxidases (MNPs) are three classes of lignin-modifying enzymes (LMEs) that are believed to be important in the degradation of lignin by white-rot fungi. There is a growing interest in LMEs because of their potential applications in a variety of biotechnological applications. These include transformation of lignocellulosic biomass to feeds, fuels and chemicals; biopulping; biobleaching of paper pulps; decolorizing and detoxifying Kraft bleach plant effluents; and degradation of highly toxic environmental chemicals such as dioxins, PCBs, various dye pollutants, and polyaromatic hydrocarbons. Basidiomycetous fungi involved in white-rot decay of wood are known to play a major role in the mineralization of the lignin polymer to carbon dioxide and water in the terrestrial environment. LMEs oxidize phenolic compounds, thereby creating aryloxy radicals. Non-phenolic compounds are oxidized via cation radicals. Laccase can only oxidize compounds with a relatively low ionization potential, whereas non-phenolic compounds with higher ionization potentials are readily oxidized by LIPs and MNPs. Our long-term research interest is to obtain a better understanding of the comparative biology of LMEs in wood-degrading basidiomycete fungi.

126. University of Michigan

Ann Arbor, MI 48109-1048
CLV Signaling in Meristem Development
Steven E. Clark, Department of Biology
$94,000

The process of cell differentiation is central to the development of specific organ and tissue types, as well as the maintenance of stem cells that retain mitotic activity. Balancing cell proliferation and differentiation is especially important in plants, because almost all plant organs (leaves, stems, petals, etc.) are formed post-embryonically.

Our approach to addressing these questions in plants has been to study the genes involved in controlling the structure and developmental patterning of the shoot meristem in *Arabidopsis*. The shoot meristem is organized in the embryo and is responsible for generating the above-ground portion of the plant. At the shoot meristem, a pool of stem cells continually provides unspecified cells for organ initiation. Our efforts have focused primarily on two genes involved in promoting organ formation, *CLV1* and *CLV2*. Each appears to encode for a receptor-like protein that should be capable of perceiving extracellular signals. CLV1 differs from CLV2 in that it also contains a functional protein kinase domain that would make it able to relay signal to the interior of the cell.

We propose to carry out a series of experiments to characterize the CLV1/CLV2 signal transduction pathway. We will also investigate the *POL* gene, which appears to be a downstream regulator of CLV1/CLV2 signaling.

This work will provide valuable information on the genes and processes controlling shoot meristem development and organogenesis in plants. This knowledge will be of great benefit to efforts to use plant material as an energy source, because all above-ground plant organs, including leaves, stems and seed-bearing flowers, are derived from the shoot meristem.

127. University of Minnesota

St. Paul, MN 55108
Metabolic Regulation of the Plant Hormone Indole-3-acetic acid
Jerry D. Cohen, Department of Horticultural Sciences
$96,000

The phytohormone indole-3-acetic acid (IAA, auxin) is important for many aspects of plant growth, development and responses to the environment. Our understanding of the biosynthesis, conjugation and degradation of auxin in plants, and the mechanisms by which plants regulate auxin levels has changed remarkably within the last few years. We showed that mutant plants that cannot make the amino acid tryptophan still make IAA, and in very high amounts. We also showed that both the traditional tryptophan pathway and a non-tryptophan pathway to IAA occur in plants, and sometimes both are used by the same plant at different times of development. An additional source of IAA in plants is the relatively large pool of IAA stored within plant cells in conjugated form. We are working on three fundamental problems related to how plants get their IAA: 1) An *in vitro* biochemical and reverse genetic approach is being used to define the tryptophan dependent and independent pathways to IAA; 2) Recent studies have shown that IAA levels change by two orders of magnitude following fertilization and remain high during the early stages of carrot zygotic embryogenesis, but return to lower levels as organized structures begin to develop. Carrot somatic and zygotic embryogenesis are being studied to determine how pathways to IAA are controlled during development and the consequences of activation of these pathways on IAA levels and embryo development; and 3) Isolation and characterization of bacterial enzymes that hydrolyze conjugates that could be useful for altering IAA metabolism in specific plant tissues and as tools for understanding the importance of IAA conjugation to specific developmental processes in plants.

128. University of Minnesota

St. Paul, MN 55108-1022
Biochemistry of Ammonia Monoxygenase of Nitrosomonas
Alan B. Hooper, Department of Biochemistry, Molecular Biology and Biophysics
$106,000

The project deals with the mechanism of oxidation of ammonia to hydroxylamine by ammonia monooxygenase (AMO) and then nitrite by hydroxylamine oxidoreductase (HAO) in the autotrophic bacterium Nitrosomonas europaea.

We have recently shown Ubiquinone and the cytochrome bc1 complex to be included in electron transfer to the terminal oxidase from the octaheme HAO monomer and its acceptor, the periplasmic tetraheme cyc554. Further the membrane-attached tetraheme cytochrome cM552 of the NirT family accepts electrons from cyc554 and is likely to transfer electrons to ubiquinone. The gene for cy cM552 and cyc554 are in the same operon. In membranes or pure form, the four hemes of cycM552 are electronically coupling as observed by integer spin EPR and, correspondingly, the Soret band of the 4 ferrous hemes shift upon binding of a CO. Hence the electron transfer through 3 proteins from hydroxylamine to ubiquinone involves 16 c-hemes which exhibit a high degree of electronic coupling.

A highly unusual periplasmic, red (lmax 390 nm, e = 4400 M-1cm-1) mononuclear copper trimeric protein, named nitrosocyanin, has been isolated. The crystal structure of the 112 residue protein was solved to 1.65 Å. The reduction potential, +85 mV, is lower than that of any blue copper cupredoxin. His, His, Cys, Glu and a solvent molecule coordinate the copper. The EPR spectrum is similar to tetragonal copper. The function, which is not known, could involve electron transfer or catalysis.

129. University of Minnesota

St. Paul, MN 55108-1095
Reverse Transposon Tagging of Maize Tubulin Genes
Susan M. Wick, Department of Plant Biology
$110,000

Tubulins comprise a group of structural proteins that help determine the shape of plant cells, and which are involved in nearly every aspect of plant growth. Tubulins assemble inside cells to make microscopic rods called microtubules. Corn plants have many tubulins, and we are trying to determine whether the various tubulins have different functions in the plant or confer different properties to microtubules. Specifically, some of the tubulins appear to be involved only during reproduction (i.e., during flower development) and some are implicated in conferring decreased sensitivity to chilling. We have identified several mutants that contain a transposon (a "jumping gene") within a tubulin gene, and are concentrating our efforts on mutants that can no longer make tubulin from the disrupted gene. We are growing several generations of the mutant plants and pollinating them to create plants that have two copies of the mutation or have mutations in more than one tubulin gene. This will allow us to analyze what effect these mutations have on growth and fertility of the plant, and on its ability to withstand chilling and grow at normal rates at temperatures that usually cause chilling stress in corn.

130. University of Missouri

Columbia, MO 65211-7411
Cellulose and the control of growth anisotropy
Tobias I. Baskin, Division of Biological Sciences
$95,000

The goal of the P.I.'s research is to understand plant morphogenesis. Morphogenesis requires the growth of cells to be different in different directions, that is, to be anisotropic. Under-standing of the mechanism controlling anisotropic growth focuses on the cellulose micro-fibrils of the cell wall, which give the wall directional reinforcement. Directional reinforcement is possible because the microfibrils are deposited in the cell wall in ordered arrays and because the microfibrils are linked to the cell wall matrix specifically. However, it is not understood how the microfibrils are deposited with such regularity, and the matrix components that mediate the required mechanical linkages have not been identified. Last year, the P. I. addressed the problem of how microfibrils become aligned by publishing a novel hypothesis that explains many seemingly contradictory observations, a hypothesis which the P. I. is testing. Additionally, the P. I. developed methods based on field-emission scanning electron microscopy to image the cell wall at high resolution in material that has been processed only minimally. This opens the way to investigate the way in which microfibrils are organized and cross-linked into the cell wall. Finally, the P. I. has nearly cloned, *RADIALLY SWOLLEN 4* (*RSW4*) and *RSW7*, two genes identified in his previous work as controlling expansion anisotropy. The genes offer an avenue for the P. I. to identify elements that cross-link cellulose into the wall and that are responsible, at least in part, for controlling the mechanical properties of the cell wall and the anisotropy of expansion.

131. University of Missouri

Columbia, MO 65211-7400
Dosage Analysis of Gene Expression in Maize
James A. Birchler, Division of Biological Sciences
$108,000

Our current work is aimed at understanding the role of dosage dependent regulatory genes in the process of hybrid vigor. Hybrid vigor is the phenomenon that individuals resulting from crosses between inbred lines have greater biomass and yield. Gene expression studies of aneuploids of maize have led to the idea that most regulatory genes exhibit a negative dosage effect and are rate limiting on vigor by their action on target loci. A study of hybrid vigor in triploid maize, where the dosage of alleles is different in the two types of hybrids, demonstrated that hybrid vigor is affected by allelic dosage. Moreover, it was discovered that the vigor of inbreds decreased with increasing ploidy, but the vigor of higher ploidy hybrids is equal to or greater than the vigor of the corresponding diploids. A molecular model of hybrid vigor has been formulated. It proposes that most regulatory molecules act as dimers and the majority act negatively. Heteroallelic multimers are less effective at their repressive functions and therefore the net effect will be increased expression of target loci and thus vigor in hybrids, but decreased expression and vigor with increasing ploidy of inbreds. Studies of global patterns of gene expression are being conducted to test this model. Experiments are also underway to understand better the relationship of aneuploid syndromes and hybrid vigor as well as to determine whether hybrid vigor results from the increased expression of many genes throughout the genome or whether it is a consequence of increased expression of a few genes that control growth.

132. University of Missouri

Columbia, MO 65211
Genetics and Molecular Biology of Hydrogen Metabolism in Sulfate-Reducing Bacteria
Judy Wall, Biochemistry Department
$96,000

Genetic and molecular tools are being applied to elucidate the electron pathways of the anaerobic sulfate-reducing bacterium Desulfovibrio desulfuricans that is considered to be a major player in biocorrosion of metals in the environment. A mutation in the gene cycA encoding the dominant c-type cytochrome, considered a major electron carrier, was constructed. This mutant is impaired in its metabolism of the carbon substrate pyruvate but not lactate. Large quantities of hydrogen are generated from pyruvate by this mutant while almost no hydrogen is made by the wild type. Thus distinct electron pathways exist from different substrates to sulfate that may suggest that different sources of carbon in the environment may alter the facility of corrosion by these bacteria. Hydrogenase mutants are being evaluated for their role in hydrogen production on pyruvate.

Regulation of the production of electron transfer components may also limit or augment the availability of pathways for biocorrosion of metals. Studies of the expression of the cycA gene have revealed an unusually strong promoter that when cloned upstream of a reporter gene decreases resources available for the cells to grow and, therefore, is not maintained in an Escherichia coli host. A single copy of the promoter fused to a reporter gene in the native Desulfovibrio background has revealed that the gene is constitutively expressed and increased by only about two fold when the cells are starved for substrate.

133. University of Montana

Missoula, MT 59812

Controls on production, incorporation and decomposition of glomalin -- a novel fungal soil protein important to soil carbon storage

Matthias C. Rillig, Division of Biological Sciences (Note: see U.S. Dept. of Agriculture, S. Wright)

$130,039 (FY 99 funds – two years)

A group of beneficial soil fungi live on carbon supplied directly to them by plant roots. The fungi are called arbuscular mycorrhizal fungi or AM fungi. These fungi have long hair-like projections called hyphae that extend several cm from the root into soil. Glomalin is a glycoprotein that is produced on AM hyphae in large amounts, is released from hyphae, and attaches to soil particles. Glomalin is important because concentrations in soil are correlated with soil aggregate stability, and large amounts of labile soil carbon are sequestered in aggregates. Plants fix more carbon under elevated CO2 than under ambient CO2, and more carbon is transported from roots to these fungi. We continue to find larger amounts of glomalin in planted soils exposed long-term or short-term to increased atmospheric CO2. Preliminary evidence indicates that warming, without increased CO2, is detrimental to aggregate stability. We found that glomalin production is influenced by plant species in the field. Laboratory studies indicated that glomalin production differed among fungal species, but not between corn and crimson clover. Incubation studies indicated that glomalin levels decline more rapidly in soils from the Midwest that have been conventionally tilled compared with no-till soils. We have evidence that glomalin makes up a large part of soil organic matter in an organic soil from Hawaii. Our work shows that glomalin is in the fraction of soil organic matter called humin – a fraction that was previously thought to be composed of undefined insoluble organic matter.

134. Mount Sinai School of Medicine

New York, NY 10029

The Respiratory Chain of Alkalophilic Bacteria

Terry Ann Krulwich, Department of Biochemistry

$115,000

Extremely alkaliphilic *Bacillus* species such as the object of these studies, *Bacillus pseudofirmus* OF4, exhibit high growth yields at extraordinarily high pH values in spite of the extra energetic costs of growth at high pH. Those extra costs include the greater energy required to synthesize ATP at elevated pH and the energy-dependence of the transport processes that enable the alkaliphile to maintain a cytoplasmic pH well below the external pH value. Studies this past year have taken advantage of the completion of the genome sequence of a slightly less alkaliphilic but related *Bacillus*, *Bacillus halodurans* C-125. It is now possible to develop primers to isolate genes encoding respiratory chain complexes of interest from both the alkaliphilic strains. In *B. pseudofirmus* OF4, the genetic tools for targeted disruptions and mutations are now available for part of the subsequent characterizations. Another major focus of the work was the major transporter that is involved in cytoplasmic pH homeostasis, i.e. the Mrp complex. This sodium-proton exchange complex has features suggesting that it may have a mode of primary energization rather than depend obligatorily on the high energy state generated by activity of the respiratory chain. Experimental approaches to possible mechanisms for primary energization are under development. Given the novel energetic phenotype of extremely alkaliphilic *Bacillus* species, the profile and modes of their energy-transducing membrane complexes is of central physiological interest.

135. NASA Ames Research Center

Moffett Field, CA 94035-1000

The molecular basis of hyperthermophily: the role of HSP60/chaperonins in vivo

Jonathan Trent, Astrobiology and Technology Branch

$100,000

The existence of organisms that live at near boiling temperatures is living proof that all of the complex biochemical machinery of life can be adapted to function under these harsh conditions. The purpose of our

research is to elucidate the role of a group of proteins known as heat shock proteins or HSP60s in this adaptation to high temperatures.

HSP60s are found in all organisms and they are among the most highly conserved proteins known. We are investigating HSP60s in an organism growing at 80°C and pH 2.0 (*Sulfolobus shibatae*). This organism produces three closely-related HSP60 proteins, referred to as HSP60 alpha, beta, and gamma. Our DOE-funded research during the last two years has focused on clarifying the role of HSP60 alpha and beta. These are among the two most abundant proteins in *S. shibatae* grown at high temperatures and significantly increase in abundance when the cells are exposed to near-lethal temperatures. We have demonstrated that these proteins protect the cells from lethal temperatures by stabilizing their membranes. During this last year we have been studying gamma, which was discovered by genome sequence analysis but nothing was known about its function. We have determined that gamma is only expressed at low temperatures, that it interacts with alpha and beta, and that it influences their ability to form higher-order structures critical to their function. We propose that gamma modulates HSP60 function at low temperatures.

The higher order structures produced by HSP60s have become the focus of a major project at NASA Ames on "Protein Nanotechnology" (see http://www.ipt.arc.nasa.gov:80/trent.html).

136. National Renewable Energy Laboratory

Golden, CO 80401

The Water-Splitting Apparatus of Photosynthesis

Michael Seibert, Photoconversion Research Branch

$134,000

The goal of this research project is to understand basic structural and functional relationships in photosystem II (PSII) with emphasis on the energy transfer, primary charge-separation, and water-oxidation processes that supply electrons from water for carbon fixation or hydrogen production. PSII is the site of one of the two light reactions of plant photosynthesis and is directly associated with water-splitting function. Primary charge separation occurs in the PSII reaction center (RC). Femtosecond transient absorption studies comparing active and inactivated isolated PSII RC complexes at 7K have identified the faster (5 ps^{-1}) component of the biphasic pheophytin Q_x band bleach kinetics as the component associated with the intrinsic rate of primary charge separation. Hole-burning studies of 5-Chl vs 6-Chl PSII RC complexes have determined that the respective energies of the Q_y states of the core pigments in the two complexes exhibit only minor differences. The RC Chl exhibiting a Q_y band at 684 nm (more prominent in 5-Chl RCs) is the peripheral Chl on the D_1 protein, probably Chl_z, associated with energy transfer into the RC and the ability to protect PSII under stress conditions. Flash-probe fluorescence methods have been used to study the interaction of iron with the high-affinity (HA), Mn-binding site of PSII, uncovered when catalytic Mn (required for photosynthetic water splitting), is removed. Both exogenous Fe(II) and Mn(II) donate electrons to Y_z, the intermediate electron carrier from water to the PSII RC, through this site. Conditions were found that block the HA Mn site, thus allowing for investigation of PSII donor sites other than the HA Mn(II) donation site without interference of the latter.

137. National Renewable Energy Laboratory

Golden, CO 80401

Regulation of H_2 and CO_2 Metabolism: O_2 Sensor Involvement in Partitioning of Photosynthetic Reductant in Green Algae

Maria L. Ghirardi and Michael Seibert, Photoconversion Research Branch

$165,000

The objective of this research is to develop fundamental understanding about oxygen regulation of photosynthetic reductant partitioning (at the level of ferredoxin) between (a) the hydrogenase enzyme pathway that produces molecular H_2 following anaerobic activation and (b) the Calvin cycle that fixes CO_2. Our approach for characterizing molecular signal mechanisms involved in this partitioning is to identify and

analyze mutants deficient in H_2 production. An insertional mutagenesis library (provided by Prof. A. Melis, University of California, Berkeley) containing genes disrupted by the insertion of a plasmid has been screened for colonies with low H_2-production activity. Four colonies have been characterized thus far. They all exhibit normal rates of photosynthesis and respiration, but have H_2-production rates approximately 25-40% of WT controls. Each mutant contains only one plasmid insert, and we are currently trying to establish genetically whether the mutation is associated with the presence of the insert. Concomitantly, we are applying different techniques to further identify the gene sequences disrupted by the inserts. The identified genes (other than that of the hydrogenase itself) will then be cloned in the bacterium, *E. coli*, the gene products will be characterized biochemically and biophysically, and their roles in regulating hydrogenase expression or activity in the alga, *Chlamydomonas reinhardtii*, will be established. This work will be important in garnering basic regulatory information that will aid in the future design of an applied algal H_2-production system based on the presence of an O_2-tolerant algal hydrogenase currently under development by the U.S. DOE Hydrogen Program.

138. University of Nebraska

Lincoln, NE 68588-0665
Regulation of nuclear response to mitochondrial dysfunction
Sally A. Mackenzie, Department of Agronomy
$94,001

The plant mitochondrial genome bears several features in structure and behavior that are distinct and poorly characterized. These include their propensity to subdivide via recombination to give rise to sub-genomic forms. The sub-genomic DNA molecules can undergo dramatic changes in their relative copy number during the plant's development. Moreover, plant mitochondria edit most of their RNA transcripts to produce functional sequences for proper protein production. Yet their unedited transcripts appear to be available for association to the protein translation machinery. These unusual features have led us to investigate three nuclear genes that regulate the necessary accommodating functions. The unusually high frequencies of recombination in plant mitochondria imply that they must have evolved novel features for mismatch repair and its regulation. So we are investigating nuclear gene MSH1 function and expression during plant development. The unusual changes in copy number for portions of the genome are influenced by the nuclear gene CHM. Finally, posttranslational regulation of mis-translated proteins would be effected, at least in part, but the LON protease. These three loci in Arabidopsis have been cloned and are the subjects of investigation for this study to understand their expression during plant development, and the consequences to mitochondrial function of their disruption.

139. University of Nebraska

Lincoln, NE 68588-0664
Plant Formate Dehydrogenase
John Markwell and John Osterman , Department of Biochemistry
$185,000 (two years)

Formate dehydrogenase (FDH) is an enzyme that occurs in plant mitochondria. This enzyme is produced in increasing amounts in response to drought, cold, wounding and other stresses that reduce yield. Such increases in FDH activity can also be produced by treating plants with one carbon molecules such as methanol. Recently, we have found that FDH is also localized in the chloroplasts of at least one plant, *Arabidopsis thaliana*. This research project will study why the FDH is targeted to both chloroplasts and mitochondria in *Arabidopsis* and examine whether this happens in other plants as well. Because the FDH may confer some degree of stress resistance to the plant, possibly by altering the metabolism of one-carbon molecules, transgenic plants will be produced with increased and decreased FDH levels to determine whether such plants have altered water use efficiency. The protein composition of chloroplasts and mitochondria treated with one-carbon compounds will be examined to identify other proteins that respond to these stress signaling molecules. A collection of mutants will be screened to see if any of them have a total loss of FDH activity. The overall goal of this study is three-fold: first to substantiate that FDH is functioning

as part of the plant stress-response system; second, to understand which other proteins with metabolically-related roles are coordinately increased during stress; and third, to determine whether the physiological role of the FDH in the chloroplast compartment is different from that of the FDH in the mitochondrial compartment.

140. University of Nebraska

Lincoln, NE 68588-0118
Protein and RNA Interactions Involved in the Pathogenesis of Tomato Bushy Stunt Virus
T. Jack Morris, School of Biological Sciences
$54,000

Our research emphasizes protein interactions important in the pathogenicity of viruses of the Tombusviridae Family. We have investigated protein interactions that occur during infection by Tomato Bushy Stunt virus (TBSV) and Turnip Crinkle virus (TCV). One aspect involving the investigation of viral genes needed for systemic spread of TBSV utilized transgenic plants expressing the movement protein of a related virus (red clover necrotic mosaic virus). These studies showed that three viral encoded genes (p22 along with CP and p19) must function coordinately to facilitate efficient systemic movement in tobacco plants. In the TCV system, we examined the role of viral proteins in systemic movement in *Arabidopsis* using GFP fusions of the movement proteins. We confirmed that plant invasion requires coordinated interaction of CP and the two virally encoded movement proteins with this virus as well. Examination of interactions between viral and host proteins focused on elucidating the role of TCV CP in inducing a hypersensitive resistance response in genetically resistant *Arabidopsis* (Dijon-17). A yeast two-hybrid screen was used to identify a host protein of the NAC family of transcription factors that interacts specifically with the viral CP. The role of CP as the elicitor of the resistance response was confirmed, and we showed that the specific interaction between CP and this host protein was essential for triggering the specific HR-mediated resistance response. Unraveling the role of this transcription factor in the signal transduction pathway leading to viral resistance in *Arabidopsis* is a primary focus of future research.

141. University of Nebraska

Lincoln, NE 68588-0664
Enzymology of Acetoclastic Methanogenesis
Stephen W. Ragsdale, Department of Biochemistry
$108,000

Methanogenesis is a microbial process that occurs in most oxygen-depleted environments, such as the digestive tract of many animals. This process generates $\sim 10^9$ tons of methane annually. Understanding the mechanism of methane formation is critical because methane is an important fuel and a potent greenhouse gas whose concentration is rising at a rate of 1% per year. We are determining the mechanisms of three key enzymes involved in methanogenesis from acetic acid. These enzymes are CO dehydrogenase/acetyl-CoA synthase (CODH/ACS), methyl-CoM reductase (MCR), and heterodisulfide reductase (HDR). We are using a battery of spectroscopic and kinetic approaches to isolate the intermediates, some of which are directly bonded to metal centers in the enzyme, in these reactions and to determine how fast the intermediates form and decay. The studies are expected to lead to important insights into how natural gas is formed in nature and into the structure and function of metals in biology.

142. University of Nebraska

Lincoln, NE 68588-0664
Role of the Rubisco Small Subunit
Robert Spreitzer, Department of Biochemistry
$95,000

The chloroplast enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) captures atmospheric carbon dioxide, thereby serving as the primary point of entry for the carbon that comprises all life on earth. The enzyme is remarkably slow, and oxygen competes with carbon dioxide at the same active site, further reducing net carbon fixation. Because Rubisco catalyzes the rate-limiting step of photosynthesis, there has been much interest in engineering the enzyme as a means for increasing agricultural productivity and renewable energy resources, as well as for decreasing the accumulation of the greenhouse gas carbon dioxide. The structure-function relationships of the enzyme's chloroplast-encoded large subunit have been studied extensively. This subunit contains the active site. Much less is known about the role of the nuclear-encoded small subunit. There is a family of small-subunit genes in land plants, making it difficult to substitute engineered small subunits into the Rubisco holoenzyme. However, a photosynthesis-deficient mutant of the green alga Chlamydomonas reinhardtii has recently been recovered that lacks both members of the small-subunit gene family. Because this mutant can be rescued via transformation with a single small-subunit gene, it is now possible to answer questions about small-subunit function. By employing genetic selection, alanine-scanning directed mutagenesis, and chimeric small-subunit genes, a region of the small subunit that resides far below the large-subunit active site has been found to influence carboxylation rate and carbon-dioxide/oxygen specificity. Further study of these distant interactions may provide a rationale for designing an improved Rubisco.

143. New York University

New York, NY 10003-6688
Asparagine Synthetase Gene Regulation and Plant Nitrogen Metabolism
Gloria M. Coruzzi, Department of Biology
$113,000

Our studies concern the structural and regulatory genes that control the assimilation of nitrogen into asparagine, a key amino acid used to transport and store nitrogen in all higher plants. Inorganic-N assimilated into glutamine in the light, is converted to asparagine in the dark. This is an energy conservation mechanism, as asparagine is a more carbon efficient N-transport amino acid, used to transport N when C-skeletons are limiting. We have shown that light represses transcription of ASN1 the major gene regulating asparagine biosynthesis in Arabidopsis, and that this repression is mediated in part by phytochrome, and by changes in levels of photosynthate. Sucrose supplied to dark-treated plants, can mimic light-repression of *ASN1.* We have begun to characterize components involved in mediating the light and carbon repression of ASN1 using molecular genetic approaches. Arabidopsis containing an ASN1 transgene have been used to drive a positive selection for mutants insensitive to light (lir) or carbon (cri) repression of ASN1. The mapping and cloning of the affected genes should define components involved in light and sugar repression of asparagine biosynthesis. We have also used a mutagenesis approach to identify binding sites in the ASN1 promoter involved in light and sugar repression and are using a yeast one-hybrid approach to clone this transcriptional repressor. As asparagine is an important amino acid used to transport nitrogen to seeds, we are also testing whether transgenic plants that constitutively express ASN1 and make higher levels of asparagine, show increased N-use efficiency, enhanced seed set, or seeds with higher N-content.

144. New York, State University of

Buffalo, NY 14260
Effects of RNA-protein Complexes on ATP Synthase Gene Expression in the Chloroplast
Margaret Hollingsworth, Department of Biological Sciences
$164,000 (two years)

In all living systems, cellular processes are governed at some level by complexes that form between particular RNAs and the specific proteins that bind them. In chloroplasts, the formation of RNA-protein complexes is an especially common mechanism for regulation of protein production. We study one type of these complexes. We hypothesize that they affect the quantity of energy-generating proteins produced by the chloroplast.

Our research project has two specific aims. The first is to directly analyze the effects of RNA-protein complexes on protein production. Results from those experiments will provide the first definitive information on the direct effects of these complexes. The second aim is to determine the elements in the RNA that are essential for interaction with binding proteins. A combination of the results from these two aims will allow us to predict how many chloroplast genes are regulated.

This research is applicable to at least two aspects of the DOE Program 00-03 on Plant and Microbial Carbon Management. First, the RNAs involved in our studies encode a key complex linking photosynthesis to carbon fixation. A thorough understanding of the processes that affect expression of this essential complex is necessary if we are to understand carbon fixation in plants and microbes. Another compelling motivation for this research lies in the fact that the proposed experiments will contribute to our understanding of the formation of RNA-protein complexes and their effects on chloroplast (and therefore plant) function. It is becoming apparent this type of RNA-protein interaction regulates the majority of chloroplast genes. Thus results from this model system will provide insights that will be applicable to expression of a wide range of chloroplast genes.

145. North Carolina State University

Raleigh, NC 27695-7612
Coordination of endoplasmic reticulum (ER) signaling during maize seed development
Rebecca S. Boston, co P.I.s Wendy F. Boss and Ralph E. Dewey, Department of Botany
$95,000

Seed storage reserves represent one of the most important sources of renewable fixed carbon and nitrogen found in nature. To improve agronomic quality of seed crops we need not only an under-standing of the metabolic pathways involved but also the ability to predict the impact of genetic alterations on lipid and protein bio-synthesis, and the packing of these reserves in membrane-bound vesicles. We are studying maize mutants that do not properly synthesize and package proteins into protein bodies. We have shown that these mutants exhibit an ER stress response associated with recognition of improperly folded proteins and an overproduction of protective proteins called molecular chaperones. In addition, we have shown that four key phospholipid biosynthetic enzymes are up-regulated in seeds of these mutants . Triacylglycerol content of endosperm is higher as is phosphoinositol accumulation. Taken together, our data are suggestive that the plant ER stress response functions in integrating ER membrane biogenesis into the broader context of energy demands with the seed. This information has increased our know-ledge of the regulation of crucial metabolic pathways of seeds and should pro-vide a framework for increasing and improving renewable energy resources.

146. North Carolina State University

Raleigh, NC 27695-7905
Proteolysis in Hyperthermophilic Microorganisms
Robert M. Kelly, Department of Chemical Engineering
$98,092

Critical to the maintenance of a functional internal environment in any cell or organism is the turnover of polypeptides for regulatory and nutritional reasons, in addition to the identification and hydrolysis of abnormal polypeptides. Whether for routine processing of proteins and polypeptides or for maintaining metabolic function under atypical conditions, cells are armed with an array of proteases that are orchestrated to handle these tasks, often by complex mechanisms. The efforts funded by this award focus on issues relate to proteolysis in hyperthermophilic microorganisms. Two model organisms, an archaeon Pyrococcus furiosus (growth Topt of 98-100°C) and a bacterium Thermotoga maritima (growth Topt of 80-85°C), are being investigated with respect to the regulation of known and putative genes encoding proteases as well as the biochemical and physiological characteristics of these enzymes. To date, approximately 40 proteases in each of these organisms (both proteinases and peptidases) have been identified from genomic sequence information and from biochemical data from previously purified proteases from native hyperthermophilic biomass. Currently, the genes for all actual and putative proteases are being cloned for the purposes of high throughput expression with the long-term objective of examining their molecular features. Also, probes based on these genes are being used in targeted DNA microarrays for physiological studies with high temperature chemostats. Among the issues studied in this period were the influence of nutritional environment (presence and absence of specific carbohydrates) and abnormal growth conditions (e.g., stress) on proteolysis patterns.

147. University of North Carolina

Chapel Hill, NC 27599-3280
Functions of the Pseudomonas syringae avrRpm1 Gene During Disease Resistance and as a Virulence Factor in Arabidopsis thaliana Cell
Jeffery L. Dangl, Department of Biology
$139,889

This project concerns the way in which bacterial pathogens infect plants and cause disease. Most bacterial pathogens of plants cause disease by robbing the plant of photosynthetic potential, and they in turn use the nutrients to feed the growing bacterial colony. We use two easily manipulable models for our research. One is Pseudomonas syringae, bacteria that causes “leaf spot” diseases on various crop plants. The other is the model plant Arabidopsis. Plant pathogens like P. syringae and pathogens of animals like pathogenic E. coli and Salmonella, use the same system to deliver proteins into cells of their hosts. In essence, they shoot in proteins which alter the behavior of the host cell to do something positive for the pathogen. We are trying to figure out tow of these proteins cause disease in susceptible plants, and how resistant plants combat them. In the last year, we made three significant contributions. First, we showed that the bacterial virulence proteins, once delivered into the plant cell, are acted upon by plant enzymes in that specifically cause them to be transported to the inside of the plant cell plasma membrane. Thus, we now know where they act. Second, we identified one plant protein which interacts physically with the bacterial proteins, and we showed that this interaction is functionally relevant. Third, we demonstrated that our bacteria have a cluster of possible virulence genes on a “pathogenicity island” which hops out of the bacterial chromosome. These advances will help us build a molecular model of how these pathogens cause disease.

148. University of North Carolina

Chapel Hill, NC 27599-3280
Characterization of Arabidopsis Genes Involved in Gene Silencing
Sarah R. Grant, Department of Biology
$97,000

Enhancer of gene silencing 1 (egs1) is an Arabidopsis mutant that enhances post-transcriptional gene silencing of a gene introduced by genetic engineering (transgene). Our goal has been to clone EGS1 based on its map position. We have not yet been able to accomplish this goal. The problem has been an unexpected tendency of the post-transcriptionally silenced transgene to switch to a more stable silenced state. Post-transcriptional silencing is reversible and we depend on reactivation of transgene in plants with a functional EGS1 gene to distinguish wild type plants from mutants in our mapping studies. This has forced us to reconsider our cloning strategy. One possibility would be to use a different transgene as the target of gene silencing. We have tested two other transgenes. Both encoded proteins unrelated to the first but they were all expressed from the same type of promoter and they all had similar tendency to become post-transcriptionally silenced. We were disappointed to find that the egs1 mutation does not enhance post-transcription silencing of the two new genes. Therefore, we could not change the target transgene for mapping. In addition to our attempts to clone EGS1, we have compared transcripts from egs1 mutants and EGS1 wild type plants in order to determine if host genes are also silenced in the egs1 mutants along with the transgenes. We are currently analyzing results from microarrays hybridized with our transcripts in collaboration with the Arabidopsis Genome Consortium.

149. University of North Carolina

Chapel Hill, NC 27599-3280 (Note: see also North Dakota State University, A.R. White)
The role of the celC gene product in cellulose synthesis by *A. tumefaciens*
Ann G. Matthysse, Department of Biology, University of North Carolina, Chapel Hill
$60,000

The long term goal of this research program is to understand the genetics and mechanism of cellulose synthesis in *Agrobacterium tumefaciens*. This is a collaborative project, in which Dr. Ann Matthysse and her research group is performing the cloning and expression of *celC* and *celC* gene products, and Dr. Alan White and his research group are doing oligosaccharide structural analysis of the enzyme reaction products of the CelC protein. Oligosaccharide structure are examined by determination of the molecular size of the products, the types of linkages between the sugars, and the arrangement of those sugars. We employ a variety of methods to accomplish those determinations including gas chromatography, mass spectrometry, high performance liquid chromatography, and capillary electrophoresis. We have made progress on this project in several ways. Shortly after funding began, we purchased a capillary electrophoresis instrument for oligosaccharide product analysis. A graduate student is currently establishing methods and preparing standard for that instrument. I have been able to hire a Postdoctoral Researcher from university funds separate from this grant. Dr. Andrew Marry arrived from the University of Glasgow, Scotland, in January 2001, and he has configured and established procedures for a new gas chromatograph, bought with funds from another grant, that will be used for linkage analysis of oligosaccharides. Ann Matthysse's group has made progress in cloning and expressing the *celC* gene products, and she has sent us the first set of product samples for analysis. Preliminary examination of those samples is currently underway.

150. North Dakota State University

Fargo, ND 58105-5517 (Note: see also University of North Carolina, A.G. Matthysse)
The role of the celC gene product in cellulose synthesis by *A. tumefaciens*
Alan R. White, Department of Biological Sciences, North Dakota State University and
$60,000

The long term goal of this research program is to understand the genetics and mechanism of cellulose synthesis in *Agrobacterium tumefaciens*. This is a collaborative project, in which Dr. Ann Matthysse and her

research group is performing the cloning and expression of *celC* and *celC* gene products, and Dr. Alan White and his research group are doing oligosaccharide structural analysis of the enzyme reaction products of the CelC protein. Oligosaccharide structure are examined by determination of the molecular size of the products, the types of linkages between the sugars, and the arrangement of those sugars. We employ a variety of methods to accomplish those determinations including gas chromatography, mass spectrometry, high performance liquid chromatography, and capillary electrophoresis. We have made progress on this project in several ways. Shortly after funding began, we purchased a capillary electrophoresis instrument for oligosaccharide product analysis. A graduate student is currently establishing methods and preparing standard for that instrument. I have been able to hire a Postdoctoral Researcher from university funds separate from this grant. Dr. Andrew Marry arrived from the University of Glasgow, Scotland, in January 2001, and he has configured and established procedures for a new gas chromatograph, bought with funds from another grant, that will be used for linkage analysis of oligosaccharides. Ann Matthysse's group has made progress in cloning and expressing the *celC* gene products, and she has sent us the first set of product samples for analysis. Preliminary examination of those samples is currently underway.

151. Ohio State University

Columbus, OH 43210

The Role of Multiple TBP and TFB in Archaeal Gene Expression

Charles J. Daniels, Department of Microbiology

$105,000

The occurrence of multiple TATA-binding protein (TBP) and transcription factor B (TFB) genes in the halophilic Archaea indicate an increased level of complexity in the transcription system of these organisms when compared to other Archaea. Preliminary observations indicate that the *tbp* and *tfb* genes of *Haloferax volcanii* are differentially expressed through the growth cycle, and at least one *tfb* gene (*tfb2*) is induced by heat shock. These findings have led us to propose a model where alternative pairing of the TBP-TFB transcription factors directs the programmed expression of specific gene families. Using an established in vivo promoter assay system we are examining the sequence requirements for the regulated expression of the transcription factor genes. These data will define both the sequence properties of the individual promoters and their responses to environmental signals. A combination of gene interruption and over-expression studies, coupled with 2D gel proteomics analyses will be used to examine the roles of these transcription factor proteins in directing global gene expression. The identification of gene networks defined by these transcription factors will aid in the assignment of physiological affiliations for proteins whose functions are not apparent from sequence data alone. Recombinant TBP and TFB proteins will also be used to develop an in vitro transcription system, which will provide a system to examine specific protein-DNA and protein-protein interactions. Results of these experiments will add to our general understanding of the molecular biology of the Archaea and their response to the environment.

152. Ohio State University

Columbus, OH 43210

Transmethylation Reactions During Methylotropic Methanogenesis in *Methanosarcina barkeri*

Joseph A. Krzycki, Department of Microbiology

$103,000

We are studying the methyltransferases involved in initiating methanogenesis from methylamines and methylated thiols in *Methanosarcina barkeri*. These methyltransferase systems are based on a family of circa 25 kDa corrinoid proteins that shuttle methyl groups from a number of substrate specific methyltransferases to a second methyltransferase that methylates Coenzyme M, forming the immediate precursor to methane. The genome sequences of many Bacteria are now revealing homologs of the methanogen corrinoid proteins, methyltransferases, and activation proteins. We are currently investigating three areas: (1) The interaction of two methyltransferases with a single corrinoid protein indicates that both methyltransferases may interact with a single methylation site, and we are studying how this might be achieved. We have recently found that association of a corrinoid protein with a methyltransferase depends

on the ligand state of the cobalt. (2) A cellular activation protein mediates ATP dependent reductive activation of cobalt (II) corrinoid protein to the active cobalt (I) state. We have now isolated the iron-sulfur protein RamM, primarily responsible for this activity and examining how it interacts with components of the monomethylamine:CoM methyltransferase system to achieve activation. Three RamM homologs are present in the *M. barkeri* genome, and we are also pursuing their functions. (3) Over 95% identical copies of some methylamine methyltransferase genes are present in *M. barkeri*. We are currently examining expression patterns of the methyltransferases in order to gain insight into the rationale for this unusual phenomenon, and have now found that they are expressed at different growth phases.

153. Ohio State University

Columbus, OH 43210

Regulation of Methane Genes and Genome Expression

John N. Reeve, Department of Microbiology

$123,000

The goals of this project are to determine how gene expression is regulated in the methanogenic archaeon Methanobacterium thermoautotrophicum, recently reclassified as Methanothermobacter thermautotrophicus (M.t.). Studies are focussed at both the level of global gene expression, and specifically on the regulation of expression of methane genes directly responsible for methanogenesis. The presence and amounts of individual mRNAs in total RNA preparations isolated from M.t. cells grown under different hydrogen-supply conditions are being determined by hydridizations to M.t. genomic DNA-microarrays. To reproduce and dissect methane gene regulation, we have established an in vitro transcription system that contains M.t. RNA polymerase and three general transcription factors, TATA-binding protein, transcription factor B, and transcription factor E (TFE). Although TFE homologs are apparently present in all Archaea, TFE function has not previously been documented. We have shown that TFE stimulates transcription in vitro from many different M.t. promoters including several methane gene promoters. In M.t. cells, genomic DNA is compacted by archaeal histones into archaeal nucleosomes, and we are now determining how positioned archaeal nucleosomes regulate gene expression. Antibiotic surveys revealed that myxin is active against M.t. and several other Archaea, and retains this activity at high temperatures, in high salt and at low pH. Therefore, myxin resistance-conferring genes, together with genes constructed to encode thermostable variants of puromycin and pseudomonic acid resistance-conferring enzymes are being used as positive selections to develop a M.t. transformation system.

154. Ohio State University

Columbus, OH 43210-1292

A Model System to Probe the Biochemistry and Molecular Control of a Globally Significant Alternative Mechanism to Sequester and Metabolize Carbon Dioxide

F. Robert Tabita, Department of Microbiology

$218,000 (two years)

This project addresses aspects of the biochemistry, enzymology, and molecular control of a key CO_2 fixation route, the reductive tricarboxylic acid (RTCA) pathway. This metabolic scheme is widespread in Nature and plays an important role in the ability of many different prokaryotic and eukaryotic organisms to remove and subsequently metabolize CO_2 from the atmosphere. We have developed the rapid-growing and genetically tractable green sulfur photosynthetic bacterium *Chlorobium tepidum* as a model system. Several of the key and important RTCA enzymes have been purified and characterized and the relationship to key ancillary proteins that relate to the specialized redox chemistry required for effective CO_2 assimilation has been established. Two different ferredoxins, and rubredoxin, have been shown to have special capabilities to facilitate catalysis by enzymes of the RTCA pathway. Likewise, we have made progress in probing the molecular basis for control at the transcription level. Mutants have been isolated and these are also currently contributing to the regulation studies. Facilitating this work has been the completed genomic sequence of *C. tepidum* by The Institute for Genomic Studies. Indeed, an unexpected finding was a putative ribulose bisphosphate carboxylase/ oxygenase (RubisCO) sequence that resembles, but thus far seems not

quite to have reached the evolutional advancement of previously described RubisCO molecules. The function of this RubisCO-like (RLP) molecule does not appear to be in CO_2 fixation per se, but it seems to play a role in sulfur metabolism and in the stress response of the cell. Finally, with the genomic sequence available, it has become feasible to approach more global regulation studies.

155. Ohio State University

Columbus, OH 43210-1292
Mechanisms of Microbial Adaptation
F. Robert Tabita, Department of Microbiology
$50,000 (FY 99 funds – 17 l/2 months)

During the summer of 2000, we hosted a 2-week workshop for 12 students. The topics of study were presented in four separate modules, each directed by a faculty member from the OSU Department of Microbiology. These included modules on Halobacterial Transcription Factors, hosted by Professor Charles Daniels; Metabolic Regulation, hosted by Professor F. Robert Tabita; *Pseudomonas* Exotoxin Synthesis, hosted by Professor Darrell Galloway; and *Rhizobium* Nodulation, hosted by Professor Michelle Rondon. Several distinguished invited guest lecturers also gave talks and interacted with the students during this time period. These guests included Stanley R. Maloy (University of Illinois), Peter J. Christie (University of Texas Health Center), Gerald L. Hazelbauer (Washington State University), Abigail A. Salyers (University of Illinois), Alicia Dombrowski (University of Texas Health Center), Rasika M. Harsey (University of Texas at Austin), James R. Brown (Smith-Kline-Beecham), Valley J. Stewart (University of California at Davis), and Judith P. Armitage (University of Oxford). Both lectures and hands-on experimental work enabled the students to gain an appreciation of the subject matter within these modules; the students also gained considerable experience with different experimental protocols and diverse instrumentation.

156. Oklahoma State University

Stillwater, OK 74078-3035
The Structure of Pectins from Cotton Cell Walls
Andrew Mort, Department of Biochemistry and Molecular Biology
$106,000

The goal of this project is to determine the remaining unknown general structural features of pectins. The functions of pectins are almost certainly related to their structures. We are attempting to isolate oligosaccharides that are small enough for complete chemical characterization from the junctions between the various classes of pectic regions. The known major regions in pectins are based on two backbones: 1) homogalacturonans (HG) and 2) rhamnogalacturonan (RG). There are at least three regions based on the HG backbone: 1) plain homogalacturonan (with varying degrees of methylesterification), 2) xylogalacturonan (XGA), which is an HG with frequent single xylosyl sidechains, and 3) rhamnogalacturonan II, which is a short stretch of HG with several different complex sidechains. Little is known about the variation on RG regions. In cotton and watermelon pectin, we always find a close association between the RG regions and XGA. We also find that about half of the xyloglucan (XG) in cotton culture cells is linked to this RG-XGA complex. We can isolate a fraction whose apparent molecular weight is around 5000 daltons but still contains linkages characteristic of XGA, RG, and XG. This complex needs to be digested further before we can completely identify the linkages between each region. We are trying (in collaboration with Rolf Prade) to clone and purify cell wall degrading enzymes from Aspergillus which can degrade this region. We are developing capillary electrophoretic methods with laser-induced fluorescence detection to allow analysis of the very small samples we sometimes have to work with.

157. University of Oklahoma

Norman, OK 73019-0245

Enzymology and Energetics of Syntrophic Benzoate Metabolism
Michael J. McInerney, Department of Botany and Microbiology
$96,000

The metabolism of benzoate, a key intermediate in anaerobic aromatic degradation, was studied in a newly isolated bacterium called *Syntrophus aciditrophicus*. A reduced ring compound called cylcohexane carboxylate along with several other compounds transiently accumulated during growth on benzoate. Cyclohexane carboxylate accumulated to a concentration of 260 µM, accounting for about 18% of the initial benzoate added. Cell-free extracts of *S. aciditrophicus* contained the enzyme activities needed to activate benzoate to its coenzyme A derivative, reduce and oxidize it to 2-ketocyclohexane carboxyl-CoA, hydrolytically cleave the ring and convert the ring cleavage product to short chain organic acids. These studies are consistent with the hypothesis that ring reduction during syntrophic benzoate metabolism involves a 4 or 6 electron reduction step and that once cyclohex-1-ene carboxyl-CoA is made, it is metabolized in a similar manner as found in *Rhodopseudomonase palustris*. Our work shows that third variation exists for anaerobic benzoate degradation.

We also report the ability of *S. aciditrophicus* to grow on benzoate in absence of hydrogen-utilizing partners or terminal-electron acceptors. This is the first report of the anaerobic growth of an organism in pure culture with benzoate in the absence of light or terminal electron acceptors such as sulfate, iron, or nitrate. In collaboration with other scientists at Oklahoma, we found that alkylbenzenes (major components of gasolines) are degraded in aquifers by an addition reaction unique to anaerobic metabolism. The intermediates generated during alkylbenzene degradation transiently accumulate in groundwater and their detection offers strong evidence for the in situ anaerobic attenuation of alkylbenzenes.

158. Oregon Graduate Institute of Science & Technology

Portland, OR 97291-1000

Biochemical Genetics of Lignin Degradation by Phanerochaete chrysosporium
Michael H. Gold, Department of Biochemistry and Molecular Biology.
$135,000

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

We are studying the structure and mechanism of lignin peroxidases (LiP) via spectroscopic, kinetic, and bioorganic methods and, in collaboration, by crystallography and other biophysical methods. Using a homologous expression system, we have expressed two different isozymes of LiP. The recombinant proteins are being isolated and characterized by kinetic, spectroscopic methods.

We are also studying the enzymes and genes involved in lignin degradation by the white rot fungus *Dichomitus squalens*, an organism which degrades lignin without producing any LiP. We have now cloned, sequenced, and heterologously expressed the *D. squalens* manganese peroxidases in *P. chrysosporium*.

We are continuing to identify and isolate proteins and genes involved in the intracellular degradation of lignin fragments and aromatic pollutants. An intracellular quinone reductase, which is involved in the metabolism of monomeric quinones, is being characterized. Finally, we are elucidating the reductive dechlorination reactions involved in the total dechlorination of pentachlorophenol by *P. chrysosporium.*

159. Oregon Graduate Institute of Science & Technology

Portland, OR 97291-1000
Cloning and Expression of Cellobiose Dehydrogenases
Michael H. Gold and V. Renganathan, Department of Biochemistry and Molecular Biology.
$92,999

Cellobiose dehydrogenase (CDH) is an extracellular hemoflavoenzyme produced by cellulose-degrading cultures of *Phanerochaete chrysosporium* and selected other fungi. A homologous expression system has been developed for CDH in our laboratory. Using this expression system and the recently published crystal structure of the heme domain, we are constructing and characterizing site-directed mutants of this enzyme. We have prepared and isolated mutants of the heme binding ligands, Met65 and His163, which demonstrate functionally that these residues are the heme binding ligands. Additional mutants in the heme domain and new mutants in the flavin domain are being analyzed. We are also continuing to study the reaction mechanism of CDH and the role of this enzyme in cellulose degradation.

160. Oregon State University

Corvallis, OR 97331-2902
Regulation of the Genes Involved in Nitrification
Daniel J. Arp, Department of Botany and Plant Pathology
$95,000

Nitrification involves the oxidation of ammonia to nitrate. This process leads to considerable losses of N fertilizers from croplands through leaching of the nitrate into ground and surface waters and through denitrification of the nitrate to dinitrogen. With this loss of N, the energy used to produce the ammonia fertilizer (primarily natural gas and H_2) is wasted. Nitrification is initiated by the oxidation of ammonia to nitrite by ammonia oxidizing bacteria. Ammonia oxidation to nitrite is catalyzed by ammonia monooxygenase, which catalyzes the transformation of ammonia to hydroxylamine, and hydroxylamine oxidoreductase, which catalyzes the transformation of hydroxylamine to nitrite. The genes for each of these enzymes have been identified and characterized in *Nitrosomonas europaea*, a well-studied ammonia oxidizer. We developed methods for the genetic manipulation of *N. europaea*, identified the transcripts expressed from the primary genes of nitrification, mapped the promoters for these genes, and examined the role of multiple copies of these genes. We are continuing to investigate the expression of these and other genes. The specific objectives are: 1) To further characterize the transcripts and their promoters for the *amo* genes, 2) to determine if there *amo* copy specific effects with regard to promoter choice or transcript production, 3) to examine how environmental parameters (ammonia concentration) influence *amo* expression, and 4) to examine the regulation of other genes (particularly *hao*, the genes for rubisco, and other genes of interest identified in the genome sequencing project).

161. University of Oregon

Eugene, OR 97403-1229
Genetic Analysis of Chloroplast Translation in Maize
Alice Barkan, Institute of Molecular Biology
$200,000 (FY 99 funds – two years)

The assembly of the photosynthetic apparatus requires the concerted action of hundreds of genes distributed between the two physically separate genomes in the nucleus and chloroplast. Nuclear genes coordinate this process by controlling the expression of chloroplast genes in response to developmental and environmental cues. However, few regulatory factors have been identified. We are using mutant phenotypes to identify nuclear genes in maize that modulate chloroplast translation, a key control point in chloroplast gene expression. This project is focused on the nuclear gene crp1, mutations in which disrupt the translation of two chloroplast mRNAs. We cloned the crp1 gene and found that CRP1 is related to fungal proteins involved in the translation of a mitochondrial mRNA. CRP1 is the founding member of a large gene family in plants, the members of which contain a repeated 35 amino acid motif called a "PPR"

motif. The PPR motif is thought to mediate intermolecular interactions, based on its similarity to the well-characterized TPR motif. However, no substrates of a PPR protein have been identified. CRP1 is present in a multiprotein complex in the chloroplast. To understand how CRP1 influences the translation of specific chloroplast mRNAs, we are identifying the components of this complex as well as molecules that interact more transiently with CRP1. We will investigate the roles of interacting proteins by identifying mutations in the corresponding genes. We are also seeking mutations in genes encoding other members of the PPR family. A large fraction of the proteins in this family are predicted to localize to mitochondria and chloroplasts, so it is anticipated that other members of the PPR family may also function in the control of organellar gene expression.

162. Pennsylvania State University

University Park, PA 16802-4500

The Characterization of Psychrophilic Microorganisms and Their Potentially Useful Cold-Active Glycosidases

Jean E. Brenchley, Department of Biochemistry and Molecular Biology

$107,000

Our objective is to explore the diversity of psychrophilic (cold-loving) microorganisms and to understand how their cold-active enzymes function at low temperatures. To accomplish this, we isolate novel psychrophiles, clone genes encoding cold-active glycosidases, and purify and characterize especially interesting enzymes. We have established a large collection of psychrophilic bacteria, some of which represent new species or genera. We have cloned genes encoding over 20 glycosidases which, based on sequence and hydrophobicity cluster analyses, belong to five different families. This work places our research in a unique position to meet the following goals. 1). Isolate additional psychrophiles growing at lower temperatures and examine the physiology and phylogenetics of those we use for cloning cold-active glycosidases. 2). Characterize novel glycosidases phylogenetically and biochemically to supplement the sequence database with functional information. 3). Use our cloned genes in directed evolution studies to identify alterations that can affect cold-activity/heat-lability of enzymes. Our integrative approach brings together information about the habitat, physiology, and evolution of the isolate with information about the functions of its glycosidases. The biochemical characterization of cold-active glycosidases not only yields insight into the structural features involved in maintaining high activities at low temperature, but some enzymes may have applications for converting saccharides found in plant biomass, paper-pulping waste, whey, etc., into sources for chemical fuels or fermentation media.

163. Pennsylvania State University

University Park, PA 16802-4500

Light-Energy Transduction in Green Sulfur Bacteria

Donald A. Bryant, Department of Biochemistry and Molecular Biology

$25,000 (funded extension – one year)

The long-term objective of this research program is to develop a detailed understanding of the structure, function, and biogenesis of the light-energy transduction apparatus found in green sulfur bacteria. Secondary goals are to analyze the transcriptional machinery of these bacteria, to develop methods for genetic analyses, and to understand the phylogenetic relationships among green sulfur bacteria and other bacteria and archaea. All ten genes encoding chlorosome envelope proteins have been cloned, sequenced, and overproduced in Escherichia coli. Each purified protein was used to raise polyclonal antibodies in rabbits. The resulting antisera have been used to demonstrate that all chlorosome proteins are localized in the chlorosome envelope and that these proteins can assemble even when bacteriochlorophyll biosynthesis is severely inhibited. Three chlorosome proteins (CsmI, CsmJ, and CsmX) have been shown to be Fe-S proteins and to contain [2Fe-2S] clusters. Our working hypothesis is that these proteins participate in the oxidation and reduction of chlorobiumquinone which is believed to control the oxygen-dependent quenching of excitation energy transfer in chlorosomes. In collaboration with The Institute for Genomic Research, the complete genomic sequence of the moderately thermophilic green sulfur bacterium Chlorobium tepidum has

been determined and annotated. Analysis of the sequence data has yielded proposed biosynthetic pathways for quinones, tetrapyrroles and chlorophylls, carotenoids, and cobalamin. A reliable method for natural transformation of this same organism has been developed and optimized, and this procedure has been used to construct knock-out mutations in the three genes encoding chlorosome Fe-S proteins. The transformation system will also be used to investigate the proposed biosynthetic pathways deduced from the genome sequence analyses.

164. Pennsylvania State University

University Park, PA 16802
The control of lignin synthesis
John E. Carlson, School of Forest Resources
$90,000

Lignin, a complex three-dimensional organic polymer, is the component of plant cell walls that provides the strength and rigidity that is characteristic of wood and of vessels in plants that transport water from roots to leaves. Lignin also plays an important role in defense of plants against attack by pests. Lignin is composed of three types of subunits (“monomers”): p-coumaryl-, coniferyl- and sinapyl-alcohols. The relative proportion of the different monomers in lignin can vary greatly, which results in differences among tree species in wood quality and in the chemistries needed to produce pulp and paper. How plants regulate the biosynthesis of lignin monomers and the mechanism by which lignin monomers are transported to the cell wall for lignin polymerization are not well understood. In this project we are testing the hypothesis that glucosylation of lignin monomers occurs within the plant cell prior to the transport of monolignols to the cell wall, followed by de-glucosylation in the cell wall by monolignol-specific glucosidase enzymes, which activates the monomers for lignin synthesis. Our preliminary results confirmed the presence of a coniferin-specific glucosidase in lignifying (wood-forming) pine tissues. We are using a combination of molecular biology, biochemistry, histology and genomics techniques to determine the structure of monolignol-specific glucosidase genes and their role in controlling lignin synthesis. This information will increase our understanding of how trees make lignin and wood, and may provide opportunities to create new tree genotypes for more environmentally friendly pulp and paper production.

165. Pennsylvania State University

University Park, PA 16802
Regulation of Plant Cell Growth: Structure and Function of Beta-Expansins in Rice and Maize
Daniel J. Cosgrove, Department of Biology
$110,000

The ability of growing plant cells to enlarge depends on cell wall rheology, which in turn is a complex function of wall structure and the activity of enzymes that weaken or strengthen the cross linking between structural polymers of the wall. We are focusing on mechanisms that rigidify and loosen cell walls. Expansins are a special class of cell wall protein that cause plant walls to extend ("creep") and undergo stress relaxation in a pH-dependent manner. In this project we have been studying the function of one sub-family of expansins, called beta-expansins, that make up a large multigene family in rice and maize. We have cloned, sequenced and characterized expression patterns for 10 beta-expansins in rice and a similar number in maize. We have been using a reverse genetic approach to identify expansin mutants in maize and to study the resulting alteration in growth and development. Additional studies are aimed at understanding the biochemical mechanism of action of expansins and identifying other enzymes that interact with the cell wall and with expansins to control cell wall enlargement. For example, we recently found a xyloglucanase that is able to cause cell wall extension, with characteristics very distinctive from expansin’s action. Moreover, alterations in pectin structure can amplify or reduce expansin’s effect on wall extension. Our long term goal is to bring together the molecular details of how specific enzymes modify the wall with a broader analysis of the important cell wall rheological properties that govern plant cell enlargement.

166. Pennsylvania State University

University Park, PA 16802-5301
Elongation Factor 1Alpha and the Plant Cytoskeleton
Richard J. Cyr, Department of Biology
$193,000 (two years)

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. 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. We have found that elongation factor 1-α behaves as a Mt associated protein serving to both stabilize, and bundle, microtubules *in vitro*. Importantly, this association can be modulated with the application of agents that acidify the cytoplasm. We are currently in the process of making several synthetic genes to explore the physiological significance of this interaction. This information will, in turn, be used to develop strategies for specifically perturbing this interaction *in vivo* thereby allowing us to directly test our hypothesis that EF-1α affects the behavior of microtubules within living cells.

167. Pennsylvania State University

University Park, PA 16802-4500
Enzymology of the Pathway for Acetate Conversion to Methane in Methanosarcina thermophila
James G. Ferry, Department of Biochemistry and Molecular Biology
$109,000

Several enzymes and proteins identified in the pathway for the fermentation of acetate to methane and carbon dioxide have been purified from *Methanosarcina thermophila* and are under investigation utilizing biochemical, biophysical and molecular genetic approaches. The mechanisms of acetate kinase and phosphotransacetylase, that function to activate acetate to acetyl-CoA in the first step of the pathway, are being determined. The crystal structure has been obtained for acetate kinase and crystals of phosphotransacetylase are being analyzed to determine the structure. Roles for arginine residues and a magnesium ion in the stabilization of a transition state supports a direct in-line mechanism for acetate kinase. Site directed mutagenesis has identified arginine residues essential for binding CoA that are also important for catalysis of the phosphotransacetylase. A novel iron sulfur flavoprotein, (Isf), first discovered in *M. thermophila*, was shown to be widespread in anaerobic procaryotes through analysis of genomic sequences and the heterologous expression and characterization of Isf homologs. The results suggest a broad electron transport function for this protein in a diversity of strictly anaerobic microbes. Site directed mutagenesis studies identified an unusually compact cysteine motif ligating the 4Fe-4S cluster in Isf from *M. thermophila*. In a separate project, the pathway for the synthesis of cysteine in the Archaea was investigated. Isolation and characterization of an O-acetylserine sulfhydrylase from *M. thermophila* has provided strong evidence for the bacterial pathway. Gene knockout experiments are underway to confirm this proposal.

168. Pennsylvania State University

University Park, PA 16802
Primary Electron Transfer in Green Photosynthetic Bacteria
John H. Golbeck, co P.I. Ilya Vassiliev, Department of Biochemistry and Molecular Biology
$94,000

The goal of our research is to identify the electron transfer cofactors and to describe their function in the photosynthetic apparatus of green sulfur bacteria. We began this work by developing a method to measure the electron acceptors in whole cells by EPR (electron paramagnetic resonance), and we succeeded in isolating a completely intact reaction center complex. This groundwork, performed in the first year of the grant, ensured success in fulfilling the aims of the grant. We made significant progress in the following areas: (1) By numeric simulation of the EPR spectra in cells, isolated membranes and reaction centers from *Chlorobium tepidum,* we obtained evidence for a [4Fe-4S] cluster that we identified as F_X. This cluster is present in the reaction centers along with the clusters F_A and F_B described earlier. (2) We developed an advanced numeric approach for analysis of transient absorbance kinetics in the near-IR. Using this approach, we were able to separate the spectra of the primary electron donor $P840^+$ and the triplet states of bacteriochlorophyll *a* in the FMO protein in the reaction center core of *Chlorobium vibrioforme*. (3) We reconstructed the iron-sulfur cluster F_X in a isolated reaction center core preparation. A kinetic analysis of F_X-depleted and F_X-reconstructed cores indicates the presence of an intermediate acceptor equivalent to A_1, which functions as an electron donor to F_X. (4) We measured the EPR spectra of isolated chlorosomes and chlorosomal proteins. The data indicate the presence of [2Fe-2S] clusters, which may be involved in regulation of the energy transfer to the reaction center. (5) We made a comparative analysis of the EPR spectra of the bound iron-sulfur clusters of green sulfur bacteria, heliobacteria and Photosystem I of cyanobacteria. The similarities and the differences between these clusters are discussed in a review which is now accepted for publication in *Biochim. Biophys. Acta*.

169. Pennsylvania State University

University Park, PA 16802-5807
Molecular-Genetic Analysis of Maize Starch Branching Enzyme Isoforms
Mark Guiltinan, co P.I.s Jack Shannon, Donald Thompson, Department of Horticulture
$95,000

Our project aims at understanding the genetic basis and molecular mechanisms of starch biosynthesis in crop plants. Starch, a major caloric source of nutrition, is also used in various industrial processes, including alcohol production. Amazingly, there is a fundamental hole in our knowledge at the mechanistic and molecular levels of the processes occurring during starch biosynthesis. This knowledge however, is essential to enable the design of rational strategies for the manipulation of carbohydrate synthesis in plant systems. For example, such knowledge may lead to approaches for production of biodegradable plastics in plants systems. Similarly, totally novel high-value carbohydrates such as cyclic-polysaccharides may be made in plant systems for use in drug delivery systems. These possibilities would lead to efficient and cheap methods for production of high value biomaterials, and help to ensure the long-term competitiveness of the U.S. Agricultural and Industrial sectors in the future. Our specific focus is on the enzymes that create branches in starch during its biosynthesis. These branches are important in determining the functional properties of starch, such as melting point, gel-strength etc. Thus, these enzymes and the branches they produce are especially important to the food processing industry, which frequently uses starches of differing properties for various applications. Our approach uses mutant corn lines, each missing individual starch branching enzymes, to probe the role of each individual enzyme in starch biosynthesis and in plant development. Studying the growth and starch produced by these mutants provides information as to the roles of the various enzymes.
Additional information

170. Pennsylvania State University

University Park, PA 16802-4500

Characterization of Lignin and Mn Peroxidases from Phanerochaete chrysosporium

Ming Tien, Department of Biochemistry and Molecular Biology

$119,000

Lignin is an aromatic polymer which constitutes up to 30% of woody biomass. Its biodegradation is predominantly through filamentous fungi. Survey of basidiomycetes has shown that this oxidative process is catalyzed by a family of extracellular enzymes, lignin peroxidases (LP), Mn peroxidases (MnP) and apparently laccases. Our research has been focused on Phanerochaete chrysosporium, which produces only LP and MnP, to determine the role of these enzymes in lignin degradation. Both LP and MnP utilize H2O2 to oxidize a variety of phenolic and nonphenolic (for LP) lignin compounds whereas laccases oxidize phenolic compounds utilizing molecular oxygen as the oxidant. Research the past 3 years has defined the nature of the substrate-binding site in LP and MnP. In MnP, we have used site-directed mutagenesis to determine the physical basis for the high reduction potential of the enzyme. Our present focus is on a continuation of these efforts along with expanding our research into characterizing the enzymology of laccases. Our work with lignin model compounds and product analyses has help map the active site of LP. Similar studies are planned with laccases. This will allow us to address the important question of substrate profile for laccases since these enzyme were originally not believed to be involved in lignin degradation due to their low reduction potential. We have also attempted to determine the role of these enzymes in lignin degradation by using antisense constructs with little success. Present efforts are now focused toward using RNA interference methods to disrupt expression of these genes.

171. University of Pennsylvania

Philadelphia, PA 19104-6018

Light Responses and Photoperiodism in Arabidopsis thaliana

Anthony R. Cashmore, Plant Science Institute, Department of Biology

$134,000

In our study of the mechanisms by which plants sense and respond to blue light, we have recently demonstrated that expression in transgenic plants of the C-terminal fragment of the Arabidopsis blue light receptor cryptochrome, confers a constitutive light response. This phenotype is similar to that of several previously described Arabidopsis mutants (the *cop* mutants) and hence these observations are both informative in terms of the mode of action of cryptochrome and are likely to facilitate the identification of cryptochrome signaling partners.

In separate studies we have identified two additional Arabidopsis genes which, like cryptochrome, affect the responses of plants to blue light. One of these genes (*ADAGIO1*) encodes a PAS-domain protein similar to white collar 1 (WC-1), a flavoprotein component of the circadian clock of Neurospora. We demonstrated that mutation of *ADAGIO1* affects the periodicity of the circadian clock in Arabidopsis. ADAGIO1 protein was shown to bind to both cryptochrome and phytochrome photoreceptors. ADAGIO1 may correspond to a new blue light receptor — alternatively, it may function in signaling and/or as a component of the Arabidopsis circadian clock.

The other gene we have identified is *NPL1*, which also encodes a PAS-domain protein, similar to NPH1 (phototropin), the photoreceptor for phototropic bending of plants toward blue light. We have shown that Arabidopsis *npl1* mutants are altered in the movement of their chloroplasts in response to blue light, and we propose that NPL1 is the photoreceptor mediating this response.

172. University of Pennsylvania

Philadelphia, PA 19104-6018
Membrane-Attached Electron Carriers in Photosynthesis and Respiration
Fevzi Daldal, Department of Biology
$117,000

The long-term goal of our project is the identification and structure-function relation as well as regulation and biogenesis of membrane-bound cytochromes that act as electron carriers in photosynthesis and respiration. We work with the facultative phototrophs of Rhodobacter species as model organisms. Our structure-function studies are focused on Rhodobacter capsulatus cyt cy that operates as a membrane-anchored electron carrier between the cyt bc1 complex and the reaction center (RC) during photosynthesis, and also the cbb3-type cyt c oxidase during respiration. This cytochrome, discovered in our group, is unique in its ability to support efficient photosynthesis by interacting with a small fraction of the RCs. We have now found its homolog in Rhodobacter sphaeroides and demonstrated that it is functional only in respiration but not in photosynthesis. The availability of two quasi-identical electron transfer proteins with different properties gives us unique opportunities to understand critical structural-functional characteristics of these membrane proteins. In our work on the biogenesis and regulation of cytochromes we have discovered several novel genes. One of the gene products is a molecular usher for delivery of apocytochromes from the protein translocation machinery to heme incorporation apparatus, and another one is a redox conveyer from the cytoplasm to the periplasm. Our ongoing analyses are directed to define their cellular location, topology, and interactions with other components of cytochrome biogenesis pathway, which is a fundamental biological process essential for efficient cellular energy transduction.

173. University of Pennsylvania

Philadelphia, PA 19104-6018
The function of the EARLY TRICHOMES gene in Arabidopsis in maize
Scott Poethig, Department of Biology
$100,000

Analysis of the function of the *EARLY TRICHOMES* (= *KANADI*) gene have shown that it plays a key role in the regulation of organ and shoot polarity in Arabidopsis. *KAN* is expressed on the abaxial side of all lateral organs in the shoot and in peripheral cells of the globular embryo. Consistent with this expression pattern, loss-of-function mutations of *KAN* eliminate some aspects of abaxial polarity in both the epidermis and internal tissue of leaves and floral organs whereas constitutive expression of KAN (driven by a *35S::KAN* transgene) produces radial, abaxialized organs, and prevents the formation of the shoot apical meristem (SAM) and the differentiation of vascular tissue in the hypocotyl. The SAM and vascular tissue in the hypocotyl arise from a central region of the embryo that is topologically continuous with the adaxial surface of lateral organs, just as the peripheral tissue of the embryo is continuous with the abaxial surface of lateral organs. Thus, we interpret the phenotype of 35S::KAN seedlings as a abaxial/peripheral transformation of adaxial/central tissue, and suggest that the adaxial/abaxial polarity of lateral organs and the central/peripheral polarity of the SAM may be specified by the same patterning system. Published data on the phenotypes and expression patterns of genes that are expressed in spatially-restricted domains of the SAM and lateral organs (e.g. AGAMOUS) support this hypothesis.

174. University of Pennsylvania

Philadelphia, PA 19104-6018
Biochemical Basis of YCF1-Dependent Vacuolar Glutathione-S-Conjugate Transport
Philip A. Rea, Department of Biology
$110,000

This program of research is concerned with several fundamental aspects of the function of vacuolar glutathione *S*-conjugate pumps, members of the MRP subfamily of ATP-binding cassette (ABC) transporters, in the yeast *Saccharomyces cerevisiae* and the model plant *Arabidopsis thaliana*. Acting

downstream of the first two phases of toxin detoxification, processes that likely converge and depend on the MRPs from plants and yeast include the detoxification of herbicides and other organic xenobiotics, the alleviation of oxidative damage, the storage of endotoxins, heavy metal sequestration and the vacuolation of natural pigments. Through research conducted under the auspices of the Energy Biosciences Program we have established the molecular identity of the principal vacuolar MRP from yeast and extended these findings to the molecular identification of its equivalents from *Arabidopsis*. Distinguished by a unique domain organization and the facility to mediate the ATP-energized transport of complex organic molecules, the discovery and characterization of these MRPs is of profound significance in two respects. First, the fact that the MRPs, like most ABC transporters, are directly energized by ATP rather than by, for example, a preformed proton gradient, has given rise to a new non-chemiosmotic perspective on the molecular basis of energy-dependent solute transport in plants. Second, the broad range of compounds that the MRPs are capable of transporting offers the prospect for manipulating and investigating many fundamental processes that have hitherto evaded analysis at the transport level.

175. Purdue University

West Lafayette, IN 47907-1155

Mechanisms of synthesis of mixed- linkage β-glucan, and the CeSA gene family of rice

Nicholas C. Carpita, Department of Botany and Plant Pathology

$100,000

We continue our investigations of the mechanism of synthesis in vitro of the maize coleoptile mixed-linkage (1-->3),(1-->4)-b-D-glucan (b-glucan). We have successfully synthesized this glucan in vitro and have characterized the fine structure of the reaction product. The synthesis occurs in such a way to favor the formation of cellotriosyl and odd numbered cellodextrin units at saturating substrate concentrations. We propose a mechanism of synthesis of b-glucan as a variation from cellulose synthesis, whereby cellotriosyl units are linked between single (1-->3)b-linkages instead of two cellobiosyl units linked by a (1-->4)b-linkage. Our hypothesis is that b-glucan synthase derived from an ancestral cellulose synthase by acquisition of an additional mode of glycosyltransferase to make cellotriose units instead of cellobiose units. We are currently testing three features of the b-glucan synthase that would shed light on the synthesis mechanism. First, we are determining the influence of cellulose synthase inhibitors on the inhibition of b-glucan synthase and in changing the ratio of the cellotriose to cellotetraose subunits. Second, we are testing sensitivity to protease digestions to determine the topological location of the synthase catalytic site. Third, we are testing acylating reagents for their ability to inhibit systhesis, an activity based on the appearance of consecutive or proximal basic amino acid residues in the catalytic site of synthesis. We have expressed truncated domains of maize CesA proteins in prokaryotic and eukaryotic expression systems in order to generate sufficient quantities of native protein to achieve crystallization. We hope this effort will yield the 3-D structure of catalytic domains of cellulose and b-glucan synthase.

Our analysis of the phylogenetic relationships among members of the CesA multi-gene families from two grass species, Oryza sativa and Zea mays, with Arabidopsis thaliana and other dicotyledonous species reveals that the CesA genes cluster into several distinct sub-classes. Whereas some sub-classes are populated by CesAs from all species, two sub-classes are populated solely by CesAs from grass species. The sub-class identity is primarily defined by the HVR, and the sequence in this region does not vary substantially among members of the same sub-class. Hence, we suggest that the region is more aptly termed a "Class-Specific Region" (CSR). Several motifs containing cysteine, basic, acidic and aromatic residues indicate that the CSR may function in substrate binding specificity and catalysis. Similar motifs are conserved in bacterial cellulose synthases, the Dictyostelium discoideum cellulose synthase, and other processive glycosyltransferases involved in the synthesis of non-cellulosic polymers with (1-->4)b-linked backbones, including chitin, heparan, and hyaluronan. These analyses re-open the question whether all the CesA genes encode cellulose synthases or whether some of the sub-class members may encode other non-cellulosic (1-->4)b-D-glycan synthases in plants, such as the mixed-linkage (1-->3),(1-->4)b-D-glucan synthase

176. Purdue University

West Lafayette, IN 47907-1153
Ferulate-5-hydroxylase: requirements for expression and activity
Clinton C. S. Chapple, Department of Biochemistry
$200,000 (two years)

Phenylpropanoid compounds participate in many plant defense responses, and absorb potentially-damaging UV-B radiation. The pathway also generates the monomers required for the biosynthesis of lignin, a polymer that provides structural rigidity to plant tissues and enables tracheary elements to withstand the tension generated during transpiration.

Our goal is to understand the enzymes responsible for the biosynthesis of phenylpropanoid compounds in plants, and to identify the regulatory factors that control their expression. To accomplish this goal, we have isolated mutants that are defective in the synthesis of sinapoylmalate, one of the major soluble phenylpropanoid secondary metabolites in Arabidopsis. We have genetically identified eight new *red-fluorescent leaves* mutants (*ref1-ref8*) that are affected in various aspects of phenylpropanoid biosynthesis. Using positional cloning methods, we have identified a small region of the Arabidopsis genome that encodes twelve genes, one of which is *REF2*. We have shown that the *ref3* mutants are defective in the gene encoding cinnamate 4-hydroxylase, and are the only mutants for this gene known in the plant kingdom. Finally, we have determined that the *ref8* mutant is defective in the gene encoding C3H, the only enzyme of the pathway that has not been characterized, and the enzyme encoded by the only gene of the pathway that has not been cloned. The study of these mutants will improve our understanding of the role of phenylpropanoid compounds, and will provide new tools for the modification of this pathway in agriculturally important plants.

177. Purdue University

West Lafayette, IN 47907-1165
Engineering Plant One-Carbon Metabolism
David Rhodes, Department of Horticulture and Landscape Architecture
$38,033
Collaborators, Institutions and their Support: Andrew Hanson [University of Florida] NSF; Hans Bohnert [University of Arizona] NSF; Douglas A. Gage [Michigan State University] NSF; Yair Shachar-Hill [New Mexico State University] NIST.

Primary and secondary metabolism intersect in the one-carbon (C1) area. Primary metabolism supplies most of the C1 units and competes with secondary metabolism for their use. This competition is potentially severe because secondary products such as lignin, alkaloids, and glycine betaine (GlyBet) require massive amounts of C1 units. Towards the goal of understanding how C1 metabolism is regulated at the metabolic and gene levels so as to successfully engineer C1 supply to match demand, we have: (1) cloned complete suites of C1 genes from maize and tobacco, and incorporated them into DNA arrays; (2) prepared antisense constructs and mutants engineered with alterations in C1 unit supply and demand; and (3) are beginning to quantify the impacts of these alterations on gene expression (using DNA arrays), and on metabolic fluxes (by combining isotope labeling, MS, NMR and computer modeling). Metabolic flux analysis and modeling in tobacco engineered for GlyBet synthesis by expressing choline oxidizing enzymes in either the chloroplast or cytosol, has shown that the choline biosynthesis network is rigid, and tends to resist large changes in C1 demand. Maize mutants defective in GlyBet synthesis show greatly reduced flux of C1 units into choline in comparison to GlyBet-accumulating wildtypes, but this is not associated with altered expression of any of the C1 genes. Control of C1 flux to choline in both tobacco and maize appears to reside primarily at the level N-methylation of phosphoethanolamine. A candidate signal for the control of this flux may be the pool size of phosphocholine.

178. Purdue University

West Lafayette, IN 47907-1392
The Impact of Environmental Stress on the Regulation of Photosynthesis
Louis A. Sherman, Department of Biological Sciences
$220,000 (FY 99 funds – two years)

The major objectives of this proposal are to understand how the redox poise of the cell regulates photosynthesis and to determine how changes in various environmental parameters affect gene expression and the assembly of the photosynthetic apparatus. The proposal utilizes the transformable, unicellular cyanobacterium Synechocystis sp. PCC 6803 for which the entire genome has been sequenced (3,168 proteins). Thus, we can also make use of genomics and analyze the impact of environmental changes on many functions simultaneously. During this project period, we have made progress on both objectives. We have detected and characterized 2 genes that act as transcription regulators and are sensitive to changes in redox poise in the electron transport chains. One of these proteins, RppA, appears to modulate the Photosystem I (PSI) and PSII proteins in opposite manners. We have also developed procedures for studying global changes in gene expression. These techniques involve the construction of an array of genes and we have constructed progressively more precise arrays. These arrays were used to analyze transcription under iron-deficient vs. iron-sufficient conditions, the wild type vs. a mutant in RppA, and high vs. low CO2. The work with changes in the concentration of iron has progressed the most. We found over 50 genes with greater than a 3-fold increase or decrease in transcription as cells undergo transition from low to high Fe concentrations or visa versa. Only 3 of these had been previously characterized. Some of the genes identified as iron-responsive include those involved in assembly of the photosynthetic membrane and the light-harvesting complexes called phycobilisomes.

179. Purdue University

West Lafayette, IN 47907-1392
Identification of Actin-Binding Proteins from Maize Pollen
Christopher J. Staiger, Department of Biological Sciences
$97,000

Diverse cellular processes like cytoplasmic streaming, tip growth and cytokinesis all depend upon the actin cytoskeleton. The goal of our research is to gain an understanding of how the organization and dynamics of the actin cytoskeleton are controlled in flowering plants, and specifically during plant reproduction. To predict how actin behavior is regulated in the living cell, we must first identify the associated proteins and characterize how they interact with actin in vitro. Other than profilin, ADF/cofilin and villin-like proteins, evidence for actin-binding proteins from pollen is rather sparse. We are using two parallel strategies to isolate and characterize actin-binding proteins from maize pollen; co-sedimentation with polymerized actin, and F-actin affinity chromatography (FAAC). Several putative actin-binding proteins have been isolated by both methods and these will be identified by peptide microsequencing and/or mass spectroscopy. Moreover, polyclonal antisera will be generated to characterize the subcellular distribution of each candidate protein and to isolate cDNAs from an expression library. At least one known protein, a villin-like protein or ABP-135 ortholog from maize, can be isolated by FAAC and confirmed by immunoblotting with a specific antiserum. We have also characterized recombinant Arabidopsis fimbrin (AtFim1), a filament crosslinking protein, and defined its biochemical properties in vitro and in living cells following microinjection. The latter studies have allowed us to develop methods and technologies that will prove beneficial for future characterization of maize pollen actin-binding proteins.

180. Rensselaer Polytechnic Institute

Troy, NY 12180-3590
A Novel, Photosynthesis-Associated Thioredoxin-Like Gene
Jackie L. Collier, Department of Biology
209,000 (FY 99 funds – two years)

The goal of this project is to elucidate the biochemical mechanism and physiological function of the cyanobacterial gene TxlA. TxlA is essential in the cyanobacterium Synechococcus PCC 7942, and has close homologues in higher plants but not in nonphotosynthetic organisms, suggesting that TxlA plays an important and fundamental role in photosynthesis. At the beginning of this project, we knew that TxlA had an N-terminal domain that might function as a signal sequence, a central domain with a high level of sequence similarity to thioredoxins, and a unique C-terminal domain. In the first year we focused on learning more about each of these three domains. An antibody raised against the C-terminal domain of TxlA recognized a soluble protein consistent with the hypothesis that the N-terminal domain of TxlA is a cleavable signal sequence that is removed from the mature TxlA product. Our molecular modeling showed that the thioredoxin-like region of TxlA was predicted to fold into the same three-dimensional structure as a thioredoxin. We expressed a number of different versions of recombinant TxlA in Escherichia coli in order to explore its biochemical characteristics. These experiments suggest that the C-terminal domain of TxlA stabilized the thioredoxin-like domain, which was insoluble when expressed alone. Biochemical analyses to date suggest that TxlA does not function in the manner typical of true thioredoxins. It may instead function in a process such as c-type cytochrome (perhaps in the b6f complex) biosynthesis.

181. Rice University

Houston, TX 77005-1892
Complex Regulatory Controls of TCH Gene Expression
Janet Braam, Department of Biochemistry and Cell Biology
$101,000

Plants are exquisitely sensitive to environmental conditions and are capable of responding in ways that make them better adapted to their local environment. The regulation of gene expression is an important aspect of the response, and this regulation can involve distinct control steps. The Arabidopsis *TCH* genes are rapidly and strongly upregulated in expression by environmental stimuli. Thus, these genes are powerful molecular tools for investigating distinct aspects of gene expression regulation in plants.

TCH expression regulation is complex in part because of the number of different, seemingly unrelated, stimuli that induce activity, including touch, darkness, temperature changes and hormones. We hypothesize that the regulatory regions of the *TCH* genes serve to integrate distinct environmental and developmental inputs. The *cis*-regulatory elements necessary and sufficient for the appropriate regulation of *TCH4* expression are being precisely defined through analyzing the activity of *TCH4*::reporter genes in transgenic plants. Components of the sensory and signaling machinery are being sought through the identification and characterization of mutants unable to regulate *TCH* expression appropriately.

Knowledge of these workings of plant cells is important for understanding the consequences of environmental conditions on plant growth. Elucidation of this basic problem in plant biology may lead to the ability to manipulate the environmental stress-induced processes such that one could activate advantageous responses even in the absence of an inducing stimulus, and, conversely, inhibit disadvantageous responses when under environmental stress. Such manipulations may lead to enhanced plant growth and production under diverse environmental conditions.

182. Rice University

Houston, TX 77005-1892

Characterization and Cloning of Sugar Insensitive (sis) Mutants of Arabidopsis

Susan I. Gibson, Department of Biochemistry and Cell Biology

$201,000 (two years)

Despite the fact that soluble sugar levels have been postulated to play an important role in the control of a wide variety of plant metabolic and developmental pathways, the mechanisms by which plants respond to soluble sugar levels remain poorly understood. Mutants of Arabidopsis that are defective in their ability to respond to soluble sugar levels have been isolated and are being used as tools to identify some of the factors involved in plant sugar response. These sugar-insensitive (*sis*) mutants were isolated by screening mutagenized seeds for those that are able to germinate and develop relatively normal shoot systems on media containing 0.3 M glucose or 0.3 M sucrose. At these sugar concentrations, wild-type Arabidopsis plants germinate and produce substantial root systems, but show little or no shoot development. Two of the mutants, *sis2* and *sis3*, have been chosen for further study. Sugar-regulated gene expression, seed lipid reserve mobilization, time to flowering and shoot to root ratios will be characterized in both of these mutants. To aid in determining the function of SIS2 and SIS3, the *SIS2* and *SIS3* genes will be cloned, sequenced and northern analyses performed to determine whether they are regulated in response to sugar, developmental stage or tissue type. Ultimately, a better understanding of plant sugar responses may allow the engineering of crop plants to partition more of their photosynthate to the harvested portions of the plant, thereby improving crop yields.

183. University of Rochester

Rochester, NY 14627-0166

The Structure-Function Relationship of the Clostridium thermocellum Cellulosomal Dockerin

J.H. David Wu, Department of Chemical Engineering

$180,000 (21 months)

The cellulosome is a large, extracellular, multi-enzyme complex capable of degrading crystalline cellulose. Incorporation of the numerous enzymatic subunits into the cellulosome occurs via binding of a highly conserved domain (dockerin) to one of the complementary receptor domains (cohesins) arranged in tandem along a noncatalytic scaffolding protein. The dockerin domain is mostly found at the C-terminus of the enzymatic subunit and consists of two 22-amino acid duplicated segments, each bearing homology to the EF-hand calcium-binding loop. To determine if the dockerin structure contains the canonical EF-hand helix-loop-helix motif, we analyzed the secondary structure of the *Clostridium thermocellum* cellobiohydrolase CelS dockerin using multidimensional heteronuclear NMR spectroscopy. In the absence of Ca^{2+}, dockerin had little secondary or tertiary structure as analyzed by 2D ^{1}H, ^{15}N-HSQC NMR spectroscopy. A titration of dockerin with Ca^{2+} resulted in changes in the NMR spectra, which indicate that Ca^{2+} induces dockerin folding into a compact domain. Sequential backbone assignments were determined for 63 out of 69 residues, and the secondary structure was determined from the NOE connectivities and the ^{1}H chemical shift index. In contrast to the helix-loop-helix motif of EF-hand proteins, each half of dockerin contains just one -helix, equivalent to the F-helix of the EF-hand motif. Thus dockerin assumes a different structure than that of the canonical EF-hand protein despite the sequence homology in the calcium-binding loop. Furthermore, the dockerin folding is mediated by Ca^{2+}.

184. The Rockefeller University

New York, NY 10021-6399

Function of Rac GTPases in Plants

Nam-Hai Chua, Lab of Plant Molecular Biology

$100,000

Small GTPases of the Rho subfamily are key regulators of the actin cytoskeleton and signal transduction in all eukaryotic cells. We have identified two Rho family members (AtRac 1 & 2) that control these processes

in Arabidopsis. In pollen tubes, AtRac1 functions through a phosphatidylinositol monophosphate kinase (PtdIns-P K) and its lipid product PIP2. The localized production of PIP2 at the pollen tube apex, may influence calcium homeostasis through a pollen-specific phospholipase C which converts PIP2 into the second messengers, IP3 and DAG. IP3 may then function to regulate the release of calcium from ER. We are cloning and analyzing the PtdIns P-K activity as well as PLC and diacylglycerol kinase (DGK) to explore their interaction with At-Rac2. In addition to polarized growth of pollen tube, rearrangement of the actin cytoskeleton is believed to underlie many dynamic processes including ABA-triggered movement of stomata. Preliminary results suggest that AtRac1 signaling is achieved through interaction with multiple lipid modifying activities. Interestingly, a possible role of the At-PIP5K in water-stress responses, as in the case for PIP-PLC and DGK, has been suggested. We confirmed that the At-PIP5K, PIP-PLC and DGK transcript levels are regulated by ABA. Using GST fusions in pull-down assays, we demonstrated that At-Rac2 physically associates with a PtdIns-P K activity. This result suggests a model in which Rac activates a cascade of lipid modifications through direct association with several lipid-modifying activities. We have mapped the binding of At-Rac1 to the plant PIP5K and show that binding occurs through a conserved stretch of basic residues.

185. Rutgers University

New Brunswick, NJ 08901-8521

Molecular Bases and Photobiological Consequences of Light Intensity Adaptation in Photosynthetic Organisms

Paul G. Falkowski, Environmental Biophysics and Molecular Ecology, Institute of Marine and Coastal Sciences

$91,218

Photoacclimation are reversible phenotypic changes in the photosynthetic apparatus that compensate for changes in irradiance. This project focuses on the underlying molecular mechanisms of the photoacclimation. We established that the redox status of the plastoquinone pool is a sensor that affects the expression of nuclear located photosynthetic gene in a eukaryotic green alga, *Dunaliella tertiolecta* (Escoubas, et al., Proc. Nat. Acad. Sci. 92:10237-41). The ongoing research effort is engaged in analyzing individual components involved in the signal transduction pathway that relays the perceived irradiance signals. The short-term research goals are to characterize the key DNA binding factors whose binding activities affect expression of nuclear photosynthetic genes and are themselves regulated by the chloroplast redox poise. We are also investigating the effect of redox modulation in the photosynthetic electron transport chain on the expression of various genes in various photosynthetic organisms that are physiologically and ecologically important, such as nitrate reductase in *Chlamydomonas reinhardtii*, and nitrogenase in a marine diazotrophic cyanobacteria *Trichodesmium* spp. This project has broad implications for understanding how environmental information is transduced to biochemical information in photosynthetic organisms, and how that information is further transmitted to nuclei, ultimately regulating the expression of targeted nuclear genes.

186. Rutgers University

Piscataway, NJ 08854-8020

Corn Storage Protein - A Molecular Genetic Model

Joachim Messing, Waksman Institute

$117,000

Corn is largely used to produce animal protein. Livestock receives its major nutrients from corn and soybean, including essential amino acids. These in turn are derived from proteins in corn and soybean meal that act as storage for amino acids. In corn, these proteins are called zeins, which are encoded by a multigene family. The organization of this gene family in the genome and its regulation by various *trans*- and *cis*-acting mechanisms provide not only an opportunity to reach a deeper understanding of the regulation of gene expression, but also new tools and products for plant genetic engineering. Multigene families are also an example of genetic redundancy that has been far more difficult to tackle from a genetic

and a molecular point of view. We have been able to clone the entire set of the 22-kDa alpha zein genes from BSSS53 due to the construction of BSSS53-specific BAC library. Twenty-two of the twenty-three genes are tandemly arrayed within a 168-kilobase region. They are contained within a 346-kilobase region, representing the largest stretch of sequenced genomic DNA from maize. A single 22-kDa alpha zein gene is located 20 cM closer to the centromere and represents the allele of the *floury-2* locus. This is the first time that we can account for all members of a complex gene family of twenty-three in plants. Sequence information and expression data of single members in normal and *o2* background reveal that transcriptional control of endosperm specific genes has a greater redundancy than previously thought.

187. The Salk Institute for Biological Studies

La Jolla, CA 92037
Signal Transduction Pathways that Regulate CAB Gene Expression
Joanne Chory, Plant Biology Laboratory
$147,500 (15 months)

The major goal of the proposed research is to define the signal(s) and signaling pathways from chloroplasts that regulate nuclear gene transcription. We have used a genetic approach in *Arabidopsis thaliana* to identify mutants that show an accumulation of *CAB* mRNA in the absence of chloroplast gene expression and development. The mutants, called *gun* mutants for *g*enomes *un*coupled, define 5 genes. Recent studies indicate that four of these genes, *gun2, gun3, gun4,* and *gun5* act in the same genetic pathway and are involved in tetrapyrrole metabolism and sensing. Thus, perturbations in the flux through the tetrapyrrole biosynthetic pathway generate a signal from chloroplasts that represses nuclear gene expression. Our current studies are aimed at understanding more precisely what these perturbations are and which molecules are involved in sensing the changes in the tetrapyrrole pathway intermediates. These studies should ultimately influence our abilities to manipulate plant growth and development, and will aid in the understanding of the developmental control of photosynthesis, a central biological process which is the source of energy for all photosynthetic organisms and, via the food chain, for almost all other forms of life.

188. The Salk Institute for Biological Studies

La Jolla, CA 92037
Molecular and Genetic Analysis of Hormone-Regulated Differential Cell Elongation in Arabidopsis
Joseph R. Ecker, Plant Biology Laboratory
$162,600 (17 months)

The hormones ethylene and auxin play an essential role in plant growth and development and in particular are required for differential cell elongation processes, such as epinasty, responses to gravity and the development and maintenance of the hypocotyl hook. The apical hook of etiolated *Arabidopsis* seedlings is an ideal structure to study the processes of hormone-regulated differential cell elongation. *HOOKLESS 1* (*HLS1)*, an ethylene-response gene, is essential for differential growth and for proper regulation of expression of primary auxin response genes, providing a molecular link between two hormone signaling pathways. Ethylene acts through proteins such as HLS, the first known plant member of the N-acetyltransferase family, to control differential cell elongation via an auxin mediated process. RNA expression profiling experiments using oligonuclotide GeneChips have revealed hundreds of genes that are regulated by HLS1 acetyltransferase. Identification and characterization of the substrates responsible for *hls1* phenotypes are an important aim of this work. A genetic screen for second-site suppressors of the *hls1-1* mutant yielded an extragenic suppressor mutation (*hss*) that partially restores the apical hook. Cloning of the *HSS* gene is in progress. Biological functions for three homologs of *HLS1* (*HLH1, HLH2 and HLH3*) are also being investigated by identifying mutations in these genes. Further analysis of *HLS1* and *HOOKLESS1-LIKE* acetyltransferases should provide significant new insight into the roles of ethylene and auxin, two critical plant growth regulators, in the control of cell elongation in all plants, including commercially importance crop species.

189. The Salk Institute for Biological Studies

San Diego, CA 92186-5800
Regulation of the floral homeotic gene AGAMOUS
Detlef Weigel, Plant Biology Laboratory
$102,000

The long-term objective of this proposal is to understand how transcriptional programs underlying organ-specific patterns of plant development are initiated. The experimental system is floral patterning in the model plant *Arabidopsis*, which is particularly suited for genetic and molecular studies because of its short generation time, small genome, and facile DNA-mediated transformation. The specific problem being investigated is how the LEAFY transcription factor interacts with other factors to regulate flower-specific gene expression.

LEAFY, which is the earliest known transcription factor to be specifically activated in floral primordia, is both necessary and sufficient for converting vegetative shoots into flowers. A unique feature of LEAFY compared to animal transcription factors is that it can be exported to adjacent cells, where it can activate direct target genes. One class of direct targets, homeotic genes that control floral organ fate, has been previously identified. These include the *AGAMOUS* gene, which confers organ identity on stamens and carpels, and which is also required to terminate floral growth. In the past year, additional factors that cooperate with LEAFY to activate *AGAMOUS* have been identified. Furthermore, a mechanism by which activity of the mobile LEAFY transcription factor can be limited to the flower has been revealed. This mechanism involves the presence of a direct repressor of *AGAMOUS* in the shoot meristem.

190. The Scripps Research Institute

La Jolla, CA 92037
Membrane Targeting of P-type ATPases in Plant Cells
Jeffrey F. Harper, Department of Cell Biology
$120,000

How membrane proteins are targeted to specific subcellular locations is poorly understood. Our long-term goal is to use P-type ATPases (ion pumps), in a model plant system *Arabidopsis*, as a paradigm to understand how different pumps can be targeted to the plasma membrane, endoplasmic reticulum (ER), or tonoplast. Our first objective is to identify targeting information in calcium pumps located in the ER (AtACA2p; *Arabidopsis thaliana* Autoinhibited Calcium ATPase, isoform 2 protein), tonoplast (AtACA4) and plasma membrane (AtACA8). The approach is to make domain swaps between these closely related pumps and determine their subcellular destinations. The second objective is to test the hypothesis that heterologous transporters can be targeted to the plant plasma membrane by fusing them to a plasma membrane proton pump. Our test case is the targeting of a sodium antiporter from yeast to the plant plasma membrane. The third aim is to use our knowledge of targeting to develop membrane specific reporters to reveal proton and calcium fluxes with subcellular resolution. The approach is to fuse a calcium-sensing or pH-sensing GFP ("cameleons and pHluorins") to calcium and proton pumps located in different membrane systems. These reporters should have wide application for monitoring calcium and pH signals in living plant cells. Together these aims are designed to provide fundamental insights into the biogenesis and function of plant cell membrane systems.

191. The Scripps Research Institute

La Jolla, CA 92037
Nuclear Genes Regulating Translation of Organelles mRNAs
Stephen P. Mayfield, Department of Cell Biology
$100,230

The purpose of this research project is to understand the basic tenants of translation in the chloroplast. Chloroplast genes are regulated primarily at the step of translation, so an understanding of chloroplast

translation is essential to understanding photosynthetic function, a requirement if we are to manipulate plant productivity. Understanding translation in plants has practical applications as well. The expression of recombinant proteins in plants has the potential to become a major source of drugs for use as human therapeutics. Among these recombinant proteins, perhaps none have seen the explosive growth in their utilization, as have monoclonal antibodies (mAbs). Because of their high degree of specificity, mAbs have given researchers new tools in their battle against a variety of maladies including cancers, bacterial infections, and even viruses. The most commonly used systems for the production of humanized mAbs rely on transformed Chinese hamster ovary cells (CHO) grown in suspension cell culture. The production costs of these systems, stemming from high capital costs as well as media and downstream processing costs, make these schemes extremely expensive. Plant expression systems have demonstrated the ability to produce highly complex mAbs, but suffer from the drawback that it can take 1.5 -3 years from the point of initial transformant generation to the actual production of a mAb. What is needed is a system combining the flexibility and speed of mammalian cell culture with the cost benefits of a plant production system. We are developing an efficient system for the production of antibodies in the chloroplast of micro algae. These transformation vectors are based on promoter and RNA elements characterized from our studies on *C. reinhardtii* chloroplast translation.

192. University of South Carolina

Columbia, SC 29208
Regulatory role of ANT in organ initiation and growth
Beth A. Krizek, Department of Biological Sciences
$96,000

Leaves and floral organs are initiated from cells within shoot meristems. While shoot meristems are indeterminate and can continuously grow and produce organs, organ primordia terminate growth after reaching a characteristic size. The manner in which cell proliferation and cell expansion are controlled within developing organs is not well understood. One gene that seems to be involved in both the initiation of organ primordia from meristems and the subsequent growth of these primordia into mature organs is the gene AINTEGUMENTA (ANT). Mutations in ANT lead to a reduction in organ number and the production of smaller organs, while ectopic expression of ANT results in the production of larger floral organs. The increased size of these organs is associated with increased cell size in some organs and increased cell number in others. We have conducted a series of studies to characterize the biochemical role of ANT in organ growth. ANT is a member of the AP2/EREBP family of transcription factors. We have demonstrated that ANT can bind to DNA and that it can function as a transcriptional activator. The domains required for each of these functions have been mapped to different regions of the protein. To further understand how ANT functions in organ initiation and growth, we are using several different approaches to identify target genes that are regulated by ANT.

193. Southern Illinois University

Carbondale, IL 62901-6508
Regulation of Alcohol Fermentation by Escherichia coli
David P. Clark, Department of Microbiology
$105,000

We are studying the regulation of fermentation in the bacterium Escherichia coli. The synthesis of alcohol is due to the AdhE protein, which is encoded by the adhE gene. This gene is expressed in response to the build-up of NADH which occurs in the absence of oxygen. The mechanism of induction of adhE has been analyzed by using gene fusions in which the regulatory region of adhE is joined to the structural gene for beta-galactosidase (lacZ). Induction is due to the AdhR regulatory protein. This binds to the DNA in the regulatory region of the adhE gene, but only in the presence of NADH. It is possible to isolate mutants of adhR that appear to make an altered regulatory protein that activates adhE even in the absence of NADH. Inactivation of the adhR gene by insertion of a kanamycin resistance cassette causes a drop in expression of adhE, lower levels of alcohol dehydrogenase and prevents cells growing by alcohol fermentation. The

synthesis of lactate is due to lactate dehydrogenase, encoded by the ldhA gene. This tends to occur later in fermentation, especially when conditions have become acidic. The mechanism of ldhA induction is also being investigated by using ldhA-lacZ gene fusions. Unlike adhE, the ldhA gene does not respond to NADH and AdhR. Instead, ldhA is under the control of several regulatory genes involved in regulating glucose uptake and sugar metabolism. The ldhA upstream region is presently being dissected by PCR to locate the promoter and regulatory sites.

194. Stanford University

Stanford, CA 94305-5020
R. meliloti-Medicago nodulation genes and signals: genetic and genomic approaches
Sharon R. Long, Department of Biological Sciences
$275,000

Nodulation Genes and Factors in the Rhizobium Legume Symbiosis
S.R. Long, Department of Biological Sciences
We use genetics and molecular biology to study the nitrogen fixing symbiosis between Sinorhizobium meliloti and its legume host, alfalfa. As a result of this symbiosis, the Rhizobium bacterial cells carry out nitrogen fixation; the plant thus can grow and produce protein without requiring nitrogen fertilizer. Because fertilizer manufacture requires intensive fossil fuel use, improvement of biological nitrogen fixation is an important strategy for maintaining productive agriculture as fossil fuels become scarce. We have previously defined and cloned the nodulation (nod) genes in Rhizobium that cause specific host plants to develop root nodules that are the site for bacterial nitrogen fixation. We have shown they act to produce Nod factors able to trigger nodule development in host roots. This year, we have finished defining the DNA sequence of the entire 1.35 million base symbiosis megaplasmid (pSymA) that includes most of the known symbiosis genes, as part of the international consortium to sequence the entire genome of S. meliloti. We have also defined early responses, including ion flux and calcium spiking, that characterize plants responding to bacterial Nod factor signals. We are pursuing the identification of plant genes for symbiosis by means of a mutational study, combined with cellular analysis and genetic positional mapping in the model legume Medicago truncatula. By understanding the genetics of both the bacterium and the plant, we will be able to find means to improve symbiosis and thus to decrease agricultural dependence on energy-intensive nitrogen fertilizers.

195. University of Tennessee

Knoxville, TN 37996
Rubisco Mechanism: Dissection of the Enolization Partial Reaction
Fred C. Hartman, Department of Biochemistry and Cell & Molecular Biology
$200,000 (FY 99 funds – two years)

The plant enzyme rubisco provides the only significant route for net synthesis of carbohydrates from atmospheric carbon dioxide (CO2) and concurrently affords the only biological means of sequestering this predominant greenhouse gas. Despite the essentiality of rubisco to life, the enzyme is very inefficient and thus a logical target for rational redesign. An improved rubisco could lead to more plentiful biomass for food, energy, and mitigation of the greenhouse effect. Our goal is to acquire mechanistic understanding of the inefficiency of rubisco as prerequisite to effecting improvements. The rate-limiting step in rubisco catalysis is enolization of the sugar phosphate (ribulose bisphosphate) which serves as the CO2 acceptor. Oddly, this step is about 1000-fold slower than chemically analogous enolizations as catalyzed by many other enzymes; ongoing work is intended to unravel this disparity. We have identified a key catalytic group (lysine166) of rubisco required for enolization. However, conflicting data have precluded assessment of whether this group functions as a general acid to activate ribulose bisphosphate or as a general base for accepting the proton released during its enolization. To resolve this issue, we have replaced Lys166 with an aminoethylated cysteinyl residue by use of site-directed mutagenesis and subsequent chemical modification. The effect of this subtle structural change is to increase the acidity of the catalytic group by about 10-fold. A comparison of the kinetic parameters of the altered rubisco with the normal form should show whether lysine 166 functions as an acid or as a base.

196. University of Tennessee

Knoxville, TN 37996-0845
Plant recognition of rhizobial Nod factors
Gary Stacey, Department of Microbiology
$90,000

We are studying the agronomically important, nitrogen-fixing symbiosis between the bacterium, *Bradyrhizobium japonicum*, and soybean. *B. japonicum* infects soybean roots and induces the formation of a nodule, a new organ, in which the bacteria reside. Organogenesis of the nodule is induced by substituted lipo-chitin molecules synthesized by the products of the bacterial nodulation genes. The potency of these molecules, as well as their high specificity, suggests the presence of plant receptors. Along with various collaborators, we have isolated cDNA clones encoding for Nod factor binding proteins (i.e., apyrases) from soybean and the model legume, *Medicago truncatula*. Recently published work indicates that genes encoding the apyrases are induced rapidly upon bacterial inoculation. Thus, these proteins can be classified as early nodulins. Our work suggests that legumes may possess more than one receptor for Nod factors. Our hypothesis is that Nod factor receptors may have evolved from a general chitin recognition pathway that exists in most, if not all, plants. Recently, we identified an 85 kDa protein in soybean membranes with the ability to bind chitin with high affinity. This protein is an excellent candidate for a chitin receptor. Plants may possess a chitin response pathway due to the presence of endogenous chitin-like molecules that could be important developmental signals. We have detected a chitinase-sensitive compound in soybean extracts using a bioassay involving the induction of the *B. japonicum nolA* gene. Currently, we are purifying this compound to determine its chemical structure and similarity to chitin. The eventual goal of this work is to elucidate the plant pathways that respond to Nod factors and their role in plant development. Detailed knowledge of legume symbioses is important for the possible extension of biological nitrogen fixation for energy conservation.

197. University of Tennessee

Knoxville, TN 37996-1100
Mechanism of Regulated Protein Transport between Nucleus and Cytoplasm
Albrecht G. von Arnim, Department of Botany
$100,000

Subcellular protein localization is a fundamental regulatory device, which plays a particularly important role in guiding the activity of many regulators of nuclear gene expression in eukaryotes. We are investigating the partitioning of proteins between nucleus and cytoplasm, using as a model system the light regulatory protein CONSTITUTIVE PHOTOMORPHOGENESIS 1 (COP1) of *Arabidopsis thaliana*. COP1 contains separable nuclear and cytoplasmic localization signals. In addition, we have recently delineated a novel subnuclear localization signal within COP1. This domain mediates the association of the COP1 protein with a specific subnuclear compartment of as yet unknown function. These and other cell biological, genetic, and biochemical data are now converging on a structure-function model for the regulated nuclear targeting of COP1. The model suggests that protein-protein contacts between two distinct domains of COP1 play a key role in regulating the activity of its nuclear and cytoplasmic localization signals in response to cues from the light environment. In the course of this work, we have examined the congruence between cell biological assays and genetic ‘in vivo’ evidence concerning the activity of nuclear localization signals; surprisingly, a commonly employed cell biological essay may underestimate the robustness of the nuclear targeting machinery under physiological conditions. Finally, we have provided proof-of-concept for the notion that nearly full protein activity in plants can be obtained by ‘fragment complementation’, i.e. the expression of a multidomain protein in the form of two non-covalently linked gene products.

198. Texas A&M University

College Station, TX 77843-1114
Novel Biomaterials: Genetically Engineered Pores
Hagan P. Bayley, Health Science Center
$160,000 (Jointly funded with the DOE Division of Material Sciences and Engineering)

My laboratory is using genetic engineering and targeted chemical modification to produce functionalized pore-forming proteins. The primary target of our studies has been staphylococcal a-hemolysin, which is a 293 amino-acid, water-soluble polypeptide that self assembles in lipid bilayers to form heptameric transmembrane pores. Recent studies have focussed on making radical modifications to the lumen of the a-hemolysin pore. Engineering the inside of a protein is an unusual venture, but we have been successful in introducing large segments of foreign polypeptide chain within the pore. In additional studies, we have begun to examine single-chain variants of a-hemolysin and related monomeric porins. 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.

199. Texas A&M University

College Station, TX 77843-3258
Regulation of Development and Nitrogen Fixation in Anabaena
James W. Golden, Biology Department
$95,000

The regulation of development and pattern formation in multicellular organisms is a fundamental biological problem. During development cells must collect and integrate external and internal information with a regulatory network that controls gene expression. This regulation is important because the decision to commit some cells to a terminal developmental pathway affects the overall fitness of the organism. The nitrogen-fixing filamentous cyanobacterium Anabaena sp. strain PCC 7120 was chosen as a simple model of microbial development and pattern formation. Anabaena PCC 7120 reduces atmospheric nitrogen to ammonia in highly specialized terminally differentiated cells called heterocysts. When grown on dinitrogen, a one-dimensional developmental pattern of single heterocysts separated by approximately ten vegetative cells is established to form a multicellular organism composed of two interdependent cell types. This is an important model system because of the multicellular growth pattern, the distinct phylogeny of cyanobacteria, the suspected antiquity of heterocyst development, and the contribution of fixed nitrogen to the environment. Several genes involved in heterocyst development have been identified but no integrated regulatory network has yet emerged. The goal of this project is to understand the signaling and regulatory pathways that commit a vegetative cell to terminally differentiate into a nitrogen-fixing heterocyst. Several genes identified by us and by others were chosen as entry points into the regulatory network. These genes will be studied and, as part of our long-term goals, used to identify additional genes in the pathway. Our research is being initially focused on transcriptional regulation by sigma factors and their regulators.

200. Texas A&M University

College Station, TX 77843
Post-transcriptional Components of psbA Expression and D1 Biosynthesis in Synechococcus
Susan Golden, Biology Department
$199,000 (two years)

The goal of this project is to understand the regulated synthesis of the proteins that make up a critical complex that is central to the process of photosynthesis-‹the photosystem II reaction center. The project uses the regulation of a set of genes in a cyanobacterium to address the question of how synthesis of a photosystem II protein is modulated by the cell to provide optimal photosynthetic function as environmental conditions change. The psbA gene family of Synechococcus elongatus PCC 7942 encodes two different forms of the D1 subunit of photosystem II. Production of D1 is affected by changes in light quality or

intensity, both through changes in activity of the psbA genes and in later steps that affect protein synthesis and insertion into the photosynthetic membrane. The specific aims for the research are: (1) to determine whether proteins that bind to the messenger RNA of the psbAI gene are responsible for modulation of the stability of that RNA; and (2) to test the model that a specific region of the messenger RNA is a site involved in targeting the message and the protein it produces to the photosynthetic membrane. If the model is correct, it will provide in the cyanobacterium a simple system for identifying the sequence information that targets photosynthetic proteins to the appropriate membrane.

201. Texas A&M University

College Station, TX 77843-2128
Regulation of Chloroplast Division in Higher Plants
John E. Mullet, Department of Biochemistry/Biophysics
$97,000

The long term goal of this research is to understand how chloroplast division is regulated in higher plants. This is important because the number and size of chloroplasts influences many functions of plants including photosynthesis. In addition, activation of chloroplast division is an early event in the differentiation of mesophyll cells. Therefore, isolation of genes regulating chloroplast division may provide insight into the early steps in chloroplast and mesophyll cell differentiation. The research focuses on a chloroplast division mutant that exhibits a dramatic decrease in chloroplast number and an increase in organelle size specifically in mesophyll cells. The gene identified by this mutation, *CDM1*, has been isolated by map-based cloning. Chloroplasts in mutant mesophyll cell are either very large or relatively small. This type of division phenotype is also observed in some bacterial cell division mutants that are impaired in the ability select the cell midpoint for cell division. This possible parallel between chloroplast and bacterial cell division is being examined in this study. Additional insight into the function of CDM1 will be obtained by characterizing CDM1 localization in cells and by searching for proteins that interact with CDM1, screening for second site suppressor mutants of *cdm1*, and determining the developmental and cell type specific expression of CDM1.

202. Texas A&M University

College Station, TX 77843
Mutants of the Legume Medicago truncatula Defective in Root Hair Development and Infection by Rhizobium
Kathryn A. VandenBosch, co P.I. Douglas Cook
$119,000

Plant growth is often limited by lack of nutrients, including nitrogen that is needed for synthesis of proteins and nucleic acids. Fertilizer application fills this requirement for crops, though legumes, including soybean, beans, alfalfa, and peanut, are able to meet their need for nitrogen through symbiosis with soil bacteria called rhizobia. We are analyzing mutants of the model legume *Medicago truncatula* to identify plant genes required for this symbiosis. Efforts this year have emphasized mutants at three loci that control early interactions with rhizobia. *DMI1* and *DMIi2* are candidates for genes that control the perception of the bacterial signal molecules by the plant. Normal function at the third locus, *PDL,* is required after rhizobia invade the roots so that infections persist to allow nitrogen fixation. Placement of the 3 loci on the *Medicago* genetic map is bringing us closer to cloning these important regulatory genes. Chromosome walking is underway for *DMI1* and *PDL*, involving sequencing of genomic DNA near the genes of interest, and development of more markers. Mapping of *DMI2* has indicated that it is near to a gene of similar function in alfalfa, called *NN1*. Colleagues in Hungary have sequenced the *DMI2* gene and determined that it is defective in the same gene as that found mutated in *nn1*. The affected gene is a likely receptor kinase that is probably needed for perception of signal molecules from the bacteria. Cloning of the genes will enhance understanding of their functions and enable improvement of nutrient utilization in plants.

203. Texas Tech University

Lubbock, TX 79409-3131
The *Dictyostelium discoideum* Cellulose Synthase: Structure/Function Analysis and Identification of Interacting Proteins
Richard L. Blanton, Department of Biological Sciences
$100,000

Cellulose is an important molecule biologically and industrially. It composes the world's most abundant renewable biomass, but we do not understand its synthesis. Cellulose synthase is the protein responsible for the linking of glucose molecules into the cellulose polymer. Other proteins have been identified as participating in the process, but we do not know how they work together. Unraveling the mechanism of cellulose synthesis will require diverse approaches, including the use of model organisms.

Dictyostelium discoideum is a microorganism that alternates during its life cycle between unicellular and multicellular phases. It has been used for decades as a model for exploring fundamental cellular mechanisms. Cellulose synthesis is an activity of multicellular development in *Dictyostelium*. There appears to be a single cellulose synthase gene in *Dictyostelium*, in contrast to higher plants, which have multiple cellulose synthase genes as well as a large group of cellulose-synthase like genes. Therefore, *Dictyostelium* presents the opportunity to explore the fundamental mechanisms of cellulose synthesis in a much less complicated context.

The goal of this project is to contribute to our understanding of the mechanism of cellulose synthesis. It is hoped that what is learned from cellulose synthesis in *Dictyostelium* will provide useful clues for understanding the mechanism of cellulose synthesis in higher plants. The project has two basic strategies: (1) identify proteins that interact with the cellulose synthase as a first step towards determining the cellular context of cellulose synthesis; and (2) to create mutant cellulose synthases to identify critical functional regions of the protein.

204. Texas Tech University

Lubbock, TX 79409-1061
Ferredoxin-Dependent Plant Metabolic Pathways
David B. Knaff, Department of Chemistry and Biochemistry
$199,700 (FY 99 funds – two years)

Thioredoxins contain a single disulfide at the active site of the oxidized proteins. On reduction, the disulfide is converted to two thiols. In plants, thioredoxins *f* and *m* are located in the chloroplasts, where they reductively regulate the activity of several enzymes in the pathway for the photosynthetic conversion of atmospheric CO_2 into organic compounds. The oxidation-reduction (redox) properties of the three regulatory disulfides of sorghum malate dehydrogenase (MDH), an enzyme that is activated by reduced thioredoxin *m*, have been characterized in detail. The effects of mutations that alter the substrate specificity of the enzyme on the redox properties of the enzyme's regulatory disulfides have been examined. Conversion of a bacterial MDH that is not redox regulated, into a redox-sensitive form, has been accomplished by mutating the enzyme so that it contains a redox-active disulfide. A series of mutants of pea thioredoxin *m* with improved ability to activate the chloroplast enzyme fructose-1,6-bisphosphatase (FBPase), an enzyme of the carbon assimilation pathway that is preferentially activated by thioredoxin *f*, have been produced. The redox properties of these thioredoxin *m* mutants and their ability to bind to FBPase have been characterized as part of a study designed to elucidate the binding domains on the two chloroplast thioredoxins for the enzymes that they regulate.

The sequence of the gene encoding the form of the enzyme glutamate synthase found in chloroplasts has been completed and a possible pathway for the evolution of this enzyme has been proposed.

205. University of Texas

Austin, TX 78712
Structural and Functional Analysis of the Cellulose-synthesizing Complex in Vascular Plants
R. Malcolm Brown, Jr., co P.I. Inder M. Saxena, Department of Botany
$100,000

During the past year, we have concentrated on purification of cellulose synthase and associated proteins from mung bean, cotton, *Arabidopsis,* and other vascular plants. The purification of the cellulose synthase associated proteins is given a priority since their isolation and identification will allow for production of antibodies that will be used for the localization of these proteins in the cellulose synthesizing complex by freeze-fracture labeling. For purification of the cellulose synthase, two different protocols are being pursued. The first one allowed partial purification of cellulose synthase activity using a Cibacron Blue 3GA-Agarose column. In this protocol, potassium iodide was used to elute the proteins that were subjected to SDS-PAGE and western blot analysis. The second protocol uses a Heparin Sepharose affinity column followed by passage of active proteins through an anion exchange Q Sepharose column. Glucan synthase activity was measured for various fractions eluted from these columns, and separation of callose synthase and cellulose synthase activities has been accomplished. Presently, it appears that some low molecular weight proteins may be involved in switching between callose synthase and cellulose synthase activity. The expression of the globular region of cellulose synthase from *Arabidopsis* and cotton as chimeric proteins is being carried out in *Acetobacter xylinum*. Chimeric proteins synthesized in *A. xylinum* are being analyzed by using an antibody to the 93-kDa from *A. xylinum* and an antibody to the globular region of cotton cellulose synthase. Membrane fractions obtained from *A. xylinum* expressing the chimeric proteins will be assayed for cellulose synthase activity.

206. University of Texas

Austin, TX 78712-1167
Phosphorylation of Plant Protein Synthesis Initiation Factors
Karen S. Browning, Department of Chemistry and Biochemistry
$96,000

Organisms use a variety of methods to regulate gene expression and function. Among the most common method is the addition or removal of a phosphate group to the hydroxyl group of the amino acids serine, threonine or tyrosine present in proteins. The addition/removal of the phosphate group may have the effect of either increasing or decreasing the function of the protein. The synthesis of proteins in mammals is known to be regulated by phosphorylation of several components of the translational machinery including initiation factors and ribosomes. However, it is not clear that plants use a similar regulatory mechanism. Our research is directed towards determining if phosphorylation is a major mechanism for regulation of plant protein synthesis or if there are alternative pathways for regulation of this vital process in the production of plant proteins. We are developing methods to radiolabel plant cells with [^{32}P] to determine if any of the components of the plant translational machinery are phosphorylated during growth or in response to environmental cues or stress. A better understanding of the regulation of protein synthesis in plants will make it easier to control the expression of desirable proteins in plants under a variety of environmental conditions.

207. University of Texas

Austin, TX 78712
Function of the Arabidopsis TIR1 gene in auxin response
Mark Estelle, Institute for Cellular and Molecular Biology
$95,999

The goal of this project is to determine the mechanism of action of the plant hormone auxin. Previous studies from several labs had shown that auxin treatment results in the rapid induction of gene expression including the transcription of a large family of genes called the Aux/IAA genes. Genetic and molecular

experiments from our lab as well and others, indicates that some members of this family act to repress transcription of downstream auxin-regulated genes. Our latest results have shown that auxin response requires the regulated degradation of members of the Aux/IAA family of proteins by the ubiquitin/proteasome pathway. We have shown that a protein complex called SCFTIR1 specifically interacts with Aux/IAA proteins and mediates their ubiquitination and degradation. The key component of SCFTIR1 is a protein called TIR1. Mutant plants that lack a functional TIR1 gene have a reduced auxin response and transgenic plants with increased levels of TIR1 protein have enhanced auxin response. Finally, we have identified the region on the Aux/IAA proteins that interacts with TIR1. Mutations in this region act to stabilize the Aux/IAA protein and prevent auxin response. In summary, our studies have shown that a network of negative regulatory proteins inhibits auxin response. Upon exposure to auxin, these negative regulators are degraded leading to activation of downstream gene expression.

208. The Institute for Genomic Research

Rockville, MD 20850-3319

Sequencing of Chromosome 10 of Rice and Validation of Annotation Methods for Rice

C. Robin Buell

$300,000 (FY 99 funds – two years)

As part of our DOE-funded project "Sequencing of Chromosome 10 of Rice and Validation of Annotation Methods for Rice", we have submitted ~15 Mb of rice genomic DNA for high throughput sequencing to our sequencing core facility. We have deposited over ~11Mb of sequence to Genbank, the public DNA database that provides access to the entire scientific community. We have identified the genes in over 3 Mb of this sequence and have also deposited this in Genbank. We have characterized repeat sequences in rice and have provided them to the public for their use. We have begun studies to determine the accuracy of our gene prediction programs for rice to further improve the annotation that we perform.

The sequence of the rice genome will serve as an invaluable resource for the public to further the understanding of plant biology. Rice is a model species for the other grasses and we have developed tools to leverage our rice sequence data to other plant species. We have provided alignments of our rice sequence with other plant species including wheat, maize, sorghum, Arabidopsis, soybean, tomato, and potato. We are currently expanding on these alignments to provide the public with an optimal resource for leveraging rice genomic sequence to important agricultural species such as cereal species. These data, along with all of our other data, are available on the TIGR Rice Genome web site at http://www.tigr.org/tdb/rice.

209. Uniformed Services University of the Health Sciences

Bethesda, MD 20814-4799

Acetyl-CoA cleavage and synthesis in methanogens: biochemistry of acetyl and carbonyl group transformations

David A. Grahame, Department of Biochemistry and Molecular Biology

$79,000 (two years)

Methanogens, microorganisms that produce methane, meet their major energy needs by using novel biochemical pathways to direct a large flux of carbon into generating methane. Our studies are centered on understanding the structure and function of a five subunit-containing multienzyme complex (the ACDS complex), which is indispensable for energy production in methanogens metabolizing acetate. Acetate is the environmental precursor of nearly two-thirds of all methane formed by methanogens in Nature, and we have shown that the ACDS complex catalyzes the critical, central reaction needed to degrade acetate, i.e., the cleavage of the acetate carbon-carbon bond. Methanogens synthesize large amounts of ACDS for energy production –as much as 25% of the total soluble protein in the cell under certain conditions. Our recent findings demonstrate that in the process of carbon-carbon bond cleavage a high energy acetyl-enzyme intermediate is formed on the beta subunit of the complex. Our results indicate that subsequent reaction of this intermediate is likely to be the step that sets the limit on the flux of methane

production, therefore controlling the overall maximal rate of energy production. We are employing techniques of biochemistry and molecular genetics to study how this acetyl-enzyme species is formed and reacts. Its relationship to the formation of other enzyme intermediates, carbonyl-enzyme, and methyl-enzyme species is also under investigation.

210. Virginia Polytechnic Institute & State University

Blacksburg, VA 24061-0308
Enzymology of Acetone-Butanol-Isopropanol Formation
Jiann-Shin Chen, Department of Biochemistry
$105,000

Acetone, butanol, and isopropanol are traditional industrial solvents. They are also important starting materials for the chemical industry and ingredients of healthcare and consumer products. In addition, they are potential additives for the transportation fuels to increase the octane number and oxygen content. These chemicals are now manufactured from petroleum, but for over half a century they were produced in the U.S. and elsewhere by a bacterial fermentation. This fermentation employed several species of air-sensitive bacteria belonging to the genus *Clostridium* to convert biomass such as corn mash and molasses into these chemicals. Although solvent fermentation was a successful commercial operation, a number of problems hinder the revival of this industry. These problems include the organism's tendency to lose productivity and our inability to coax the cell into early solvent production or to alter the product ratio for better market value. To help alleviate these problems, we study the enzymes and genes that are crucial to solvent production. We are now characterizing the genes for the different forms of the enzyme alcohol dehydrogenase to determine their relative importance to butanol production. To study the regulation of solvent production, we use the nitrogen-fixation activity to manipulate the availability of reducing power for butanol and isopropanol production. We have cloned and analyzed the sequence of all of the clustered solvent-production and nitrogen-fixation genes in the genome of *Clostridium beijerinckii.* The control mechanisms for solvent production and nitrogen fixation will be the focus of our ensuing research.

211. University of Virginia

Charlottesville, VA 22903-2477
Protein Structure in Catalytic Function of NADPH: Protochlorophyllide Oxidoreductases
Michael P. Timko, Department of Biology
$98,000

NADPH: protochlorophyllide oxidoreductase (EC 1.3.1.33, abbreviated POR) catalyzes the light-dependent reduction of protochlorophyllide (PChlide) to chlorophyllide (Chlide), a critical step in the biosynthesis of chlorophyll in vascular plants, green algae, and cyanobacteria. In etiolated plant tissues, the enzyme, along with its substrate (PChlide) and cofactor (NADPH) forms a stable ternary complex that accumulates to high levels in the prolamellar bodies (PLB) of etioplasts. Upon exposure to light, the enzyme rapidly converts the bound pigment to Chlide, an event concomitant with the dispersal of the PLB and formation of the thylakoid membranes. During the past several years we have learned a considerable amount about how POR expression is regulated during plant development and the role PChlide formation and reduction play in plastid differentiation and overall photomorphogenesis. Our studies have also begun to illuminate the basis for the light-dependency of the POR catalyzed reaction and the details of the reaction mechanism. Using an efficient bacterial (*E. coli*) over-expression system we developed to prepare milligram quantities of wild-type and mutant POR proteins, we have carried out kinetic analysis, chemical modification studies, and begun the preparation of high quality crystals for X-ray diffraction analysis. We have determined the residues/domains within POR required for PChlide and NADPH binding and structural substituents of the protein, pigment, and cofactor required in the assembly of photoactive POR-PChlide-NADPH ternary complexes. We determined the active site residues necessary to stabilize substrate and cofactor in a conformation that permits their specific interaction upon illumination and elucidated the reaction mechanism leading to Chlide formation. Our experiments point to critical roles for Tyr-275, Lys-279, and Cys-281 in the reaction mechanism, as well as additional residues within POR necessary for the formation of the enzyme

photoactive state and required in photoreduction. We have analyzed two-dimensional (2D) crystals of POR grown on lipid monolayers and determined their spatial grouping and structure. We have also begun to define conditions for the growth of 3D crystals in solution suitable quality for X-ray diffraction analysis. Our studies completed thus far have provided significant new information on structure-function relationships in an enzyme mediating one of the most crucial biosynthetic steps in the development of photosynthetic organisms.

212. Washington State University

Pullman, WA 99164-6340
Lipid Signaling and Membrane Function in Mutants of Arabidopsis
John A. Browse, Institute of Biological Chemistry
$112,000

Our investigations of the desaturases involved in the synthesis of polyunsaturated lipids in plants have been expanded by the isolation and cloning of genes that encode desaturases in the model nematode *Caenorhabditis elegans*. This has allowed us to study additional desaturation reactions within this large class of enzymes and provided further tools to alter plant membrane lipid composition. One of the most important roles of polyunsaturated fatty acids in plants is to act as precursors of oxylipins, which are signaling and defense chemicals. Jasmonic acid (JA) and its precursor 12-oxo-phytodienoic acid (OPDA) act as plant growth regulators and mediators of environmental responses. To investigate the role of these oxylipins in anther and pollen development, we characterized a T-DNA-tagged, male sterile mutant of *Arabidopsis*, *opr3*. The *opr3* mutant plants are sterile, but can be rendered fertile by exogenous JA but not by OPDA. Cloning of the mutant locus indicated that it encodes an isozyme 12-oxo-phytodienoate reductase, designated OPR3. All the defects in *opr3* are alleviated by transformation of the mutant with an *OPR3* cDNA. Our results indicate that JA and not OPDA is the signaling molecule that induces and coordinates the elongation of the anther filament, the opening of the stomium at anthesis and the production of viable pollen. Just as importantly, our data demonstrate that OPR3 is the only isoform of OPR capable of reducing the correct stereoisomer of OPDA to produce JA required for male gametophyte development.

213. Washington State University

Pullman, WA 99164-6340
Regulation of Terpene Metabolism
Rodney Croteau, Institute of Biological Chemistry
$114,000

Terpenoid oils, resins and waxes from plants are important renewable resources with a range of industrial uses, as well as pharmaceutical, food and agricultural applications. The long-term goal of this project is to improve terpenoid production in plants by targeting key regulatory, biosynthetic and secretion processes for genetic engineering to increase the yields and expand the types of terpenoid natural products that can be made available for commercial exploitation. The immediate goal of the project is to understand the developmental organization and regulation of terpenoid metabolism in plant secretory organs using monoterpenes (C_{10}) as a model, with specific focus on the biochemical, cellular and molecular characterization of (–)-menthol production in mint. Genes encoding enzymes of the pathway responsible for the supply of terpenoid precursors (the mevalonate-independent pathway), and those encoding the catalysts of monoterpene biosynthesis, have been isolated using a broad range of molecular biological techniques. Studies at the whole plant, tissue, and cell-free level have indicated that terpene yield is largely controlled by biosynthetic capacity that is developmentally regulated at the level of gene expression. Initial genetic engineering experiments to enhance steps of the precursor supply pathway have demonstrated substantial terpenoid yield improvement. Similar transgenetic approaches to suppress specific monoterpene biosynthetic steps have shown the feasibility of altering terpenoid oil composition by this means. Continuation of these studies will provide an important theoretical and experimental base for the rational manipulation of the complex metabolic and secretory pathways responsible for plant terpenoid production and accumulation.

214. Washington State University

Pullman, WA 99164-4234

Functional Analysis of Vegetative Storage Protein Proteolysis in Specialized Leaf Vacuoles

Howard D. Grimes, co P.I. Andreas M. Fischer, Department of Genetics and Cell Biology

$190,000 (two years)

This research focuses on understanding the mechanisms of protein turnover in plant leaves and how this turnover contributes to crop yield by supplying the resulting nutrients to developing soybean seeds. We have established that a novel cell layer, termed the paraveinal mesophyll (PVM) cell layer, is the principal site of both protein storage, lysis in specialized vacuoles, and mobilization to the phloem for long-distance nutrient transport to the seed. In the latest report period (during which we were funded four months), we have initiated research in two directions. First, we have manipulated the growing conditions of soybeans to maximize our control over protein storage (sink regulated) and protein lysis (source regulated). While technically difficult, we have identified conditions that allow reversibility of these physiological states. Thus, protein storage can be facilitated in the PVM cell layer by sink removal and protein lysis can be initiated by allowing developing sink tissues to mature and become source tissues. Second, a complete battery of antibodies and antibody conjugates were prepared to lipoxygenase isoforms and to a common epitope. These antibodies are being analyzed for their ability to quantitatively report the relative rate of specific lipoyxgenase turnover under conditions of storage and lysis using ELISA assays. As these analyses continue, they will provide the foundation for our proposed research identifying the mechanisms of protein turnover in soybean leaves. The ability to manipulate the physiological transitions where proteins are stored *vs* lysed is essential to further work identifying the vacuole-specific proteases involved in this turnover.

215. Washington State University

Pullman, WA 99164-6340

Carbon Metabolism and Dinitrogen Reduction in Symbiotic Nitrogen Fixation

Michael L. Kahn, Institute of Biological Chemistry

$99,000

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 by exchanging photosynthetically derived carbon compounds for nitrogen compounds 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 breakdown of photosynthate. We are investigating the genetics and physiology of symbiotic carbon metabolism in both *Sinorhizobium meliloti*. 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. By investigating *S. meliloti* TCA cycle mutants, including those with defects in citrate synthase (CS), isocitrate dehydrogenase (ICDH) and oxoglutarate dehydrogenase we are probing the relationship between the TCA cycle and nodule development and metabolism. We have recently been able to show that bacteria with temperature-sensitive alleles of CS form effective nodules at low temperature but are rapidly degraded when the temperature is raised. This indicates a role for CS in maintaining nodules in addition to the role we previously discovered in establishing nodules. Other work has shown that an NAD-dependent ICDH will substitute in symbiosis for the NADP-dependent ICDH normally found in *S. meliloti*.

216. Washington State University

Pullman, WA 99164-6340
The Energy Budget for Steady-State Photosynthesis
David M. Kramer, co P.I. Gerald E. Edwards, Institute of Biological Chemistry
$98,000

The photosynthetic apparatus of higher plants and algae is an extremely efficient energy conversion system, and provides the vast majority of energy for powering the planet's ecosystem. It is also the only pathway by which CO_2 (a major greenhouse gas) is taken up from the atmosphere into organic matter (or biomass) and O_2 is released into the atmosphere. Because the photosynthetic reactions involve highly reactive intermediates, plants must continuously balance efficiency with protection against photo-damage. The ability of a plant to regulate photosynthesis in this way is critical to its survival or productivity in a particular environment. The major photo-protective pathways are triggered by light-induced acidification of the chloroplast lumen, and are thus the subject of intense interest. However, our understand of the relationships among photosynthetic energy conversion, photo-protection and downregulatory processes is far from complex. The goal of our research is to delineate the processes by which plants store photosynthetic energy and how these processes are regulated to efficiently meet the needs of the plant while avoiding photodamage. We have developed a series of new techniques to probe the function of specific photosynthetic partial reactions in living plants under steady-state conditions (this work has resulted in three patents). One of our major discoveries is that of a new step in the regulation of photosynthesis, wherein the plant can alter the relationship between photosynthetic energy storage and lumen acidification, thus altering the sensitivity of photo-protection. We have also shown that these same spectroscopic tools can be used to monitor the physiological status of crop plants (because photosynthetic reactions must be regulated to reflect the needs of the plants) and thus should be useful for improving directed farming practices.

217. Washington State University

Pullman, WA 99164-6340
A New Perspective on Phenoxy Radical Coupling Reactions in vivo and Phenylpropanoid Pathway Regulation
Norman G. Lewis, Institute of Biological Chemistry
$109,500

Free-radical processes are often viewed as being uncontrolled and/or degenerative in biological systems, resulting in, for example, degradation of lignocellulosic material or causing the onset of different malignancies. Yet the deployment of free-radical biochemistry was of critical importance to the successful colonization of land by all vascular plants. That is, evolution of free-radical (phenolic) biochemical coupling processes resulted in: formation of the lignins for cell wall reinforcement thereby enabling plants to stand upright and conduct water/nutrients; formation of various layers such as cutins, suberins in bark for protection against the desiccating environment on land, as well as providing a plethora of (poly) lignan substances for plant defense. Thus, the integrity and distinctive qualities of all vascular plants, whether for wood type and performance, or for ornamental purposes, or for edible foodstuffs, depend integrally on these free-radical biochemical systems.

In the current research study, we have discovered and characterized the very first proteins (so-called dirigent, Latin: *dirigere* to guide or align) responsible for controlling the outcome of free-radical coupling processes with monolignols (precursors of lignins and polylignans). These biochemical functions apparently only evolved with the colonization of land, and this control was necessary for the elaboration of all of life's vascular plants, as well as for the species (including humans) that depend upon them. A number of dirigent proteins have been isolated and their physiological functions identified (i.e. we have determined where they are deposited in developing plant tissues, and their modes of coupling). We have established that for a particular coupling mode, the entire plant body is differentially regulated in order to control the outcome of coupling in the different tissues and cell types, i.e. via a so-called multidimensional network. In this way,

plant species can differentially control the color, durability, structural integrity and quality of the various tissues, whether for wood development, edible grains or other tissue forms.

218. Washington State University

Pullman, WA 99164-6340
Enhancement of Photoassimilate Utilization by Manipulation of ADPGlucose Pyrophosphorylase
Thomas Okita, Institute of Biological Chemistry
$98,000

ADP-glucose pyrophosphorylase plays a pivotal role in controlling the flow of carbon into starch in photosynthetic leaves and developing sink organs such as seeds, tubers and fruits. The objective of this project is to control the activity of this enzyme as a means to regulate starch synthesis and, in turn, plant productivity and yields of harvestable organs. To achieve this objective, the structure-function relationships of this enzyme must be understood not only at the amino acid level but also at the physiological level during normal plant growth and development. Using a powerful mutagenesis-bacterial complementation system, we have demonstrated that the two subunits, which constitute the oligomeric enzyme, play different roles in catalysis and allosteric regulation. The large subunit displays only regulatory properties whereas the small subunit has both catalytic and regulatory roles. The allosteric site of the large subunit was mapped by the isolation of mutants showing reduced (down) or enhanced (up) regulatory responses using a reverse genetic approach. The corresponding region of the small subunit was studied by a DNA shuffling approach. In both instances, the allosteric response requires both the N- and C-terminal regions. Several of the up-regulatory large subunit mutant sequences have been transferred into the model plant Arabidopsis and model crop plant rice. These transgenic plants will be evaluated for enhanced leaf starch production during the photoperiod and the impact of the size of this transient sink on photosynthetic capacity, growth and productivity.

219. Washington State University

Pullman, WA 99164-6340
Targeting and Processing of the Thiol Protease Aleurain
John C. Rogers, Institute of Biological Chemistry
$99,000

Our work centers on understanding how a plant cell decides to send a protein in the secretory pathway to a lytic (digestive) vacuole rather than to a storage vacuole. Proaleurain, the precursor form of the cysteine protease aleurain, is recognized and bound by a sorting receptor that is present in the membrane of the Golgi complex. This sorting receptor causes proaleurain and other ligands to be directed into vesicles that carry the receptor plus ligands to a prevacuolar organelle where proaleurain is proteolytically processed to its mature form. The prevacuolar organelle then delivers aleurain to the lytic vacuole. To understand more about how the receptor recognizes ligands, we have expressed the lumenal portion of the prototype of these receptor proteins, pea BP-80, in Drosophila suspension culture cells and purified it to homogeneity. Additional forms of the protein that had been truncated to remove, sequentially, three C-terminal epidermal growth factor repeat units were also expressed and studied. Our results indicate that the N-terminal "unique" domain of the protein contains the binding site responsible for recognizing the conserved Asn-Pro-Ile-Arg (NPIR) motif present in proaleurain and other ligands. The three epidermal growth factor repeats affect the conformation of this unique domain in such a way that allows the ligand binding site to be accessible to ligands. Additionally the presence of the repeat units allows recognition of a broader range of sequences beyond NPIR. Ongoing and future work is directed towards defining the crystal structure of BP-80, and towards experiments with other isoforms of the protein so that we can better understand how numerous different protein ligands are recognized and directed to the lytic vacuole.

220. Washington University

St. Louis, MO 63130-4899
Biogenesis of Photosystems in Synechocystis 6803, a cyanobacterium
Himadri B. Pakrasi, Department of Biology
$199,000 (FY 99 funds – two years)

Photosystems I and II are two light-driven molecular machines responsible for the conversion of light energy to biochemical energy during oxygenic photosynthesis. The principal goal of this project is a careful dissection of the initial steps of biogenesis of PSI and PSII in the widely-studied cyanobacterium *Synechocystis* 6803. In the prokaryotic cyanobacterial cells, the thylakoid membrane is topologically distinct from the plasma membrane. We have developed a two-dimensional separation procedure to purify thylakoid and plasma membranes from this *Synechocystis* 6803. Surprisingly, the plasma membranes contained protein components closely associated with the reaction centers of both photosystems. An exciting finding during the past year was that these proteins in the plasma membrane are assembled in pigment-protein complexes. The activity of CtpA, a carboxyl-terminal processing protease for the D1 reaction center protein of PSII, is essential for the formation of a tetramanganese cluster that is the catalytic center for oxidation of water to molecular oxygen. A critical finding was that the CtpA enzyme is localized exclusively in the plasma membrane fraction. Our data indicate that the plasma membrane, and not the thylakoid membrane, is the site for the initial steps of biogenesis of the photosynthetic reaction centers in cyanobacteria. During this year, we have also developed for the first time, a refined technique to purify plasma membranes with either inside-out or right side-out orientations. Such preparations are currently being used to examine the orientations of various protein components of the partially assembled photosystems in the plasma membrane of *Synechocystis* 6803.

221. University of Washington

Seattle, WA 98195-1750
Genetics in Methylotrophic Bacteria
Mary E. Lidstrom, Department of Chemical Engineering
$106,000

In the future, environmental concerns will mandate that manufacturing processes shift towards the use of renewable resources and the minimization of wastes, especially hazardous wastes. One-carbon compounds are of interest as feedstocks for synthesis of chemicals and materials, because they represent a relatively inexpensive, abundant and renewable resource. In addition, the environmentally-benign characteristics of microbial processes make them of interest as part of a long-term waste-minimization strategy for industry. The concept that methylotrophic bacteria could serve as non-polluting multistage catalysts to generate chemicals and materials using C_1 compounds as feedstocks is a highly attractive one. In order to develop production strains of methylotrophs, it is necessary to understand and manipulate central methylotrophic pathways. One of the most important of these is the methanol oxidation, or Mox system. In this project, we are studying the promoters and transcriptional regulation of this 26-gene system in *Methylobacterium extorquens* AM1, a facultative methanol-utilizer. We have shown that the 14-gene *mxa* cluster is a single cotranscribed operon, with a strong methanol-inducible promoter. Another gene, *mxaW*, is transcribed in the opposite direction, with a low-level methanol-inducible promoter. The transcriptional start sites have been mapped. We are now carrying out a mutational analysis of putative sigma factor genes identified from the genome sequence of this bacterium, to determine if any are involved in Mox transcription. This work is providing the foundation for development of methylotrophic strains to convert methanol into higher value added products.

222. Wisconsin, Medical College of - Milwaukee

Milwaukee, WI 53226
Enzyme Regulation and Catalysis in Carbon Fixation Metabolism
Henry M. Miziorko, Department of Biochemistry
$198,000 (two years)

Microorganisms and plants control carbon assimilation by a variety of mechanisms, including regulation of key enzymatic steps in carbon fixation metabolism. The long term objective of this program is the elucidation of molecular events accounting for activation and catalysis of these regulated reactions. Efforts aimed at such an objective seem quite relevant, given the potential impact on energy (biomass) production. The reaction catalyzed by phosphoribulokinase, an early and irreversible step in Calvin's reductive pentose phosphate pathway, is an important control point in CO2 assimilation. For this reason, phosphoribulokinase is the focal point for the proposed studies.

This project will combine chemical, physical, and molecular biology approaches in an enzymological investigation of purified R. sphaeroides phosphoribulokinase. Molecular biology approaches support production of point mutants useful in identification of active site amino acids and eventual functional assignment of these residues. Functional assignments will also involve the use physical (spectroscopic) techniques to study model complexes of substrate(s) or substrate analog(s) with wild-type enzyme and selected mutants. Functional assignments may also require the use of chemical approaches which involve either direct modification of active site amino acids or the preparation of reactive substrate analogs that modify the active site and allow target identification by protein sequence analysis approaches.

223. University of Wisconsin

Madison, WI 53706-1569
Identification and characterization of Arabidopsis thaliana cell-plate proteins
Sebastian Y. Bednarek, Department of Biochemistry
$77,001

The proper regulation and execution of the assembly of the cell-plate, which mediates plant cell division, is critical for normal plant growth and development. As an initial step toward discerning the dynamic membrane processes involved in cell-plate biogenesis, we have begun to characterize the function of AtCdc48p, an ortholog of the yeast and mammalian AAA family ATPases Cdc48p and p97, respectively. Cdc48p/p97 has been demonstrated to regulate the homotypic fusion of membranes through its interaction with secretory compartment-specific SNAREs. Given that cell-plate membrane assembly appears to involve extensive homotypic membrane fusion we have hypothesized that AtCdc48p would likewise function in its formation. By subcellular fractionation, membrane-associated AtCdc48p was found to cofractionate with the putative ER-to-Golgi intermediate compartment t-SNARE, AtSed5p, and the cell-plate t-SNARE, Knolle. Likewise, AtCdc48p, AtSED5p and Knolle colocalize at the cell-plate in dividing Arabidopsis cells as demonstrated by immunofluorescence microscopy. In vitro however, AtCdc48p bound specifically to AtSed5p but not with Knolle. Preparative affinity chromatography and MALDI-TOF mass spectrometry analysis revealed several novel Arabidopsis proteins, including two UBX domain containing proteins, p52 and p28, that in addition to AtCdc48p bound to AtSed5p. Interestingly, the mammalian protein, p47, which functions as an adapter protein to promote the association of p97 with the t-SNARE, syntaxin 5, contains a UBX domain. Based on this similarity, we hypothesize that p52 and p28 act as cofactors to regulate and specify the target of AtCdc48p and have begun to analyze their function using standard biochemical, morphological and reverse genetic approaches.

224. University of Wisconsin

Madison, WI 53706

Genetic Analysis of Ethylene Perception and Signal Transduction in Arabidopsis
Anthony B. Bleecker, Department of Botany
$102,000

Ethylene is a regulator of growth and development in higher plants There are five isoforms for ethylene receptors in the model plant, *Arabidopsis thaliana*. We have generated mutants of these receptors individually, and in various combinations to try to understand the role that each has in ethylene signalling. From this work, we have determined that two of these, ETR1 and ERS1 are required for ethylene signalling. Plants that are mutated for both of these are stunted and have other, severe, negative characteristics. Another direction my research has taken is to characterize a class of proteins called receptor-like kinases. There are more than 613 members of this gene family in Arabidopsis which we have categorized into 35 subfamilies. We find that all plant receptor-like kinases share a common origin. We have also been characterizing one member of the plant receptor-like kinases, TMK1. Because TMK1 is part of a subfamily that includes three other kinases, we are in the process of making mutants in these other kinases so that we can determine the role that these play in plants.

225. University of Wisconsin

Madison, WI 53706-1567

Molecular Genetics of Ligninase Expression
Daniel Cullen
$111,000

In addition to playing a key role in the carbon cycle, lignin-degrading fungi have demonstrated potential in a number of emerging technologies. These include energy saving biomechanical pulping processes, bleaching and otherwise improving chemical and mechanical pulps, converting lignin to useful chemicals, effluent treatments, and remediation of organopollutant contaminated soils. The mechanism(s) involved in these processes are poorly understood, and this represents a barrier to further development. This research seeks to elucidate the basic genetics and physiology of the model ligninolytic fungus, *Phanerochaete chrysosporium*. Our long term objective is the development of environmentally friendly bioprocesses by exploiting the ligninolytic system of *P. chrysosporium* and related fungi. Toward this end, novel genes and enzymes were discovered during FY2000. Several of these have considerable potential in enzymatic bleaching and modification of fibers. In addition, a genetically altered fungal strain was isolated and shown to greatly simplify genetic analyses and to increase energy savings in biomechanical pulping. The strain has been widely distributed and has become central to large scale genome investigations.

This research furthers our understanding of mechanism(s) involved in the degradation of lignin and related aromatic compounds. In collaboration with other DOE-funded laboratories, these studies are providing insight into gene organization and regulation in lower eukaryotes with particular emphasis on complex gene families. The identification of key genes and production of recombinant enzymes provide the framework for the development of energy efficient bioprocesses.

226. University of Wisconsin

Madison, WI 53706

Microbial Formaldehyde Oxidation
Timothy J. Donohue, Department of Bacteriology
$96,000

Our research seeks to determine how cells generate energy from the oxidation of formaldehyde. Formaldehyde is a toxin, potent mutagen and possible carcinogen that is produced naturally, chemically or by metabolism of a wide variety of methyl-containing compounds. Our immediate goals are to identify how cells sense the presence of this toxic compound and determine how they generate energy and nutrients

from the oxidation of formaldehyde. This research capitalizes on the known roles of the *Rhodobacter sphaeroides* glutathione-dependent formaldehyde dehydrogenase (GSH-FDH) in formaldehyde oxidation under respiratory and photosynthetic growth conditions. This enzyme is part of a formaldehyde oxidation pathway that is apparently found in a wide variety of microbes, plants and animals, so our findings will illustrate what is required for a large variety of cells to metabolize this toxic compound. A second major focus of our research is to determine how cells sense the presence of this toxic compound and control the expression of gene products required for its detoxification. From this work, we expect to develop novel ways in which bacteria could be used to sense formaldehyde in the environment and efficiently remove this toxic compound from industrial or natural sites that routinely contain this to this chemical.

227. University of Wisconsin

Madison, WI 53706-1567
One-electron oxidative mechanisms for lignocellulose decay by fungi
Kenneth E. Hammel, Department of Bacteriology
$170,000 (two years)

Most terrestrial biomass consists of or is derived from dead plants. These recalcitrant lignocellulosic residues have to be recycled to keep the earth's carbon cycle in operation. Certain fungi, some of whose habitats are endangered, are the organisms chiefly responsible for this key step. Despite the critical role these fungi play, we lack an understanding of how they degrade lignocellulose. One longstanding, unproven hypothesis is that decay fungi employ highly reactive oxygen radicals for this purpose. Results from this project confirm that a wide variety of wood decay fungi produce an extracellular oxidant with properties similar to those of the hydroxyl radical, which is probably the most reactive biological oxidant known. Work on one of these fungi, Gloeophyllum trabeum, showed that it produces hydroxyl radicals by secreting hydroquinones that reduce ferric iron. This reaction yields ferrous iron and hydrogen peroxide, two species that react to give hydroxyl radicals, and it oxidizes the hydroquinones to give the corresponding quinones. G. trabeum then returns these quinones to their reduced state by the action of an intracellular quinone reductase. This enzyme has been purified to homogeneity and characterized. Portions of its amino acid sequence have been obtained, and cloning of the gene that encodes it is underway.

228. University of Wisconsin

Madison, WI 53706-1574
Epigenetic Silencing of the Maize r Gene
Jerry L. Kermicle, Laboratory of Genetics
$90,000

The maize *r* gene product activates expression of structural genes in the 3-hydroxy anthocyanin pathway. Different *r* alleles confer pigmentation on different plant parts. In some strains multiple *r* genes are clustered on chromosome *10* forming haplotypes. The level of *r* action is heritably silenced (paramutated), in certain heterozygotes. We want to know how sensitivity of *r* genes to paramutation is organized and whether silencing is facilitated when the inciting and responding genes are in the same rather than in homologous chromosomes. And, what other genes, acting in *trans*, are necessary for paramutation to occur? Sensitive *r* genes typically confer a blotchy distribution of pigment in the kernel's aleurone layer after pollen transmission, but uniform, intense pigmentation after ovule transmission (genomic imprinting). We want to learn what *r* sequences distinguish alleles showing this behavior and to identify genes that regulate expression of this class of alleles. Our overall strategy is to dissect paramutation and imprinting genetically, then characterize selected aspects molecularly.

229. University of Wisconsin

Madison, WI 53706-1544
The Biochemistry, Bioenergetics, and Physiology of the CO-Dependent Growth of Rhodospirillum rubrum
Paul W. Ludden, Department of Biochemistry
$104,000

The photosynthetic bacterium Rhodospirillum rubrum is capable of growth under a wide range of conditions. Among its capabilities is the ability to grow with carbon monoxide as the carbon and energy source anaerobically, in the dark. The key enzyme which allows this process is carbon monoxide dehydrogenase, a nickel-iron-sulfur-containing enzyme. The goals of this project are to understand the metal clusters of this carbon monoxide dehydrogenase (CODH) and to determine how the cell accumulates nickel and builds the nickel-iron-sulfur cluster at the active site of CODH. Genes encoding carbon monoxide dehydrogenase and the nickel processing enzymes have been identified in the Rhodospirillum rubrum genome. The structure of carbon monoxide dehydrogenase from Rhodospirillum rubrum has been determined, and mutant forms of the enzyme with amino acid substitutions at the site of metal cluster ligation have been constructed and are being analyzed to learn about the enzyme. Likewise, mutant forms of the nickel processing enzymes are being investigated to learn about their roles in nickel processing and nickel-iron-sulfur synthesis.

230. University of Wisconsin

Madison, WI 53706-1381
Starch Conversion to Sucrose in Plant Leaves
Thomas Sharkey, Department of Botany
$243,000 (FY 99 funds – two years)

Chloroplasts are the organelles in which sugars are produced during photosynthesis. During the day sugars are exported as triose phosphates or stored as starch. We have shown that altering the balance between triose phosphate export and starch formation can increase plant yield. However, there is an optimum ratio of daytime sugar export to starch formation, and exceeding this optimum depresses yields. To better understand this phenomenon we are working to understand starch mobilization pathways in leaves. At night chloroplastic starch is broken down. We have shown that the nighttime pathway of sugar export from chloroplasts differs from the daytime pathway. Specifically, glucose and maltose appear to be the primary exported sugars at night. Maltose export might lead to increased energy efficiency at night but the reactions involved in converting maltose to sucrose are not yet known. We are using metabolite analyses and nonaqueous fractionation to determine how much maltose is present and where it is located in plant leaves. We are using transgenic plants with various enzymes missing to determine which enzymes are important during starch breakdown. These techniques are also being used to determine the regulatory control over the nighttime carbon pathway. This past year we have made an intense effort at measuring maltose in small plant samples. Most enzymatic methods are not specific for maltose and HPLC methods are subject to coeluting amino acids. We have also started searching for a cytosolic starch phosphorylase knockout *Arabidopsis* plant.

231. University of Wisconsin

Madison, WI 53706-1580
Molecular Mechanism of Energy Transduction By Plant Membrane Proteins
Michael R. Sussman, Director, Biotechnology Center
$114,000

The proton pump (H+-ATPase) is the primary active transporter in the plasma membrane of higher plants and its function is thought to be essential for creating the protonmotive utilized by all other transporters at the surface membrane of plant cells. This enzyme is encoded by a family of a dozen genes (collectively called AHA's, for Arabidopsis H+-ATPase) in the genome of Arabidopsis. My laboratory has been using molecular biology and genetic techniques to understand the precise in planta function played by each of the

pump enzymes encoded by the AHA genes. In particular, we have been using reverse genetic techniques to create 'knockout' mutant plants in which a particular pump gene is misfunctioning. From a collection of such mutants we have recently discovered that homozygous diploid mutants for AHA-3, a phloem specific pump, are embryo lethal, i.e., at least one copy of a functional AHA3 gene is essential for development and/or growth of a live plant embryo. We have also been investigating the structure and function of a gene encoding a plasma membrane transporter that helps the plant accumulate large amounts of sugar in the phloem. This enzyme is thus a major 'consumer' of the protonmotive force generated by AHA3. Using our knockout approach, we have found that embryos and seeds can form without this sugar transporter, but the germinated seedlings cannot grow without additional sucrose. These knockouts provide an excellent resource for obtaining definitive information on the precise in situ physiological functions of membrane proteins in higher plants.

232. University of Wisconsin

Madison, WI 53706-1590

Post-Translational Regulation of Phytochrome Action

Richard Vierstra, Department of Horticulture and the Cellular and Molecular Biology Program

$112,000

Plants use the phytochrome family of red/far-red light photoreversible chromoproteins to optimize photosynthesis and to adapt their growth and development to the ambient light environment. To help understand how phytochromes perceive light and translation this information into biochemical signals, we are investigating how the amount and activity of phytochromes are regulated at the post-transcriptional level. In particular we are studying how the chromophore is synthesized, how the activity of the photoreceptor is modulated, and how the molecule is selectively degraded upon conversion to the biologically active form. Initial studies revealed that that the first step in chromophore biosynthesis requires a heme oxygenase that converts heme into a linear bilin precursor. We have discovered that plants contain multiple heme oxygenase (*HO*) genes and that mutants in specific *HO*s are responsible for some of the well characterized photomorphogenic mutants. Analysis of the protein revealed that phytochrome A contains a domain near the N-terminus that down-regulates its activity. Given that this region is rich in serines, we are investigating whether phosphorylation of one or more of these residues is responsible. Phytochrome A is also subjected to rapid degradation upon photoconversion to Pfr. Previous studies showed that the ubiquitin/26S proteasome proteolytic pathway is involved. Through site-directed mutagenesis and the analysis of phytochrome A/B chimeras, the domain responsible for proteolytic recognition is being defined. Completion of this work will reveal the many steps that control phytochrome assembly and activity that ultimately can be used to manipulate phytochrome action for agricultural benefit.

233. University of Wisconsin

Milwaukee, WI 53211

Anaerobic Fe(III) reduction by Shewanella putrefaciens: Analysis of the electron transport chain

Daad Saffarini, Department of Biological Sciences

$89,994

Iron is an essential nutrient for almost all organisms. Iron containing proteins catalyze a wide variety of reactions such as heme and iron-sulfur proteins which are involved in electron transfer and respiration. Additionally, some bacteria use iron oxides as electron acceptors during anaerobic respiration. The process of iron respiration by bacteria has a major impact on both carbon and metal cycles in aquatic environments. In such environments, organic carbon is oxidized at the expense of iron reduction. Since these bacteria metabolize large amounts of organic carbon, they have obvious potential as agents of bioremediation in these anaerobic environments. In fact, many bacteria have been isolated that can couple the reduction of Fe(III) to the oxidation of hydrocarbons such as benzene and toluene.

The process of metal reduction is poorly understood. The enzymes that are responsible for this process are thought to be on the outer membrane of the cell, in contrast to other respiratory enzymes that are usually

found in the inner membrane. This unusual location is thought to be an advantage for organisms that use highly insoluble iron oxides for respiration. This also poses the question of how these organisms are able to generate energy for locomotion and growth. The proposed work will investigate the location and function of proteins or other components of the electron transport chain that leads to iron reduction. The proposed work will help elucidate the molecular mechanisms of metal reduction, and the information can be applied to enhance microbial bioremediation activities.

234. University of Wyoming

Laramie, WY 82071-3165

Analysis of genes that regulate cell division and expansion patterns during maize leaf morphogenesis

Anne W. Sylvester, Department of Botany

$193,000 (two years)

Cell division is carefully regulated during normal development of all organisms. We have discovered that plants have a unique ability to withstand defects in cell division or cell expansion. Mutations in corn that alter the pattern or timing of cell division can have surprisingly little impact on the overall growth of corn leaves. We have taken a genetic, molecular and cellular approach to understand how normal development is maintained despite obviously derailed cell cycles. Our observations suggest that neighboring plant cells can adjust for sporadic cellular defects, thereby maintaining overall balanced leaf growth. To test this idea, our funded research focuses on a group of genes that we identified by screening for cell pattern mutants. These cell pattern mutants have enlarged or disorganized cells in the otherwise highly ordered corn leaf. Using methods of transposon tagging, we identified that one of these genes likely encodes an important signaling protein, which is similar to proteins required for normal cell growth in other organisms. One of our research objectives is to confirm the identity of this gene and then to discover how this gene and its product function during normal development. Another objective is to characterize newly identified mutants and to investigate their genetic interactions. The results from this research will help to clarify the function of these cell pattern genes and should provide further understanding of how plants are uniquely able to tolerate abnormal cell division and expansion.

235. Xavier University of Louisiana

New Orleans, LA 70125

Molecular Characterization of Bacterial Respiration on Minerals

Robert Blake II, College of Pharmacy

$106,463

Certain chemolithotrophic bacteria inhabit ore-bearing geological formations and obtain all of their energy for growth from oxidation-reduction reactions with insoluble minerals. The purposeful exploitation of these bacterial activities to extract base metals, principally gold and copper, for commercial gain is a growth industry that now exceeds $1 billion/year. The long term objective of this project is to achieve a detailed understanding of respiration on minerals at the molecular level. One goal is to elucidate the molecular principles whereby these bacteria recognize and adhere to their insoluble mineral substrates. The specific, high affinity adhesion of *Thiobacillus ferrooxidans* to pyrite was recently shown to be mediated by the *apo* form of a blue copper protein (rusticyanin) located on the outer surface of the bacterial cell. Efforts are in progress to identify, isolate, and characterize other mineral-specific receptors expressed in different genera of the bacteria that respire on minerals. A second goal is to identify and characterize the redox-active cellular components necessary for respiration on mineral substrates. Each phylogenetically distinct group of chemolithotrophic bacteria expresses one or more unique acid-stable, redox-active biomolecules in conspicuous quantities during respiration on minerals. Current structural and functional studies focus on two acid-stable molecules (a *c*-type cytochrome and an iron-sulfur protein) produced in large amounts by *T. ferrooxidans* as it respires using ferric ion as the electron acceptor. Stopped flow spectrophotometric studies indicate that both redox-active proteins readily transfer electrons to both soluble and insoluble iron. The aim of these studies is to determine the role of each protein in the iron respiratory chain. It is

anticipated that this project will provide useful information toward manipulating *T. ferrooxidans* and related organisms for commercial use.

236. Yale University

New Haven, CT 06520-8114
Essential Cysteine Metabolism in Archaea
Dieter Soll, Department of Molecular Biophysics and Biochemistry
$113,000

Asparagine is one of the twenty canonical amino acids required for translation in all known organisms. It is commonly accepted that this amino acid is formed via the well-understood biosynthetic pathway resulting in the conversion of free aspartate into asparagine.

In *Escherichia coli* and *Bacillus subtilis* this amidation of aspartate catalyzed by two different asparagine synthetase enzymes encoded by *asnA* and *asnB* or related genes. However, our recent genomic, genetic and biochemical analyses with *Deinococcus radiodurans* and an analysis of all known bacterial genomes suggest that most bacteria lack the pathway found in γ-proteobacteria. Instead they use exclusively a transfer RNA-dependent route, the conversion of Asp-$tRNA^{Asn}$ to Asn-$tRNA^{Asn}$ by a novel and still poorly understood enzyme, aspartyl-$tRNA^{Asn}$ amidotransferase. This enzyme is essential in many bacteria and provides Asn-tRNA for protein synthesis. This link between amino acid and protein biosynthesis may be a remnant of an earlier closer connection between intermediary metabolism and macromolecular synthesis.

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