Summaries of FY 2001 Activities

Energy Biosciences

August 2002

ABSTRACTS OF PROJECTS SUPPORTED IN FY 2001

(NOTE: Dollar amounts are for a twelve-month period using FY 2001 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, USDA-ARS and Department of Plant Biology, University of Illinois at Urbana-Champaign
$72,666 (21 months)

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 proton-sucrose symporter that mediates long-distance sucrose transport. Research this year showed that symporter protein and transcripts turn-over with half-lives of about 2 hr and, therefore, sucrose transport activity and phloem loading are directly proportional to symporter transcription. Moreover, we showed that sucrose is a transcriptional regulator of symporter expression. We concluded from those results that sucrose-mediated transcriptional regulation of the sucrose symporter plays a key role in coordinating resource allocation in plants.

2. U. S. Department of Agriculture

Raleigh, NC 27695-7631

Molecular Analysis of the Role of Sucrose Synthase in Sugar Sensing and Assimilate Partitioning
Steven C. Huber, USDA/ARS and Departments of Crop Science and Botany, NCSU
$111,000

The overall goals of the project are to determine the molecular bases for the localization of sucrose synthase (an important enzyme of sucrose utilization) within heterotrophic cells, the role of phosphorylation of specific sites on the enzyme, and to identify the requisite protein kinases that phosphorylate sucrose synthase. It is known that sucrose synthase is phosphorylated on a single major site close to the N-terminus (serine-15). Studies in the past year suggest that phosphorylation of serine-15 affects either the conformation or accessibility of the N-terminus of the protein. This structural change may underlie effects on activity and/or localization. In addition, we have obtained first evidence for phosphorylation of a second residue—serine-170. Both sites appear to be phosphorylated by calcium-dependent protein kinases (CDPKs). Phosphorylation-state specific antibodies that recognize phospho-serine-15 or phospho-serine-170 have been produced to follow the phosphorylation status of these two sites. Results to date suggest that phosphorylation of serine-170 (but not serine-15 as originally thought) might be part of the mechanism that controls the membrane association of sucrose synthase. Identifying the mechanisms that control the intracellular localization of sucrose synthase is important because its localization may control how plant cells use sugars for different processes such as respiration, cell wall synthesis or starch production.

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
$61,968

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
$95,000

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. This research explores the alterations to lignin's structure and properties in a variety of natural mutant and transgenic plants; many are just becoming available as researchers seek to alter the lignin biosynthetic pathway. 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.

Our work is revealing that deprivation of a plant's ability to produce lignin precursors can result in increased incorporation of other plant components (phenolics) into the lignin. Some of the incorporated components were unexpected and not normally associated with the biosynthetic pathway. Others are producing new structures in the lignin that alter the pulping properties, for example. We have identified marker compounds for many of the gene deficiencies that are useful to researchers trying to assess how heavily their plants have been affected. The large compositional shifts that are possible indicates 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.

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)

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 (FY00 funds - two years)

Our long-term goal is to understand how photosynthetic organisms 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 made mutations of amino acids in the close vicinity of the phylloquinones, targeting several amino acids that were most likely to be involved. Our identification of a specific tryptophan residue as an important part of the quinone site was recently confirmed by the high-resolution PS1 crystal structure. We have built upon this by making new mutations of residues now known to be near the quinones. The effect of the tryptophan mutations upon electron transfer kinetics was striking: a 3-5 fold decrease in the rate of electron transport from the nearby quinone to the next cofactor in the chain. This effect has also been extended to two glutamate residues; their conversion to glutamine slowed the reoxidation of the respective nearby quinone. These results have allowed us to make the unexpected conclusion that both branches of cofactors are active. Furthermore, although the environments of the two phylloquinones are very similar , they must be sufficiently different to produce an order of magnitude difference in the rate of electron transport from them. Several new mutations have been made in an attempt to discover the nature of these differences.

7. Arizona State University

Tempe, AZ 85287-1604

Structure, Function and Reconstitution of Antenna Complexes of Green Photosynthetic Bacteria
Robert E. Blankenship, Department of Chemistry and Biochemistry
$246,000 (two years)

All photosynthetic organisms contain chlorophyll pigments that function as an antenna, absorbing light and transferring excitations to a photochemical reaction center where energy storage takes place by a series of chemical reactions. The green photosynthetic bacteria are characterized by large antenna complexes known as chlorosomes, 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

Regulation of Chlorophyll a and b Biosynthesis

Willem F.J. Vermaas, Department of Plant Biology

$120,000

The tetrapyrrole biosynthesis pathway leads to the formation of cofactors such as heme and chlorophylls. This pathway is tightly regulated because several products and intermediates are toxic in the light in the presence of oxygen due to formation of singlet oxygen and superoxide. In photosynthetic organisms, chlorophyll is the most abundant product of the tetrapyrrole biosynthesis pathway. Using deletion mutagenesis in the cyanobacterium *Synechocystis* sp. PCC 6803, we have observed that small proteins resembling parts of LHCII are involved with the regulation of early stages of the heme/chlorophyll biosynthesis pathway. These proteins appear to bind chlorophyll, and may be sensors for the amount of chlorophyll available in the membrane, upregulating the early steps of the tetrapyrrole biosynthesis pathway if the chlorophyll supply is insufficient. We have further modulated chlorophyll biosynthesis in *Synechocystis* by introduction of higher-plant *cao* and *lhcb* genes, coding for chlorophyll *a* oxygenase and LHCII, respectively. This mutant incorporates chlorophyll *b* into photosystem II, replacing chlorophyll *a* at more than half of the chlorophyll-binding sites; note that in all natural systems chlorophyll *b* occurs in the antenna only, and is absent from photosystems. Even in the reaction center complex itself at least one chlorophyll *a* has been replaced by chlorophyll *b*, slowing down primary charge separation in the reaction center. However, energy transfer characteristics are rather normal. This molecular-genetic approach allows an in vivo replacement of pigments, broadening the opportunities for modulating the reaction center and antenna pigment composition in photosynthetic systems.

9. Arizona State University

Tempe, AZ 85287-1601

An Integrative Approach to Energy Carbon and Redox Metabolism in the Cyanobacterium Synechocystis sp. PCC 6803

Willem F.J. Vermaas and Robert W. Roberson, Department of Plant Biology; in collaboration with Kym F. Faull (University of California, Los Angeles)

$353,003

The goal of this multidisciplinary and multi-institutional Microbial Cell project is to help provide integrated insight into subcellular structures, protein components, and fundamental metabolism involving photosynthesis, respiration, and other energy-related and redox processes in the cyanobacterium *Synechocystis* sp. PCC 6803. This goal will be achieved by using wild-type and mutant cells of this organism to analyze changes in proteome composition, metabolic function, metabolite levels, localization/abundance of specific proteins, and cell structure upon genetic deletion of specific processes or upon changes in environmental conditions. A large number of targeted deletion and site-directed mutants altered in particular open reading frames or with newly introduced genes related to photosynthesis and respiration have been created. Striking ultrastructural differences between wild type and mutants already have been observed (for example, a mutant lacking the terminal oxidases has a greatly increased number of polyhydroxybutyrate inclusions, suggesting PHB to be a main fermentative product in this cyanobacterium). Moreover, detailed proteomic and metabolic analysis has been initiated. On the basis of the multidisciplinary information gathered in the project, we aim at developing a comprehensive metabolic model of *Synechocystis* sp. PCC 6803, focusing on photosynthesis, respiration, and related processes.

10. 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

$100,000

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 90 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. Using selective excitation of the reaction center chlorophylls with spectrally narrow laser pulses we have identified strong excitonic interaction between the six chlorophylls of the electron transfer chain. This interaction considerably broadens the initial absorption spectra and may be important for providing spectral overlap with antenna pigments, thus increasing energy transfer efficiency. In mutants where the connecting chlorophylls have been modified, we find impaired excitation energy trapping consistent with their role in excitation energy transfer. We also detect a novel 1-2 ps energy transfer event at 10 K that may represent fast initial electron transfer to the primary acceptor.

11. University of Arizona

Tucson, AZ 85721-0088

Restructuring Metabolism for Photosynthesis Protection

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

$103,000 (FY 00 funds)

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 T_0 and T_1 generations but in later generations and increasing number of genes, apparently randomly, appears to be silenced. Analyses are ongoing.

12. University of Arizona

Tucson, AZ 85721-0036

Role of Root Tip Polysaccharide Solubilizing Enzymes in Root Development Structure and Function

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

$102,000

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.

13. University of Arizona

Tucson, AZ 85721-0036

Systemic RNA silencing paramutation and epigenetic control of gene expression patterns
Richard A. Jorgensen, Department of Plant Sciences
$96,000

RNA silencing is a sequence-specific RNA degradation process directed by short double-stranded RNA (dsRNA) molecules, known as short, interfering RNA (siRNA). siRNA molecules are products of enzymatic cleavage of longer dsRNA molecules; thus, any process producing dsRNA molecules may lead to siRNA-directed silencing of an homologous gene. Plants encode an RNA dependent RNA polymerase (RdRP) that can produce complementary RNA from mRNA molecules, potentially resulting in dsRNA and RNA silencing. In petunia, chalcone synthase transgenes appear to trigger RNA silencing by this pathway when present as a single copy. We have previously shown that this depends on two properties of the transgene: 1) the transgene must be transcribed by a strong promoter and 2) the transgene must be translated fully, as evidenced by the fact that premature nonsense codons block RNA silencing by single copy transgenes. The latter suggests that RdRP-triggered RNA silencing may be associated with the translation process, and/or that a high level of mRNA (i.e., the mRNA is not degraded by the process of nonsense-mediated decay) must accumulate in order for the RdRP to use it as a template to make a copy RNA. Using RNA sequences and a protein obtained from brome mosaic virus, we are attempting to distinguish between these possibilities. The BMV protein 1a is capable of stabilizing RNA molecules containing a short RNA sequence from the BMV genome and reducing its translation. This rare property creates a unique tool with which to investigate the mechanism of induction of RNA silencing by mRNA molecules.

14. 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 (FY 00 funds)

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 zein-containing 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 distribution of zein mRNAs on endosperm rough endoplasmic reticulum (RER) membranes and the interactions between zein proteins, as each of these could influence the structure of protein bodies. Based on *in situ* hybridization, mRNAs encoding the 22-kD α- and 27-kD γ-zeins are randomly distributed on RER; hence, mRNA targeting does not appear to influence the formation of protein bodies. Investigation of the interactions between zein proteins (α, β, γ-, δ) with the yeast two-hybrid system showed that interactions between the 19- and 22-α-zeins are relatively weak, although each of them interacted strongly with the 10-kD δ-zein. Strong interactions were detected between the α- and δ-zeins and the 16-kD γ- and 15-kD β-zeins; however, the 50-kD and 27-kD γ-zeins did not interact detectably with the α- and δ-zein proteins. The NH_2- and COOH-terminal domains of the 22-kD α-zein were found to interact most strongly with the 15-kD β- and 16-kD γ-zeins, suggesting the 16-kD and 15-kD proteins bind and assemble α-zeins in protein bodies. Additional evidence supporting this hypothesis was obtained by showing that the starchy endosperm mutant, *Mucuronate*, appears to result from a defective 16-kD γ-zein protein.

15. University of Arizona

Tucson, AZ 85721-0036
Regulation of DNA Endoreduplication in Maize Endosperm
Brian Larkins, Department of Plant Sciences
$121,000 – six months

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. 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-dependent mechanism. By expressing a mutant Cdc2 gene in maize endosperm, we significantly reduced the degree of endoreduplication. Experiments are in progress to characterize development of these mutant kernels and the role of genes regulating the activity of the S-phase kinase in the endosperm.

16. 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
$94,000

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 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 the mosses *Physcomitrella patens* and *Funaria hygrometrica* to establish a role for calcium in hormone-induced morphogenesis and to identify transporters responsible for increasing cytosolic calcium levels during this process. Using 1,4-dihydropyridines (DHPs), molecules that block calcium movement through voltage-dependent channels in animal cells, we have shown that calcium is important early in the transition from filamentous to meristematic-like growth that occurs in response to the plant hormone cytokinin. 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 currently using tandem mass spectrometry of the partially purified DHP-binding proteins to identify the putative calcium channel and provide sequence information. Our ultimate goal is to understand channel expression, regulation, structure, and function during development. In addition, we are using random insertional mutagenesis to identify other components of the mechanism underlying this developmental process. We are currently characterizing two mutants, both of which remain primarily filamentous. One mutant is impaired in a cell differentiation process, while the other is unable to respond to exogenous cytokinin.

17. University of Arizona

Tucson, AZ 85721-0036
Manipulation of Phytoalexin Biosynthesis: Effects on Plant-Microbe Interactions
Hans D. VanEtten, Department of Plant Pathology
$101,000

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.

18. 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.

19. University of Arkansas

Fayetteville, AR 72701

Protein Targeting to the Chloroplast Thylakoid Membrane: Structure and Function of a Targeting Complex
Ralph L. Henry, Department of Biological Sciences
$95,002

Chloroplast thylakoid membranes are the site of photosynthetic electron transport and therefore represent the primary energy generating membranes in plants and algae. Consistent with this function, nearly 50% of the chlorophyll in mature thylakoids is associated with light harvesting chlorophyll a/b-binding proteins, the LHCs. We are investigating the mechanism by which these chlorophyll-binding proteins are targeted and assembled in the thylakoid. Understanding of this process may ultimately lead to the ability to manufacture artificial membranes that capture light energy for energy generation/conversion. Because LHCs are targeted and integrated into thylakoids by protein machinery similar to that found in bacteria, we also anticipate that our study of targeting and assembly mechanisms will lead to findings that are broadly applicable to understanding how bacterial pathogens facilitate the disease state in animals and humans.

In this context, we are initiating studies to examine the structure and function of cpSRP, a protein that binds and targets LHCs to the thylakoid. By identify the functional regions of cpSRP and coupling this with high-resolution crystallographic (structural) data of these protein regions, we hope to understand how cpSRP holds LHCs in a form suitable for integration and assembly into thylakoids.

20. Boston College

Chestnut Hill, MA 02467
Osmoregulation in Methanogens
Mary F. Roberts, Department of Chemistry
$100,000

This general goal of our research is to understand all the different facets of the osmotic response in these methanogens During the past year, our studies concentrated on *Methanococcus jannaschii*. Using NMR methods, we monitored the time-dependent response of intracellular organic osmolytes □- and □-glutamate to altered NaCl. Protein synthesis after osmotic shock was also examined using ^{35}S-uptake and SDS-PAGE analyses. As observed previously for *M. thermolithotrophicus*, there are a few rapidly labeled bands suggestive of molecular chaperones. The osmolytes used by methanogens (and archaea more generally) are often different from those of other organisms (e.g., in archaea there is a predominance of anionic solutes, □-amino acids, charged carbohydrates, unusual phosphodiesters, etc.). To understand why such different molecules are used in archaea, we have started to evaluate how archaeal osmolytes affect stability and dynamics of the same enzyme derived from archaeal and bacterial (or eukaryotic) sources. The thermal stabilities of recombinant glutamine synthetase, aspartate transcarbamoylase, and protein isoaspartyl methyltransferase from *M. jannaschii* have been examined using CD techniques and compared with similar activities from nonarchaeal organisms. Differences in the pattern of solutes that act as thermoprotectants correlate somewhat loosely with the solutes accumulated by the particular organism (e.g., K^+-glutamates are often the most effective thermoprotectants for the archaeal proteins). The last area examined during the past year has been a more detailed structural analysis of the MJ0109 gene product – a dual activity inositol monophosphatase / fructose bisphosphatase that potentially links gluconeogenesis with response to osmotic stress.

21. Boyce Thompson Institute for Plant Research

Ithaca, NY 14853
Post-transcriptional Gene Regulation in Chloroplasts
David B. Stern, Plant Molecular Biology Program
$191,000 (two years)

The chloroplast is the site of photosynthesis, an indispensable process which captures light to support life. The chloroplast arose through the colonization of an ancient nonphotosynthetic organism, and in derives most of its ~3,000 proteins from nuclear genes. Chloroplasts also retain about 100 genes, however, and their expression is also essential for photosynthesis. This project is geared towards elucidating two mechanisms by which the nucleus regulates chloroplast gene expression. The first concerns the function of a nucleus encoded chloroplast protein, CSP41, which is thought to be involved in RNA processing or degradation, and is related to an ancient class of proteins called nucleotide-sugar epimerases. The second revolves around the regulation of protein synthesis in chloroplasts. Most proteins involved in photosynthesis assemble into large complexes, and the synthesis of the different components is often coordinately regulated.

In the CSP41 project, transgenic tobacco plants are being used to determine the consequences of increased or decreased expression. To increase expression, the gene has been moved from the nucleus to the chloroplast, where it appears to have important consequences for plant development. Overexpression of proteins in chloroplasts is important in general, since it is an increasingly popular way to confer new functions on plants which cannot be transmitted through pollen. Decreased expression has been achieved by an “antisense” approach, and yields altered patterns of chloroplast RNA degradation. The protein synthesis project is testing sensing mechanisms for coordinate synthesis of complexes, and whether bacterial ribosome binding sites are functionally conserved in chloroplasts.

22. Brookhaven National Laboratory

Upton, NY 11973
Molecular Plant Genetics
Benjamin Burr and Frances Burr, Biology Department
$340,000

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.

23. Brookhaven National Laboratory

Upton, NY 11973
Regulation of Energy Conversion in Photosynthesis
Geoffrey Hind, Biology Department
$314,000

This project addresses DOE's strategic goal of securing the nation's energy supply. Understanding how land and marine plant life responds to environmental stress will suggest ways to maximize biofuel production and carbon sequestration using marginal lands and the oceans We will elucidate molecular mechanisms that protect photosynthetic tissues from stress-related damage in these environments.

Protein kinases protect against stress-induced photoinhibition by regulating excitation energy distribution between the photosystems. Sequence analysis reveals ten candidate precursors for thylakoid kinases in the Arabidopsis genome; biochemical and immunological studies will begin to characterize these, and their individual substrates. The phosphorylation substrate PsbZ will be investigated as a putative linchpin between photosystem II and light-harvesting complexes. Substrate light-harvesting complexes will be examined by scanning transmission electron microscopy to discover the role of protein phosphorylation and specific membrane lipids in their light/dark reversible monomer/trimer transition.

Mineral nutrient (Fe,P,N,Si) deficiencies are stress factors for oceanic microalgae, potentially leading to photoinhibition. The study of surface proteins in microalgae by the recently developed technique of whole-cell mass spectrometry is a taxonomic tool that also can reveal biomarkers for stress, and information on novel biochemical reactions at the cell surface. These processes, and proteins that are preferentially expressed under nitrogen, iron or silicon deficiency will be investigated by serial analysis of gene expression, in view of the planned sequencing of a diatom genome by DOE's Joint Genome Institute. These analyses will identify genes significant to understanding diatom bloom dynamics and to discovering mechanisms for the nanofabrication of diatom silica structures.

24. Brookhaven National Laboratory

Upton, NY 11973
Modification of Plant Lipids
John Shanklin, Biology Department
$386,000

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.

25. Brown University

Providence, RI 02912

The Magnesium Branch of the Chlorophyll Biosynthetic Pathway
Samuel I. Beale, Division of Biology and Medicine
$114,000 (FY 00 funds)

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.

26. 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
$147,000

Plants decide when it is time to flower based on internal cues, such as age, and external cues, such as temperature and day length. In the laboratory plant *Arabidopsis thaliana*, the earliest known result of the decision to flower is upregulation of the transcription factor LEAFY; after upregulation LEAFY acts to maintain its elevated expression and to activate the genes that control flower development. One of the direct LEAFY targets is the gene *APETALA1*. The other targets, and cofactors that act along with LEAFY, are generally not known. In the past year we have completed experiments that have revealed one of the cofactors, which is a chromatin remodeling ATPase. The implication of the finding is that some of the environmental factors that lead to flowering may act by causing changes in chromatin conformation, thereby allowing low levels of LEAFY to activate flower development. Higher levels (such as are seen in older plants) may be required for flowering when environmental conditions are inappropriate. We have also performed studies to find the genes activated after LEAFY upregulation, taking time points after LEAFY activation in shoot-forming callus and assessing the nature of the newly upregulated RNAs. This has allowed us to start cataloguing the genes that respond to LEAFY, that is, the genes that lead eventually to formation of flowers. One practical result of this work should be the ability to better control flowering in crop plants, which will suit them better to their latitude and to growth conditions.

27. 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 (FY 00 funds)

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.

28. 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)

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.

29. University of California

Berkeley, CA 94720

A Chloroplast Immunophilin and Its Targets in the Electron Transport Chain
Sheng Luan, Department of Plant and Microbial Biology
$101,500

Immunophilins are defined as receptors for immunosuppressive drugs including cyclosporin A, FK506, and rapamycin. These receptor proteins not only mediate the drug action in immunosuppression but also play an important role in protein folding and other cellular processes. We have identified an immunophilin family from Arabidopsis. In particular, two of the family members are localized in the chloroplast. One of them is AtFKBP13 that is targeted to the thylakoid lumen. Interestingly, AtFKBP13 specifically interacts with two putative components of photosynthetic electron transfer chain. A major objective of this proposal is to determine the function of FKBP13 and its target proteins in photosynthetic electron transport using both biochemical and genetic approaches. Finding interaction between AtFKBP13 and electron transport components has connected immunophilin function with photosynthesis. A key question to understand this connection is to demonstrate a functional relevance of AtFKBP13 interaction with its targets, which is what we propose to do in the second objective. The third objective is to further dissect the molecular basis for AtFKBP13 interaction with its target proteins.

This project provides a unique opportunity to study immunophilin function in photosynthesis. In particular, AtFKBP13 may serve as a novel regulator of photosynthetic electron transfer chain. Confirmation of this hypothesis will contribute significantly to the understanding of energy transfer process in the chloroplast.

30. University of California

Berkeley, CA 94720

Phytochrome from Green Plants: Properties and Biological Function
Peter H. Quail, Department of Plant and Microbial Biology
$109,000

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 phyC using Arabidopsis as a model system. Previously, we showed that photoactivated phyB binds to a basic-helix-loop-helix transcription factor, PIF3, bound in turn to a DNA-element found in the promoters of various light-regulated genes. Using deletion and point mutations, we have now mapped domains within the phyB and PIF3 molecules important for this protein-protein interaction. These include the amino- and carboxy-termini of the photoreceptor, and a PAS-like domain within PIF3. We have also determined that phyB binds stoichiometrically to PIF3 at an equimolar ratio, suggesting that this complex is the unit active in transcriptional regulation at target promoters. For the purpose of investigating sequence, structural and expression pattern conservation among *PHY* genes, we have recently cloned the *PHYC* gene from rice, the first such gene from a monocot.

31. 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
$115,000

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.

32. University of California

Davis, CA 95616

The Phosphate Starvation Response Pathway in Arabidopsis thaliana
Steffen Abel, Department of Vegetable Crops
$90,000

Plants 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 nucleolytic enzymes 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 previously

isolated 22 conditional mutants that show reduced growth on medium containing DNA as the only source of phosphorus, but which recover in high Pi. Characterization of 9 lines demonstrated their inability to utilize either DNA or RNA, suggesting that these plants are defective in Pi signaling. During the past year, we have focused on a detailed genetic and molecular characterization of two most interesting mutants, termed *pdr1* and *pdr2* (*phosphate deficiency response*), which appear to affect components of separate Pi signaling pathways. The recessive *pdr1* mutation disrupts induction of various Pi-starvation inducible genes and causes typical phenotypes characteristic of Pi-deprived plants in low Pi conditions. A second recessive mutation, *pdr2*, does not affect Pi-responsive gene expression but inhibits cell division in primary root tips and subsequent root growth. Interestingly, unlike in wild-type plants, cell cycle arrest in primary roots of *pdr2* is only observed in low Pi concentration and not in Pi-sufficient conditions. Both *pdr* mutations have been mapped, and their positional cloning is underway. The *PDR1* gene is expected to function upstream of Pi-starvation inducible gene expression, whereas the *PDR2* gene is proposed to be necessary for Pi-responsive cell division during the adaptation of root system architecture to Pi limitation.

33. University of California

Davis, CA 95616

Developmental Genetics of Nectaries in Arabidopsis and Gossypium
John L. Bowman, Section of Plant Biology
$102,000

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 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.

34. 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
$100,000

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.

35. University of California

Davis, CA 95616-8535

Nodulation genes of Medicago truncatula

Douglas Cook, Department of Plant Pathology; in collaboration with Kathryn A. VandenBosch (University of Minnesota)

$121,000 (two years)

Nodulation mutants of *Medicago truncatula* that are defective in infection by the nitrogen-fixing bacterium *Sinorhizobium meliloti* are the topic of this renewal proposal. We have investigated the inheritance of mutations at 3 loci, and the phenotypes of *dmi1* and *dmi2* have been extensively characterized. Both mutants respond briefly to rhizobia through loss of polar growth of root hairs, a phenotype that is mimicked by application of the actin depolymerization drug cytochalasin. Downstream responses to *S. meliloti* do not occur, including root hair curling and infection, cell cycle activation in the root cortex, and induction of nodulin gene expression. The mutant phenotypes indicate that the affected genes may function in signal transduction. Positional cloning of the *dmi1* locus is underway. We have identified two tightly linked markers and have begun chromosome walking to this gene. We will also initiate cloning of the other two loci by using the same map-based approaches. Secondly, we will identify genes whose expression is dependent upon *DMI1*, *DMI2*, and *NSP* by employing microarray analysis of cDNA clones identified through an independent genomics project. Expression analysis will emphasize genes likely to function in signal transduction and cytoskeletal control of cell architecture. Genes whose action is inferred to reside downstream of the mutant genes will provide targets for more extensive analysis of function. Thus, the research is part of a larger, long term strategy for defining the molecular, genetic and cell biological control points for symbiotic interactions.

36. University of California

Davis, CA 95616

The Mechanism and Regulation of Cellulose Syntheses in Plants

Deborah P. Delmer, Section of Plant Biology

$120,000

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.

37. University of California

Davis, CA 95616-8535

Structure, Function and Assembly of the Clostridium cellulovorans Cellulosome
Roy H. Doi, Section of Molecular & Cellular Biology
$124,000

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. We are currently taking several approaches for analyzing the cellulosome. We have been able to demonstrate by cloning and expressing the genes that code for cellulosomal subunits that the cellulosome can degrade not only cellulose, but also xylan, mannan, and pectin, and that the cellulosome is an efficient plant cell wall degrading enzyme complex. We have also shown that the composition of the subunits of the cellulosome is regulated by the carbon substrate on which C. cellulovorans is grown. For instance xylan grown cells had much higher xylanase activity. In order to construct designer cellulosomes with more efficient activity, we have expressed the engB gene in B. subtilis and obtained complete EngB and have been able to make a mini-cellulosome comprised of mini-CbpA and EngB in vitro. We have shown that EngE, which contains three tandem surface layer homology (SLH) domains at its N-terminus, can bind to the cell surface and to the CbpA thus linking the cellulosome complex to the cell surface and to the cellulose substrate. These studies are leading to a much better understanding of cellulosome function.

38. University of California

Davis, CA 95616-8537

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

Many cell types of a higher plant can be induced to undergo embryogenesis. Although much is known about the treatments needed to induce embryo formation, the mechanisms by which cells are reprogrammed to undergo embryogenesis remain to be determined. To address this problem, we have been studying Arabidopsis *LEAFY COTYLEDON1* (*LEC1*). *LEC1* is required to specify embryonic organ identity and to initiate and/or maintain embryo maturation. Ectopic *LEC1* expression induces somatic embryo formation from vegetative cells, suggesting that LEC1 is sufficient to establish a cellular environment that promotes embryo development.

A major objective is to define how LEC1 promotes embryo formation at a mechanistic level. *LEC1* encodes one of a family of Arabidopsis HAP3 subunits of the CCAAT transcription factor. Functional analyses showed that LEC1 and the HAP subunit most closely related, LEC1-LIKE (L1L), are sufficient to induce embryonic environments in vegetative cells and to suppress the *lec1* mutation. Other HAP3 subunits do not have these functions. We identified specific amino acid residues that are shared between LEC1 and L1L but differ from conserved residues found in all other HAP3 subunits. Site-directed mutagenesis experiments have shown that some of these residues are required for the ability of LEC1 and L1L to induce embryonic environments. Thurs, we have defined specific amino acid residues that underlie LEC1 function. This information enables other experiments to define other proteins that interact with LEC1 in the CCAAT binding transcription factor.

39. University of California

Davis, CA 95616-8537

Cytoskeletal Organization in Cotton Fiber Growth: Roles of Microtubule-Based Motor Enzymes
Bo Liu, Section of Plant Biology
$90,000

Plant cell growth involves cytoskeletal elements of microtubules and actin filaments. The cotton fiber provides an excellent model for studies of cell growth. Microtubule-based motor enzymes play roles in

dynamic rearrangements of microtubules and actin filaments, which are essential for cell growth. These motors render motile activity upon hydrolyzing ATP. We have identified two novel motors expressed in the cotton fiber. They are kinesin-like calmodulin-binding protein (KCBP) which renders motor activity in a Ca^{++}/calmodulin-dependent manner, and kinesin-related protein 1 (KRP1) which contains a calponin homology (CH) domain, which is often found among actin-binding proteins. The proposed studies focus on elucidating roles of these two motors by testing two hypotheses: KCBP involves in the reorganization of microtubules, and KRP1 involves in reorganization of actin filaments following the microtubule pattern. Experiments will be carried out to test whether KCBP directly interacts with the microtubule organizer protein γ-tubulin, and to examine whether ectopic expression of KCBP in living cells will affect microtubule organization. Because KRP1 contains a CH domain, we will test whether it binds to actin. The function of KRP1 on actin reorganization will be tested in living cells upon ectopic expression of this novel protein. Finally, subcellular localizations of these two motor proteins will be examined in cotton fibers. Very little is known about how microtubules and actin filaments are reorganized in plant cells. Results garnered from our studies will shed light on mechanisms underlying spatial organization of cytoskeleton and cell wall patterning during plant cell growth and morphogenesis.

40. University of California

Davis, CA 95616-8537

Cellular and Molecular Characterization of Vascular Plasmodesmata
William J. Lucas, Section of Plant Biology
$128,000

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.

41. University of California

Davis, CA 95616-8665

Physiology and Genetics of Energy Conservation in Chemoautotrophic Sulfur-oxidizing Bacteria
Douglas C. Nelson, Section of Microbiology
$182,000 (two years)

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.

42. University of California

Davis, CA 95616-8665
Genetic Control of Nitrate Assimilation in Klebsiella oxytoca
Valley Stewart, Section of Microbiology
$90,000

Klebsiella oxytoca, a soil bacterium, is a genetically-amenable close relative of *Escherichia coli* that has been extensively studied with respect to dinitrogen fixation and other aspects of nitrogen assimilation and metabolism. Our previous DOE-supported studies focused on the genetics and regulation of nitrate assimilation. We have now turned our attention to other pathways for nitrogen utilization, with emphasis on purine (hypoxanthine) utilization. We had previously isolated and characterized transposon insertion mutants unable to use as sole nitrogen source the early intermediates of purine catabolism: hypoxanthine and xanthine, uric acid, and allantoin. Our in-progress studies aim to characterize the structural and regulatory genes responsible for these steps. We have isolated recombinant DNA clones that complement *hyxC* and *hyxD* mutants unable to use urate and allantoin, respectively. We have subjected these clones to transposon mutagenesis in order to identify genes required for *hyxC* and *hyxD* function. DNA sequence analysis of these insertions has revealed genes with similarity to purine permeases, hydantoin racemase, and fungal uricase. We have just begun to use allelic exchange to cross these insertions into the *K. oxytoca* chromosome in order to determine their resulting phenotypes. At this early stage of our analysis, it appears that gene clusters for xanthine, urate and allantoin catabolism, although linked, are separated from each other by genes of unrelated function. In other studies, we are continuing our studies of genetic map and genome structure in *K. oxytoca* to better understand metabolic and evolutionary relationships among the enterobacteria.

43. University of California

Davis, CA 95616-8537
Protein Import and Assembly in Chloroplasts
Steven M. Theg, Section of Plant Biology
$101,000

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.

44. 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.

45. University of California

La Jolla, CA 92093-0116

The Signal Transduction Pathway of the Unfolded Protein Response
Maarten J. Chrispeels, Department of Biology
$107,000

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 string 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.

46. University of California

La Jolla, CA 92093-0116

Physiology and Regulation of Calcium Channels in Stomatal Guard Cells
Julian I. Schroeder, Division of Biology
$161,000

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.

47. University of California

La Jolla, CA 92093-0116

Analysis of the Localization and Function of TANGLED a Protein Required for Spatial Control of Cytokinesis in Plant Cells

Laurie G. Smith, Division of Biology

$100,000

Plant cells are surrounded by walls that fix their positions within tissues and define their shapes. New cell walls are formed during cytokinesis, the process that physically separates daughter cells following division of the nucleus. Orientation and localization of new cell walls in dividing cells is critical for the development of functional plant tissues because of its impact on cell shapes and arrangements. The cytoskeleton plays crucial roles in the formation and localization of new cell walls in dividing cells, but the molecular mechanisms governing these processes are poorly understood. Previously, we showed that the Tangled gene (Tan) of maize encodes a novel, highly basic protein required for proper orientation of cytoskeletal structures involved in the formation and positioning of new cell walls during the development of leaves and other organs. The goal of our DOE-funded project (initiated 6/01) is to elucidate the localization of TANGLED protein (TAN) in dividing cells, its interaction with other proteins, and ultimately to understand how TAN promotes properly oriented cell divisions. Transgenic maize expressing an epitope-tagged form of TAN will be used to determine its intracellular localization, and to identiry other proteins with which TAN interacts. Two putative kinesins, molecular motors that move cell components along microtubules, have already been identified as candidate TAN-interacting proteins in a yeast two hybrid screen. Antibodies specifically recognizing these kinesin-like proteins will be used in a variety of ways to investigate their potential to interact with TAN in vivo, and to understand what the functional significance of such interactions might be.

48. University of California

Los Angeles, CA 90095-1606

An Integrative Approach to Energy Carbon and Redox Metabolism in the Cyanobacterium Synechocystis sp. PCC 6803

Kym Francis Faull, Department of Chemistry and Biochemistry; in collaboration with Willem F.J. Vermaas and Robert W. Roberson (Arizona State University)

$199,997

The goal of this multidisciplinary and multi-institutional Microbial Cell project is to help provide integrated insight into subcellular structures, protein components, and fundamental metabolism involving photosynthesis, respiration, and other energy-related and redox processes in the cyanobacterium *Synechocystis* sp. PCC 6803. This goal will be achieved by using wild-type and mutant cells of this organism to analyze changes in proteome composition, metabolic function, metabolite levels, localization/abundance of specific proteins, and cell structure upon genetic deletion of specific processes or upon changes in environmental conditions. A large number of targeted deletion and site-directed mutants altered in particular open reading frames or with newly introduced genes related to photosynthesis and respiration have been created. Striking ultrastructural differences between wild type and mutants already have been observed (for example, a mutant lacking the terminal oxidases has a greatly increased number of polyhydroxybutyrate inclusions, suggesting PHB to be a main fermentative product in this cyanobacterium). Moreover, detailed proteomic and metabolic analysis has been initiated. On the basis of the multidisciplinary information gathered in the project, we aim at developing a comprehensive metabolic model of *Synechocystis* sp. PCC 6803, focusing on photosynthesis, respiration, and related processes.

49. University of California

Los Angeles, CA 90095-1606

Suspensor Differentiation During Early Plant Embryogenesis
Robert B. Goldberg, Department of Molecular, Cell and Developmental Biology
$109,000

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 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. Deletion experiments have identified a "box" that is required for transcription within the suspensor. 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 are regulated coordinately during plant embryo development, and, eventually, to identify regulatory networks in the plant genome that are responsible for allowing a plant to "make a seed."

50. 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
$113,000

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.

51. University of California

Los Angeles, CA 90095-1606

Sensory Transduction of the CO2 Response of Guard Cells
Eduardo Zeiger, Department of Biology
$104,000

Ambient CO_2 concentration is a key environmental signal sensed by guard cells. DOE-sponsored research in our laboratory has shown that stomata from growth chamber-grown leaves of *Vicia faba* have an enhanced CO_2 response, as compared with stomata from green house-grown leaves. Stomata from leaves transferred between the two environments acquire the CO_2 sensitivity characteristic of their new

environment over the course of a week, indicating that the shift in CO_2 sensitivity is an acclimation response to growth conditions.

We now find that manipulation of the relative humidity of the growing environment changes the stomatal sensitivity to CO_2. Relative humidity was low in the green house and high in the growth. Misting increased the relative humidity in the green house, and stomata acquired an enhanced CO_2 sensitivity. Shifting the plants from misted to non-misted conditions, caused the same acclimation response than observed when leaves were transferred from the growth chamber to the green house. Leaves grown in growth chambers in which the relative humidity has been reduced had stomata with a reduced CO_2 sensitivity. We conclude that relative humidity is the key environmental factor responsible for the acclimation of the stomatal response to CO_2.

Regulation of the CO_2 response of stomata by relative humidity could function as a signal for leaves inside dense foliage canopies, promoting stomatal opening under low light, low CO_2 conditions. The acclimation properties of the CO_2 response of guard cells also have implications for the understanding of stomatal function under predicted increases in atmospheric CO_2.

52. 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

$87,000

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.

53. 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 (FY 00 funds)

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.

54. Carnegie Institution of Washington

Stanford, CA 94305-4101
Genetic Engineering of Biomaterials
Chris Somerville, Department of Plant Biology
$468,551

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.

55. Carnegie Institution of Washington

Stanford, CA 94305-4150
Powdery Mildew Disease Resistance
Shauna C. Somerville, Department of Plant Biology
$102,000

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.

56. University of Chicago

Chicago, IL 60637

Cell-cell Interactions pollen tube growth in Arabidopsis
Daphne Preuss, Department of Molecular Genetics and Cell Biology
$105,000

Gamma –aminobutyric acid (GABA) is a four-carbon amino acid that is ubiquitous in organisms ranging from prokaryotes to vertebrates. It is one of the few non-protein amino acids that mediate several biological processes both within and between cells. In animal nervous system, it functions as a inhibitory neurotransmitter, an inhibitor of cortical neuron cell divisions, and a chemoattractant for brain neuron migration. In prokaryotes and fungi, GABA is used as a nitrogen and/or carbon source. Although the role of GABA in plants is not clear, several line of evidence suggest that it is involved in pH regulation, nitrogen storage, plant development and defense against pathogens. Using an Arabidopsis mutant (pop2) that displays random growth of pollen tubes in the neighborhood of egg cell and consequent fertilization failure, we have demonstrated that optimal levels of GABA is essential for proper pollen tube growth. This concentration dependent pollen tube growth is strikingly similar to the guidance of post mitotic spinal neuroblasts, which exhibits random motility upon exposure to high concentrations of GABA. Localization experiments in wild type female tissues revealed abundance of GABA along the path of pollen tubes, especially in the vicinity of egg cell. However, in pop2 mutant female tissues, many cell types around the egg cell exhibit increased accumulation of GABA, perhaps resulting in random growth of pollen tubes. Conservation of GABA's role in cell migration suggest that it originated from a primitive mechanism that predates the divergence of animals and plants.

57. Cold Spring Harbor Laboratory

Cold Spring Harbor, NY 11724

The Iojap Gene in Maize
Robert A. Martienssen
$102,000 (FY 00 funds)

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.

58. Colorado State University

Fort Collins, CO 80523

Functional Analysis of Novel Serine/Arginine-rich Proteins that Interact with U1-70K in Basic and Alternative Splicing of Pre-mRNAs
A.S.N. Reddy, Department of Biology
$191,000 (two years)

Most protein coding genes in eukaryotes contain introns that are excised in the nucleus in a large multicomponent complex called the spliceosome. Little is known about the mechanisms that regulate basic and alternative splicing in plants. Using the full-length and C-terminal region of U1-70K in the yeast two-hybrid system we have isolated four cDNAs encoding novel serine/arginine-rich proteins that interact with

U1-70K. Two of these proteins (SRZ21 and SRZ22) interact with the full-length U1-70K whereas other two proteins (SR33 and SR45) interact with the full-length as well as C-terminal arginine-rich region of U1-70K. To study the function of SR45 in basic and alternative splicing of pre-mRNAs *in vivo* we generated transgenic lines expressing SR45-GFP. The SR45 is localized in certain regions (speckles) within the nucleus. These transgenic lines expressing SR45 will allow us to study the effect of increased levels of SR45 on basic and alternative splicing of several pre-mRNAs using RT-PCR. To generate knockout mutants of SR45 we screened T-DNA insertion lines and identified a mutant. Our *in vivo* approaches on the role of SR45 in splicing should help elucidate its role in basic and alternative splicing processes in plants. Furthermore, these studies should provide the basis for future investigations on plant-specific mechanisms that control RNA splicing in plants.

59. University of Colorado

Boulder, CO 80309-0215
Microbial Production of Isoprene
R. Ray Fall, Department of Chemistry and Biochemistry
$94,000

As petroleum availability declines, there is increasing interest in finding alternative sources of useful hydrocarbons, especially those larger than methane. We have discovered that soil bacteria produce and emit the hydrocarbon isoprene (2-methyl-1,3-butadiene). If isoprene-producing enzymes and their genes can be harnessed, useful hydrocarbon-producing systems might be constructed. We are focusing our efforts on *Bacillus subtilis*, since it produces isoprene in relatively large amounts, it is a 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 release is controlled by carbon assimilation rate, but not by the concentration of its immediate precursor, dimethylallyl diphosphate. We have partially characterized the enzyme responsible for isoprene formation, and demonstrated that its activity rises and falls in parallel with isoprene release. This suggests that isoprene formation is tightly regulated, possibly related to metabolic signaling processes. Our recent results demonstrate that wild type *B. subtilis* isolates are attached to plant roots and form prolific biofilms. Experiments in progress are aimed at relating isoprene formation to intercellular signaling (i.e. quorum sensing) in biofilms, or to extracellular signaling in the rhizosphere. Our work is also directed at 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, and c) establish if isoprene is a metabolite that can be overproduced.

60. University of Connecticut

Storrs, CT 06269-3125
Molecular characterization of catabolite repression by succinate in the nodulating symbiotic bacterium Sinorhizobium meliloti
Daniel J. Gage, Department of Molecular and Cell Biology
$189,949 (two years)

Bacteria belonging to the genera Sinorhizobium, Rhizobium, Bradyrhizobium and Azorhizobium grow in the soil as free-living organisms, but can also live as nitrogen-fixing symbionts inside root nodules of plants belonging to the legume family. Once a symbosis is established, infected plants are able to grow in nitrogen poor environments, and don¹t require the additional of fertilzers for vigorous growth. Free-living Rhizobia can utilize a wide variety of compounds as sources of carbon for growth. Such compounds include sugars, amino acids and TCA cycle intermediates. The TCA intermediates succinate, fumarate and malate are of special importance for both the free-living and symbiotic forms of Rhizobia. A large body of evidence indicates that these organic acids are used to fuel and provide reducing equivalents for nitrogen fixation by S. meliloti. Succinate is a favored carbon and energy source for free-living S. meliloti and is used in preference to other carbon sources. This phenomenon, called succinate mediated catabolite repression, is well documented but the molecular mechanisms of its operation remain to be elucidated. We have described genes in S. meliloti required for the uptake and utilization of a-galactosides. These genes are induced by a-galactosides, but they are not induced by these sugars in the presence of succinate. Using the succinate-repressed genes of the a-galactoside utilization system as an entry point we will identify and

characterize the genes and proteins that mediated catabolite repression in S. meliloti. From this we expect to gain insights into how high-order gene expression is regulated in this species.

61. University of Connecticut

Storrs, CT 06269-3125
Genetic Analysis of Sugar Nucleotide Interconversions in Arabidopsis
Wolf-Dieter Reiter, Department of Molecular and Cell Biology
$97,000

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-rhamnose, 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 and functionally characterized an *Arabidopsis* enzyme that catalyzes the conversion of UDP-D-glucuronate to UDP-D-galacturonate. Experiments are currently underway to determine the functions of related proteins by expression in the yeast *Pichia pastoris*. 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.

62. Cornell University

Ithaca, NY 14853-2703
Intracellular Dynamics of Energy-Transduction Organelles
Maureen R. Hanson, Department of Molecular Biology and Genetics
$110,000

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 clels 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 these organelles in certain tissues. Furthermore, we have observed that tubules emanate from plastids, sometimes connecting one plastid to another, surrounding another organelle, or lying in close proximity to another subcellular structure. Measurements of the rate of movement of green fluorescent protein (GFP) 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. We have also observed that GFP can move within plastid tubules by an energy-requiring process, suggesting that the tubules may have a role in long-distance distribution of molecules within the cell. We are exploring the role of tubules further by labeling plastid proteins and protein complexes with GFP in transgenic plants in order to assess the movement of genuine plastid proteins through tubules by fluorescence correlation spectroscopy. In addition to movement, another possible role of the tubules is to increase the surface area of the envelope membrane to facilitate import of proteins and other molecules. By confocal and epifluorescence microscopy, we are also performing additional developmental studies of tubule size, number, location, and morphology in different tissues and in transgenic plants carrying fluorescent tags on other organelle types.

63. Cornell University

Ithaca, NY 14853-8101

Regulation of Denitrification in Rhodobacter sphaeroides
James P. Shapleigh, Department of Microbiology
$96,908

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.

64. 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 (FY 00 funds)

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.

65. 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
$97,000

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.

66. University of Delaware

Lewes, DE 19958
Plant Growth with Limited Water
John S. Boyer, College of Marine Studies
$110,004

Plant growth is frequently limited by water. The project objective is to identify the underlying molecular mechanisms causing decreased growth when water is limited. Studies so far showed that 1) turgor pressure is required for elongation, 2) despite high turgor, elongation ceases when water is in short supply, 3) water potential gradients are significant in tissues, 4) the gradients are locally disrupted when water is in short supply, and 5) the disruption prevents water from flowing into the elongating cells and thus blocks growth. The gradients were found to originate from wall yielding that prevented turgor from being as high as it otherwise would. This sequence of events was followed by a loss in the extensibility of the cell walls, changes in gene expression, increases in certain wall proteins, decreases in wall biosynthesis but increases in wall mass. Xyloglucan endotransglycosylase (XET) activity was related to the lower extensibility and increased wall mass. These gradient concepts were further explored in fully established maize plants. Evidence was found that the sequence of events constitutes a signal transduction path beginning with a change in xylem water potential followed by a disruption of the Ψ_w gradient that immediately inhibits enlargement after which unused solute accumulates, the osmotic potential of the cells adjusts downward, and changes in gene expression and wall extensibility follow. Because it was shown that certain growth regulators affect wall yielding and thus the water potential gradient, it may be possible to alter the growth response to limited water.

67. 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
$105,000

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.

68. 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
$87,147

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.

69. Florida State University

Tallahassee, FL 32306-4370

Role of Sucrose in Modulating Stomatal Aperture
William H. Outlaw, Jr., Department of Biological Science
$84,000 (FY 00 funds)

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.

70. 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 from the middle east. 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 our 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 the processing of crop residues. Improvements in tolerance to these compounds will offer an opportunity to reduce the number of steps in a lignocellulose-to-ethanol process, reducing the costs of fuel ethanol production and improving efficiency.

71. University of Florida

Gainesville, FL 32611-0690
Genetic Control of Abscisic Acid Biosynthesis in Plants
Donald R. McCarty, Horticultural Sciences Department
$107,000

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.

72. University of Georgia

Athens, GA 30602-7229
The Metabolism of Hydrogen by Hyperthermophilic Microorganisms
Michael W. W. Adams, Department of Biochemistry & Molecular Biology
$115,000

Hyperthermophiles are a recently discovered group of microorganisms that have the remarkable property of growing optimally near and even above 100°C. They have enormous biotechnological potential in chemical conversions. 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. If elemental sulfur is added, the organism produces hydrogen sulfide. Several unusual oxidoreductase-type enzymes have been purified from Pf that in concert couple substrate oxidation during fermentation to hydrogen production and sulfur reduction. These include three types of aldehyde-oxidizing enzyme that contain tungsten (W), an element seldom used in biology. Recent studies have cast considerable doubt over the pathways that were presumed to lead to hydrogen production. They include the characterization of a membrane-bound hydrogenase that appears to have a respiratory function, the purification of a second cytoplasmic hydrogenase and of a fourth tungsten-containing enzyme, and the identification of a new type of enzyme system involved in sulfur reduction. All of this information is being assembled into a genome-centered database for Pf that will encompass biochemical, metabolic, and structural data coupled with results from enzymatic, DNA microarray and proteomic studies. The long term objective of this research is to assess the utility of hyperthermophilic enzymes in industrial energy conversions involving hydrogen and sulfur.

73. University of Georgia

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

The polysaccharide-rich cell walls of plant hosts and fungal pathogens are directly involved in many attempted infections. Plants and fungi both secrete *endo*glycanases that fragment the polysaccharides present in the cell walls of the interacting adversaries. The result of *endo*glycanase action on cell wall polysaccharides can include weakening of the wall, penetration of host cells by the pathogen, solubilization of carbohydrate nutrients, and formation of oligosaccharins (oligosaccharides with regulatory function) that can regulate developmental processes of the plant and stimulate the activities of plant defenses. The oligosaccharins may have equally important effects on the ability of pathogens to attack plants. This research emphasizes three separate projects that involve studies of protein/protein and protein/carbohydrate interactions that we hypothesize have profound effects on the pathogenicity of fungal pathogens. The three projects involve: (i) the interactions between fungal *endo*polygalacturonases and plant polygalacturonase-inhibiting proteins; (ii) the interactions between plant *endo*glucanases and fungal *endo*glucanasae-binding proteins; and (iii) the interactions between fungal *endo*xylanases and plant arabinoxylans. Our results to date have made us optimistic that these studies will substantiate our working hypothesis that the outcome of attempted pathogenesis can be determined by biochemical interactions in the extracellular matrices of the host and pathogen, and that complex carbohydrates can be important components of these interactions.

74. University of Georgia

Athens, GA 30602-4712
Center for Plant and Microbial Complex Carbohydrates
Peter Albersheim and Alan Darvill, Complex Carbohydrate Research Center
$625,000

The DOE-sponsored Complex Carbohydrate Research Center (CCRC) of the University of Georgia operates as a national resource for the study of complex carbohydrates. The DOE Center grant enables the CCRC to provide service and training to academic, government, and industrial researchers who study the complex carbohydrates of plants and of microbes that interact with plants. The grant funds enable a variety of services to be offered to academic, government and industrial researchers. Scientists who request analytical services receive a written report containing a description of (i) the analytical procedures used, (ii) publishable quality results (data) of the analyses of their samples, and (iii) an authoritative interpretation of the results. CCRC personnel also provide collaborative service by becoming deeply involved in scientific research projects with individuals from other laboratories. CCRC personnel consult with external scientists via e-mail and telephone, helping the scientists address specific analytical problems or interpretation of data. The CCRC's web site (www.ccrc.uga.edu) provides freely accessible, Internet-searchable databases assisting in aspects of carbohydrate science and a scheduler for shared use of instrumentation. The CCRC, upon request, provides researchers with chemical and biological samples. Training students and scientists

in all aspects of carbohydrate science is a very important part of CCRC's mission. Much of the training occurs when undergraduate students, graduate students, postdoctoral fellows, and visiting scientists undertake research projects with or take formal courses from CCRC faculty and staff. In addition, annual one-week training courses are offered for individuals from academic institutions, government laboratories, and private industry.

75. University of Georgia

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

The primary cell wall is a dynamic structure that has many functions in plants including determining the shape of cells, contributing to the control of cell growth, and as a barrier to potential pathogens. The major structural elements of primary walls include crystalline cellulose embedded in a matrix of non-crystalline polysaccharides. The wall polysaccharides predominantly exist in two domains, a cellulose/hemicellulose domain and a pectin domain. This grant funds research to identify and characterize matrix polysaccharides and to characterize enzymes and the genes that encode them that have key roles in cell wall metabolism. The research includes determining in arabidopsis and tomato the structures of complex carbohydrates, characterization of wall-localized enzymes, determination of the specific effects on structure and function of wall-associated mutants, and characterization and localization of epitopes of monoclonal antibodies that bind to cell walls. The research emphasizes studies on the pectic polysaccharide rhamnogalacturonan II (RG-II) and the hemicellulose xyloglucan (XG) that have important functions in the control of growth and development. Specifically, our studies include determining: (i) the function of the covalent borate cross-link between two RG-II molecules including its role in controlling cell growth, (ii) the function of the three structural and physiological domains of XG and the metabolism of these domains in plants subjected to various physiological conditions, and (iii) the location of specific wall polysaccharide epitopes in individual cells and tissues with an emphasis on RG-II. The results from this research will contribute to a detailed molecular description of the structure and function of primary walls and their role in plant growth and development.

76. University of Georgia

Athens, GA 30602-2152
Jeffrey F.D. Dean, School of Forest Resources
Structure-Function Relationships in Plant Laccases
$93,000

Laccase-type multicopper oxidases (LMCOs) exist as gene families of moderate complexity in plants having received extensive study. Phylogenetic analyses have demonstrated that plant LMCOs may be classified into at least four major groups, and at least three of these predate the evolutionary split between the angiosperms and gymnosperms. Analyses of LCMO gene expression in Arabidopsis found a variety of expression patterns with some genes being expressed constitutively while others were only expressed in certain tissues at specific stages of development. Few Arabidopsis LMCO genes displayed expression patterns that were closely correlated with tissues undergoing active lignification, and several were expressed in tissues that are not thought to contain lignin. These results suggest that plant LCMOs may not be critical catalysts for the monolignol coupling process leading to lignin deposition. However, preliminary results have suggested an alternative physiological function for these enzymes. An Arabidopsis laccase that is strongly and specifically expressed in elongating roots, a tissue containing little or no lignin, has been cloned and expressed in E. coli. Current work is focused on enhancing the heterologous expression of this LMCO in order to generate antibodies and carry out detailed enzymatic analyses. Work is also underway to characterize the phenotypes associated with Arabidopsis lines having T-DNA inserts in various LCMO genes.

77. 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
$202,000 (14 months)

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.

78. University of Georgia

Athens, GA 30602-2605

Roles of the Metal-Binding Protein Nickelin in Symbiotic Nitrogen Fixation
Robert J. Maier, Department of Microbiology
$135,000 (fifteen months)

The process of biological N_2 fixation, whereby atmospheric N_2 gas is reduced to ammonia so it can be assimilated into amino acids by organisms, is a key process for the positive input of N on earth. The N_2 fixing legumes harbor bacteria (in root nodules) that carry out this process, resulting in plants that do not require N-fertilizer for growth and that yield high N-containing products. The symbiotic bacteria are uniquely adapted to carry out the N_2 fixation process. In particular, they are able to sequester the metals needed for the manufacture of enzymes crucial for N_2 fixation. One nickel-containing enzyme that aids in the efficiency of symbiotic nitrogen fixation by several mechanisms is hydrogenase. It uses molecular H_2 and couples its oxidation to production of readily available energy, enabling the bacteria to glean otherwise lost energy that can be used to further the main (N_2 fixation) process. Our work has focussed on the role of a nickel-binding/nickel storage protein called nickelin; it sequesters nickel for the N_2 fixing symbiont of soybean, *Bradyrhizobium japonicum*. Nickelin was found to be able to sequester nickel for nodulated soybean plants in low nickel supplement conditions, so that the nickel-dependent hydrogenase activity could be maintained at high rates. The Ni-sequestering portion of nickelin that aided in symbiotic Ni-hydrogenase activity and in the retention of nickel in the root nodules was localized to a histidine-rich portion of the protein. This histidine-rich area was found to be able to sequester other divalent ions as well, and may play a physiological role as a metal-reservoir in root nodules for additional metals. The mechanistic characteristics of another nickel-binding protein in permitting hydrogenase synthesis only in conditions when hydrogenase is useful to the symbiosis (such as when both H_2 and nickel are available) is also being determined.

79. University of Georgia

Athens, GA 30602-7271

Genetic Analysis of Polyamine Synthesis in Arabidopsis
Russell L. Malmberg, Department of Botany
$109,000

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. We have isolated both regulatory and structural gene mutations in the model plant Arabidopsis thaliana. We have also generated a set of monoclonal antibodies that allow us to study the localization and expression of two of the proteins that are important in polyamine synthesis. We are using these mutants and antibodies to study the function of polyamines, and the regulation of polyamine synthesis. 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.

80. University of Georgia

Athens, GA 30602-7223

Mechanisms and Determinants of RNA Turnover: Plant IRESs and Polycistrons for Metabolic Engineering
Richard B. Meagher, Department of Genetics
$123,000

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

81. University of Georgia

Athens, GA 30602-7271

Identification of Novel Cell Wall Components
Michelle Momany, Department of Botany
$172,000 (FY 00 funds - 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.

82. 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)

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.

83. 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 (FY 00 funds)

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.

84. University of Georgia

Athens, GA 30602-2605

Biochemistry and Genetics of Autotrophy in Methanococcus
William B. Whitman, Department of Microbiology
$211,000 (FY 00 funds - two years)

In nature, the methanogenic fermentation of organic matter to CH_4 and CO_2 requires a complex consortium of microorganisms. The methanogenic archaea, which catalyze the terminal step in this process, have a very narrow substrate specificity and can not directly oxidize organic compounds. Instead, they utilize H_2 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 would have a great utility in this regard. One goal of our studies is to understand the physiological basis for this narrow substrate specificity of the methanogens. For instance, we have shown that although the methanococci contain the enzymatic machinery to oxidize organic substrates such as pyruvate, this activity is not utilized during normal growth. Based upon these studies, we have hypothesized that two unique genes associated with the genes for the enzyme that synthesizes pyruvate is responsible for preventing pyruvate oxidation. We have now mutagenized these genes and found that the mutants possess the expected properties. Current studies are underway to further examine the role of these unusual genes. In addition, genetic methods have been developed to identify genes necessary for the autotrophic fixation of CO_2 to acetate, and mutations at several loci are currently being characterized. In these environments, CO_2 fixation is an anomaly and the reverse of the fermentation of organic matter to CH_4 and CO_2.

85. University of Georgia

Athens, GA 30602-2605

Global Regulation in the Methane-Producing Archaeon Methanococcus maripaludis
William B. Whitman, Department of Microbiology; in collaboration with John Leigh (University of Washington) and Dieter Soll (Yale University)
$418,082 (two years)

In the microbial fermentation of organic material to methane gas, about 95 % of the combustion energy of complex substrates is retained in the methane formed. This process has great potential in converting biomass to a fuel because it is CO_2 neutral and does not contribute to the net evolution of radiatively important trace gases. It can also be applied to a wide variety of organic wastes. The hydrogenotrophic methane-producing archaea play critical roles in this process. They produce about one-third of the methane formed. By maintaining a low partial pressure of H_2 gas, they also prevent the accumulation of toxic intermediates that would otherwise poison the fermentation. *Methanococcus maripaludis* is typical of many hydrogenotrophic methanogens. Because of its rapid growth and the availability of a well developed genetic system, this organism is especially suitable for detailed studies of this type of physiology. The proposed research will delineate the global regulatory systems and the molecular mechanisms that govern them using *M. maripaludis* as a model organism. Special emphasis will be given to regulatory systems that are key to the methanogenic physiology or are universally important but poorly understood in the Archaea. For example, the regulatory response to hydrogen levels and to nutrient-limited variations in growth rate will be investigated. A combination of experimental approaches will be utilized based upon the genome sequence of *M. maripaludis*, the proven genetic methodology, and a practical understanding of *M. maripaludis* physiology. Expression array analysis will be complemented by proteomic and metabolic analyses.

86. University of Georgia

Athens, GA 30602-2605

Novel Reversible Phenolic Carboxylase Family Shared by Members of the Domains Bacteria and Archaea
Juergen Wiegel, Department of Microbiology
$200,000 (two years)

Arylcarboxylase activities and non-oxidative hydroxy arylcarboxylic decarboxylase (HO-aryl-COOH DC) have been shown to be present in several bacteria and fungi. Surprisingly little is known about these ATP-,

biotin-, and thiamin pyrophosphate-independent enzymes with respect to their roles as carboxylases and/or decarboxylases (DCs) of hydroxy aromatic compounds, both in anabolic and catabolic pathways. Even less is known about their genes, phylogenetic distribution, structural properties and catalytic mechanism. We purified and characterized the first 4-HO- and 3,4-di-HO benzoate DCs from *Clostridium hydroxybenzoicum*, which we believe function physiologically as phenol carboxylases in the sequential multi-bacteria, anaerobic degradation chain of chlorophenols under methanogenic conditions. The deduced amino acid sequence exhibits high identity values and similarities to many hypothetical proteins derived from genome sequences, but little to other known carboxylases and decarboxylases. These findings lead to our working hypothesis: these hydroxybenzoate decarboxylases belong to a so far unrecognized family of reversible hydroxyaryl decarboxylases (hydroxyaryl carboxylases) involved in the anabolism and catabolism of mainly hydroxylated aromatic compounds. Our long term goals are to provide in depth information on this proposed new family and their physiological roles and include elucidation of the reaction mechanism and X-ray crystal structure.

87. 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 (FY 00 funds)

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.

88. 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

$91,792

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.

89. University of Hawaii

Honolulu, HI 96822

Xanthophyll Cycle and Photoprotective Systems in Higher Plants
Harry Y. Yamamoto, Department of Molecular Biosciences and Biosystems Engineering
$111,000

Photosynthesis captures the sun's energy and generates the oxygen and food that sustains plant and animal life on earth. Paradoxically, sunlight is also potentially damaging to plants when it exceeds their photosynthetic capacity. To guard against potential "sunburn," plants have evolved protective systems, one such system being dissipation of the excess energy as heat through a xanthophyll-cycle=dependent system. The cycle, first discovered about 40 years ago, has been found in all higher plants examined to date and comprises of light-induced reversible changes in the carotenoids, violaxanthin, antheraxanthin and zeaxanthin. The protective role of the xanthophyll-related energy dissipation is well documented but, surprisingly, recent evidence has shown that plants can survive in its absence. It appears that plants have alternative protective systems that work in parallel or sequentially with the xanthophyll-dependent one. The objectives of this research are to characterize the xanthophyll independent system in greater detail to better understand its mechanism and the functional relationship between the xanthophyll-cycle system. This project will use tobacco and *Arabidopsis* that we have developed in which the xanthophyll cycle has been suppressed or enhanced by antisense and sense violaxanthin de-epoxidase transformations. *Arabidopsis* mutant plants, developed by others, with deletions in essential components of the protective system will also be used. 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.

90. University of Illinois

Chicago, IL 60612-7344

Molecular Genetics of the Arsenite Oxidase of Alcaligenes faecalis strain NCIB8687
Simon Silver, Department of Microbiology & Immunology
$115,000

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.

91. University of Illinois

Urbana, IL 61801-3364

Studies on Cytochrome bo_3 from *Escherichia coli*

Robert B. Gennis, Department of Chemistry

$272,000 (two years)

The long range goal of the work supported by this grant is to better understand the structure and function of the cytochrome bo_3 quinol oxidase from *Escherichia coli*. When the bacterium grows under conditions of high aeration, this is the primary respiratory oxidase. The enzyme catalyzes the 2-electron oxidation of ubiquinol-8 in the cytoplasmic membrane and the 4-electron reduction of O_2 to 2 H_2O. The free energy made available from this reaction is coupled to the generation of a transmembrane voltage and proton gradient (a proton motive force) across the membrane. This proton motive force is subsequently used to make ATP and carry out many energy-requiring functions within the cell. Work in the past year has focused on the quinol binding site(s). It has been speculated that there are two quinol binding sites, one with a high affinity which stabilizes a semiquinone species formed during catalysis, and the second site which has a weak affinity for the quinol substrate which is oxidized and released into the membrane. A low resolution X-ray structure of cytochrome bo_3 was reported from the group of Dr. So Iwata (Uppsala, Sweden), and it was hypothesized that one of the quinol binding sites was located in a cavity within subunit I. We tested this by making mutations in key residues that were thought to be hydrogen bonded to the quinol. Indeed, several of these enzymes resulted in inactive enzyme. In addition, the stabilitiy of the bound ubisemiquione was also examined by EPR spectroscopy. It was found that mutations in R71 and H98 abolished the ubisemiquinone, and that the EPR signal from the ubisemiquinone was substantially altered by a mutation in D75. It was concluded that this site is the “high affinity” site which stabilizes the semiquinone species that is essential for catalysis and that R71, H98 and D75 are essential for semiquinone stabilization.

92. University of Illinois

Urbana, IL 61801

Studies on the Microbial Formation of Methane

Ralph S. Wolfe, Department of Microbiology

$84,626 (FY 00 funds)

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.

93. Indiana University

Bloomington, IN 47405-3700

Regulation of Plastid Development During Embryo Maturation and Seed Germination
Roger P. Hangarter, Department of Biology
$191,000 (two years)

We have identified a novel Arabidopsis mutation, named *spd1* (seedling plastid development), that exhibits defective chloroplast and amyloplast development upon germination in the light which results in white cotyledons and agravitropic hypocotyls. However, plastids apparently develop normally in cells produced by the shoot apical meristem so that leaf and shoot tissues are green. Cells in the flowering stem also contain amyloplasts and the flowering stems display normal gravitropism. The *spd1* phenotype suggests that it specifically affects plastid development during embryo maturation and/or early germination. The overall goal of this project is to isolate and characterize the *SPD1* gene and determine its function during embryo and seedling development. The specific goals are to isolate the *SPD1* gene by positional cloning and chracterize the wild-type and mutant sequences, characterize the physiology and developmental biology of *SPD1* function and the nature of the *spd1* mutant phenotype, characterize plastid fate during embryo maturation and early germination in wild type and *spd1*, and isolate additional *spd* mutants and mutant suppressors of *spd1*. Our investigations of the *spd1* mutant will lead to new insights into fundamental molecular mechanisms that regulate plastid differentiation during an important developmental period. In addition, this work is of agronomic significance since plastids in developing embryos affect seed storage deposition and, in oil seeds, oil quality can be reduced significantly by chlorophyll contamination if chloroplasts fail to degreen properly. Ths *spd1* mutation offers a unique opportunity to gain new insights into the mechanisms controlling plastid development during the critical stages of late embryogenesis and early seed germination.

94. 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
$96,000

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. 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.

95. Iowa State University

Ames, IA 50011-3211

Mechanism of Methane Oxidation in Cells Expressing the Membrane-Associated Methane Monooxygenase
Alan A. DiSpirito, Department of Microbiology
$86,839

Methanotrophs utilize methane as a sole carbon and energy source. The first enzyme in the pathway, methane monooxygenase (MMO), catalyzes the energy dependent oxidation of methane to methanol. In some methanotrophs, the cellular location of the MMO is dependent on the copper concentration during growth. At low copper to biomass ratios, the enzyme activity is observed in the soluble fraction and referred to as the soluble MMO (sMMO). At higher copper to biomass ratios, methane oxidation activity is observed in the membrane fraction and the enzyme referred to as the membrane-associated or particulate MMO

(pMMO). The main objective of this project is to characterize the mechanism of methane oxidation in methanotrophs expressing the pMMO. Two objectives of this projects, one involving improvements to the purification procedure, and the second identification of the physiological reductant of the pMMO , were completed this year. Current purified preparations of pMMO show activity levels representative of physiological rates (100 – 300 nmol•min^{-1}•mg protein^{-1}). Improved culture conditions, identification of the physiological reductant (ubiquinone-8), and simplification of the purification procedure were responsible for the higher activity preparations. Improvements to growth conditions focused on the rate and levels of copper addition, which was the key determinate in the initial stabilization of the enzyme in cell free assays. In addition to enzyme stabilization, studies this year have shown that the pMMO transcripts and polypeptides as well as the concentration of fatty acids (indicator of cell membrane content) are regulated by the concentration of copper in the culture medium copper.

96. 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 (FY 00 funds - 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.

97. Iowa State University

Ames, IA 50011-1020

Acetyl-CoA: precursor for an alternative biotic source of hydrocarbons
Basil J. Nikolau, Department of Biochemistry, Biophysics & Molecular Biology
$102,000

Acetyl-CoA is the biochemical precursor of a large number of phytochemicals that have utility as sources of renewable energy-rich products of agriculture (e.g., fats, oils, waxes, bioplastics). In particular, in specific tissues or in response to developmental or environmental cues, acetyl-CoA is channeled towards the biosynthesis of specific hydrocarbons such as isoprene, natural rubber, or alkanes. We seek to better understand the genetic and biochemical mechanisms of acetyl-CoA generation and its conversion to energy-rich alkanes. These data are critical for the metabolic engineering of plants to produce “large” quantities of alkanes, which could serve as a renewable, environmentally friendly source of hydrocarbons to supplement petroleum-derived hydrocarbons.

To achieve these objectives we have isolated genes coding for five enzymes that can produce acetyl-CoA in plants: pyruvate decarboxylase, acetaldehyde dehydrogenase, acetyl-CoA synthetase, plastidic pyruvate dehydrogenase, and ATP-citrate lyase. These genes are expressed in different spatial and temporal compartments of plants. Hence, they are probably involved in gnerating different pools of acetyl-CoA. We

propose to elucidate the role of each isolated gene in the biogenesis of different acetyl-CoA pools by using our existing collection of mutant plants that are deficient in each acetyl-CoA-producing enzyme. These plants will be characterized to ascertain the effect of each mutation on the synthesis of acetyl-CoA-derived phytochemicals. These data will identify the molecular details of how different acetyl-CoA pools are generated in plants, which will provide the fundamental scientific framework for improving the production of a large number of energy-rich acetyl-CoA-derived phytochemicals (i.e., fats, oils, hydrocarbons). In addition, we will test the hypothesis that the cloned *CER1* gene of Arabidopsis codes for an aldehyde decarbonylase, one of the two enzymes required for the conversion of fatty acids to alkanes. Together, these data will generate the fundamental scientific basis for designing a rational biotechnological approach for maximizing the flux to acetyl-CoA, and diverting its metabolism to the biosynthesis of alkanes.

98. Iowa State University

Ames, IA 50011-1020

Regulation of Carotenoid Biosynthesis: The *immutans* Mutant of *Arabidopsis*
Steven R. Rodermel, Department of Botany
$91,000

The *immutans (im*) variegation mutant of *Arabidopsis* has green and white leaf sectors due to the action of a nuclear recessive gene. The white sectors of *im* accumulate the noncolored carotenoid, phytoene. We have cloned IM and found that it is a chloroplast homolog of the alternative oxidase (AOX) of the inner mitochondrial membrane. *In vitro* studies using the purified IM protein have shown that IM has quinol oxidase activity. Our studies are consistent with the idea that IM serves as a redox component in phytoene desaturation, transferring electrons from the quinone pool to molecular oxygen. We have compared the sequence of IM with that of mitochondrial AOX and identified residues that are likely important for catalysis. This has been confirmed by *in vitro* mutagenesis experiments. 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. 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. The green sectors also have enhanced Calvin Cycle activity and enhanced production of starch and sucrose. 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.

99. University of Iowa

Iowa City, IA 52242-1109

Molecular Biology of Anaerobic Aromatic Biodegradation
Caroline S. Harwood, Department of Microbiology
$95,000

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.

100. 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 (FY 00 funds)

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.

101. KAIROS Scientific Inc.

Santa Clara, CA 95054
Macromolecular Scaffolds for Energy Transfer
Douglas C. Youvan
$138,000

The Green Fluorescent Protein (GFP) from the jellyfish *Aequorea victoria* provides an almost singular example of a protein that can convert part of its own primary structure (Ser-Tyr-Gly) into a fluorescent heterocycle. The GFP structure, in which eleven β-strands surround a central α-helix (which bears the fluorophore) has been described as "paint in a can". We are applying the techniques of directed protein evolution to construct minimized versions of genetically-encoded fluorescent proteins. The path through sequence space traveled by the fluorescent protein during the minimization process will be of interest to theorists modeling directed evolution and protein structure. A minimized GFP would have a number of advantages for applications in energy conversion, enzymology, sensor technology, and cell biology.

102. Keck Graduate Institute of Applied Life Sciences

Claremount, CA 91711
Regulation of Gene Expression by Methanol in the Yeast *Pichia pastoris*
James M. Cregg
$85,000

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 required for gene expression in

response to methanol. 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. Recently, we discovered that Mxr1p specifically binds to sequences in this *AOX1* promoter region, thus providing direct evidence that Mxr1p is a DNA-binding transcription factor.

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.

103. 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 (FY 00 funds - 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.

104. University of Kentucky

Lexington, KY 40546-0091

Molecular Regulatory Mechanisms of Two Senescence-Specific Genes in Arabidopsis

Susheng Gan, Department of Agronomy

$95,000

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.

105. 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

$88,808

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 that 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.

106. 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

$147,000

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.

107. Lawrence Berkeley National Laboratory

Berkeley, CA 94720

Energy Conversion in Photosynthesis - Photosynthetic Light Reactions

Kenneth Sauer and Vittal K. Yachandra, Physical Biosciences Division

$247,000

Light absorption by the chlorophyll or phycobiliprotein pigments of photosynthetic organisms determines their efficiency of growth and ability to adapt to different environments. Small changes in the molecular

surroundings of the light absorbing groups in pigment proteins like phycocyanin provide a means for optimizing the wavelength and energy-transfer properties that are important for increasing photosynthetic efficiency. [Pizarro and Sauer (2001)] Green plants and algae generate almost all of the oxygen present in the atmosphere by the oxidation of water. This light-requiring reaction is catalyzed by a four-manganese atom cluster associated with Photosystem II. In our ongoing investigations of the oxidation-state changes, the structure and the associated cofactors Ca and Cl, we have made extensive use of X-ray and EPR spectroscopy. As the membrane-associated complex containing the Mn_4 cluster is advanced through its intermediate states (S-states) by light absorption, the X-ray spectroscopy indicates that Mn undergoes an increase in oxidation state between S0 and S1 and between S1 and S2, but not between S2 and S3. This latter conclusion, which had been questioned by others, is now confirmed by a new and more definitive X-ray spectroscopy involving K□ fluorescence. [Messinger, et al (2001)] The properties of model compounds containing Mn provide essential background information for interpreting the behavior of the biological systems. One challenge has been to obtain relevant compounds and compare their properties in more than one oxidation state. We have used electrochemistry together with Mn K-edge absorption and K□ fluorescence to characterize the changes that occur when synthetic complexes containing Mn_2 pairs bridged by O atoms undergo successive 1-electron oxidation-state changes. [Visser, Anxolabéhère-Mallart, et al (2001)] We are also exploring new methods to provide complementary information on these issues. Infrared spectroscopy is nicely sensitive to the structural changes associated with oxidation-reduction chemistry. We have designed a new electrochemistry/infrared interface that allows small samples of electrochemically active materials to be monitored by attenuated total reflection, Fourier-transform infrared (ATR-FTIR) spectroscopy. [Visser, Curtright, et al (2001)] By making application of new spectroscopic methods, we establish a basis for applying them to the biological materials that form our principal focus. In this way we hope to elucidate not only the structure of the complex, but also the mechanism of the photosynthetic water oxidation process. The insights gained from plant photosynthesis can then be applied directly in the design of artificial solar-energy based devices to split water into oxygen and hydrogen fuel as well as fuel cells that use hydrogen or other transportable fuels to produce electricity directly.

108. Lawrence Berkeley National Laboratory

Berkeley, CA 94720

Vanadium Haloperoxidase: Functional organization and regulation of catalysis in Fucus zygote adhesion

Valerie Vreeland, Division of Materials Science

$132,000

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.

109. 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

$194,000 (two years)

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 which are present on the CP47 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 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. Additionally, in collaboration with Dr. Cindy Putnam-Evans at East Carolina University, we have recently identified residues in the CP43 protein which also modify the chloride requirement for PS II. We are currently in the process of examining in detail the defects in these CP47 and CP47 mutants.

110. Marquette University

Milwaukee, WI 53201-1881

K. Dale Noel, Department of Biology

Lipopolysaccharide structures and genes required for root nodule development

$96,000

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. More recently, 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. Past results supported the idea that the lipopolysaccharide nonspecifically protects the bacteria from toxic compounds that are part of plant defenses. Discovery of the importance of specific structures and modifications suggest that it also acts as a signal in which specific portions of its structure interact with recognition molecules on the plant cell surfaces. Current investigations of the sequences and regulation of the genes that control these structures may ultimately be important in the fine-tuning of legume nodule development to the benefit of both agronomic yields and environmental quality.

111. University of Maryland

Baltimore, MD 21202

Physiology and Genetics of Aceticlastic Catabolism in the Methanogenic Archaea

Kevin R. Sowers, Center of Marine Biotechnology

$196,000 (two years)

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.

112. 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

$99,000

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.

113. 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 (FY00 funds)

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.

114. 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

$97,000

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.

115. University of Maryland

College Park, MD 20742-5815

Regulating Intracellular Calcium in Plants: From Molecular Genetics to Physiology

Heven Sze, Department of Cell Biology and Molecular Genetics

$101,000

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. Over 10 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 15 Ca^{2+}-ATPases, only a few have been functionally characterized, though the activity modulation, and the physiological roles of each protein are not understood. At**E**CA1 and At**A**CA2, serve as models for '**E**R'-type and '**A**utoinhibitor'-type pumps, respectively, as they were the first plant Ca^{2+} pumps to be functionally characterized by heterologous expression in yeast. ACA2 on the ER is calmodulin-stimulated and autoinhibited by a novel N terminal regulatory domain; but nothing is known about the modulation and roles of 'ER'-type pumps. The central goals are to understand the in vivo roles of selected pumps by analyzing T-DNA insertional mutants, and determining their spatial and temporal expression. eca1-1 mutant grew poorly on medium with low Ca^{2+} or high Mn^{2+}. Failure to elongate root hairs in mutants indicates that a reduction in Ca^{2+} and Mn^{2+} pumping into the ER impaired tip growth processes. Studies are underway to determine if stress-induced Ca^{2+} transients are altered in mutants. Thus despite the potential redundancy of active Ca^{2+} transporters, each ECA may be required to support growth under conditions of nutrient stress or environmental stress.

116. 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 on our planet. 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 cellulose-decomposing microbial communities, and to discern the nature of key interactions among community members contributing to the efficient degradation of biomass. 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, and it is the most abundant nitrogen-bearing organic compound in nature. Chitin is widely distributed in soils and sediments where it is present in proximity to cellulose, and some organisms have evolved the ability to degrade both insoluble biopolymers. Our research program is directed toward advancing understanding of processes involved in the anaerobic decomposition of cellulose and chitin, the biology of the cellulolytic and chitinolytic microbes involved, their community structure, 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. This information will be invaluable in the development of practical applications, including bioconversion of cellulose- and chitin-containing wastes to fuels such as ethanol.

117. University of Massachusetts

Amherst, MA 01003
Mechanisms for Microbial Reduction of Humics and Structurally Related Compounds
Derek R. Lovley, Department of Microbiology
$96,000 (FY 00 funds)

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.

118. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824
Molecular Mechanisms That Regulate the Expression of Genes in Plants
Pamela J. Green
$275,769

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*, *dst2, and dst3* that are defective in an mRNA decay pathway mediated by the mRNA instability sequence DST. Current efforts focus on mapped-based cloning of the *DST* 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 on a global scale. Other projects include the characterization of intra- and extracellular ribonucleases for their roles in mRNA decay and other processes, and the study of genes that encode RNA as their final product.

119. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824
Molecular biology of plant-bacterial interactions
Sheng Yang He
$260,769

Plant disease is a major 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 it 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 that directs 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.

120. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824-1312
Molecular and Biochemical Basis of Induced Resistance
Gregg A. Howe
$255,769

The long-term goal of our research is to elucidate the signal transduction pathways that control plant defense responses to herbivores. Emphasis is on understanding the role of systemin, a peptide signal, and jasmonic acid (JA), a fatty acid-derived signal, in coordinating defense responses that are induced systemically upon herbivore attack. Tomato is being used as a model system to identify, through forward genetics, genes involved in JA biosynthesis and perception, and in systemin perception. We are conducting grafting experiments to determine the role of these genes in long-distance signaling triggered by herbivore attack. Work is also progressing toward isolating the genes defined by mutational analysis. A second project is to characterize a gene family (CYP74) that encodes cytochrome P450 enzymes involved in the biosynthesis of JA and related defensive compounds. This research led to the identification of a gene encoding divinyl ether synthase (DES), which produces compounds implicated in plant defense against a broad spectrum of pests. Additional members of the CYP74 gene family have been identified and are being characterized with respect to their biochemical and physiological function. This research will provide new

insight into the processes by which plants recognize and respond to invading pests, and may facilitate the development of new strategies for crop protection.

121. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824
Plastid Biogenesis
Kenneth Keegstra
$285,769

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 are directed to one of 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. We have isolated and are now characterizing mutants containing T-DNA inserts in genes encoding selected components of the transport apparatus. 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 of a unique outer membrane protein that contains a targeting sequence.

122. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824
Studies on Hormone Action in Vegetative Growth
Hans Kende
$275,769

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. Three plant hormonesethylene, abscisic acid (ABA), and gibberellin (GA)participate in regulating growth of deepwater rice internodes. The immediate growth-promoting hormone is GA. We have investigated the effect of GA on gene expression in deepwater rice internodes and the roles of their protein products. *OsGRF1* encodes a protein that has all the hallmarks of a transcription activator. We showed that it is targeted to the nucleus and that it activates transcription in a yeast assay. *OsGRF1* belongs to a family of 10 other similar rice proteins. We are complementing our work on rice *OsGRF1* with experiments in Arabidopsis, which has nine OsGRF-like proteins (AtGRL). They function as transcriptional activators and interact in the yeast two-hybrid system with At-SYT1 and AtSYT2, proteins that interact with a human protein motif similar to the N-terminal region of AtGRL.. Because single T-DNA insertional mutants of *AtGRL* genes have not yet yielded a phenotype, we are constructing multiple mutants of *AtGRL* genes. Expression of genes encoding - and - expansins is stimulated in the rice internode by gibberellin. To determine whether expansins are, indeed, involved in regulating growth, we have expressed *OsEXP4* sense and antisense constructs under control of an inducible promoter in rice. Overexpression promoted growth of the coleoptile and mesocotyl, whereas antisense expression reduced growth.

123. 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
$245,769

Extensive physiological evidence indicates that flowering is hormonally controlled. In plants exposed to inductive photoperiods, a floral stimulus or florigen is formed in the leaves, then translocated to the shoot apical meristem. The chemical nature of the floral stimulus remains one of the unsolved problems in plant

biology. Using HPLc and MALDI-MS, we are investigating the nature of the floral stimulus by analyzing phloem exudate from vegetative and flowering *Perilla* plants for possible differences in peptide composition. Sixteen peptides have been purified and sequenced. Three of these peptides were detected only in exudates from induced plants. The genes encoding these peptides are being sequenced and their expression will be analyzed by northernblots. 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 and lupine. Recently, we have begun to label and characterize the various mRNAs present in sap from flowering plants. We hope to determine whether specific size classes of RNA are present as part of the flowering process.

124. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824
Interaction of Nuclear and Organelle Genomes
Lee McIntosh
$265,769

Plant organelles, chloroplasts and mitochondria, mediate energy transduction and thus control the biosynthesis of carbon intermediates, leading to plant yield and responses to stress. Recently, we have shown that plant mitochondria also have roles in environmental sensing and apoptosis. To understand the regulatory roles of plant mitochondria, we need to elucidate the pathways of mitochondrial /nuclear communication, especially mechanisms that allow the plant to survive environmental stress. The mitochondrial mediating signals (and receptors) that influence nuclear gene expression are virtually unknown in plants. Exploring how this communication is linked to cellular function, with emphasis upon biochemical/physiological consequences to the organism, is a primary goal of our group. To delineate these signal systems we are employing three approaches: 1) characterization of mitochondrial/nuclear signal transduction mutants in Arabidopsis, 2) genomic approaches to expression of nuclear genes regulated by mitochondrial signals, and 3) defining the plant mitochon-drial proteome.

Photosystem I (PS I) produces reduced NADPH and is closely linked to many important anabolic, biosynthetic, reactions. It contains three iron-sulfur clusters: FX, FA, and FB. There are essential genes involved in iron-sulfur cluster assembly: cysteine desulfurization, Fe and S mobilization, and regulatory genes overlaying these functions. We have isolated a number of the cyanobacterial Fe-S cluster assembly genes and are investigating their functions. Our recent use of cyanobacterial suppressor mutations also allowed us to isolate a transcription suppressor, sll0088, active during oxidative stress conditions. These investigations led to a multi-dimensional approach to PSI complex assembly.

125. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824
Molecular Mechanisms of Protein Trafficking Through the Secretory System
N. V. Raikhel
$275,769

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 used genetic and biochemical approaches to address the functional complexity of Arabidopsis syntaxins, a subgroup of SNAREs. Syntaxins comprise a large group of proteins found in all eukaryotes; they are involved in the fusion of transport vesicles to target membranes. Twenty-four syntaxins grouped into ten gene families are found in the model plant *Arabidopsis thaliana*, each group containing one to five paralogous members. To organize the nomenclature, we have developed a system of naming the plant syntaxins using the name syntaxin of plants (SYP). The Arabidopsis SYP2 and SYP4 gene families contain three members each that share 60 to 80% protein sequence identity. Gene disruptions of the yeast orthologies of the SYP2 and SYP4 gene families (Pep12p and T1gp, respectively) indicate that these syntaxins are not essential for growth in yeast. However, we have isolated and

characterized gene disruptions in two genes from each family, finding that disruption of individual syntaxins from these families is lethal in the male gametophytes of Arabidopsis. Complementation of the *syp21-1* gene disruption with its cognate transgene indicates that the lethality is linked to the loss of the single syntaxin gene. Thus, it is clear that each syntaxin in the SYP2 and SYP4 families serves an essential, non-redundant function. We also used biochemical and proteomic approaches to characterize other SNAREs.

126. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824
Cell Wall Metabolism
N. V. Raikhel, K. Keegstra, H. Kende, J. Walton
$215,769

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 the Plant Research Laboratory (Raikhel and Keegstra labs), 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 reverse 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 family of Golgi-associated 40-kD proteins that are postulated to have a role in cell wall polysaccharide biosynthesis. We have isolated mutants with disruptions in two of the genes encoding these proteins and will use these mutants to investigate the role of the 40-kD proteins, if any, in polysaccharide biosynthesis.

127. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824
Molecular Basis of Environmental Stress Tolerance
M. F. Thomashow
$255,769

Our overall interest is to understand the mechanisms that plants have evolved to tolerate environmental stresses. Our studies focus primarily on the cold acclimation response, the process whereby plants increase in freezing tolerance in response to low, nonfreezing temperatures. We recently established that cold acclimation in Arabidopsis involves rapid cold-induced expression of the CBF transcriptional activators followed by expression of CBF-targeted genes that increase freezing tolerance. We have now found that components of the CBF response pathway are conserved in flowering plants and are not limited to those that cold acclimate. We have shown that canola (*Brassica napus*), wheat, rye and tomato encode CBF-like genes that are induced in response to low temperature and that expression of Arabidopsis CBF genes in transgenic canola increases the freezing tolerance of both nonacclimated and cold-acclimated plants. Additional studies provide insight into how CBF might act to stimulate transcription. We have found that the ability of CBF1 to function in yeast is dependent upon the activities of key components of the Ada and SAGA complexes: the histone acetyltransferase (HAT) Gcn5 and the transcriptional adaptor proteins Ada2 and Ada3. Further, we have found that Arabidopsis has a homolog of the *GCN5* gene and two homologs of *ADA2* and that CBF1 physically interacts with these proteins. We conclude that Arabidopsis encodes HAT-containing adaptor complexes that are related to the Ada and SAGA complexes of yeast and propose that the CBF1 transcriptional activator functions through the action of one or more of these complexes.

128. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824

Biochemical and Molecular Aspects of Plant Pathogenesis

J. D. Walton

$275,769

Pathogenic microorganisms and plants exchange many signals that determine the outcome of their interactions. The goal of our research is to elucidate the biochemical mechanisms by which fungal pathogens successfully invade plants and the mechanisms by which plants resist attack. We study cell- wall-degrading enzymes as examples of non-specific virulence factors, including the genes that regulate enzyme production and virulence. We study host-selective toxins as examples of chemical agents of specificity and virulence. Work in our lab has elucidated the molecular genetics of HC-toxin biosynthesis and the basis of HC-toxin resistance in maize. Most recently, our work on the mode of action of HC-toxin has led to a study of the target enzyme, histone deacetylase, and the relevant genes in plants and fungi.

129. Michigan State University-DOE Plant Research Laboratory

East Lansing, MI 48824

Developmental Biology of Nitrogen-Fixing Cyanobacteria

C. Peter Wolk

$255,769

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 ecological importance. A fusion of *Anabaena variabilis* gene *avaK* to *gfp*, encoding green fluorescent protein, showed that *avaK* can serve as a molecular marker of akinete formation. We have begun to use *avaK* fusions to identify other genes that are required for the development of akinetes. A gene whose product, expressed in *Escherichia coli*, lyses walls of *Anabaena* PCC 7120, was shown to be needed for normal induction of *hepA*, a gene required for synthesis of heterocyst envelope polysaccharide. This result suggests that degradation or reconstruction of a vegetative-wall layer is required for heterocyst maturation. Inactivation of genes whose products resemble a proteinase and an endoglucanase and bind sequence-specifically to a sequence upstream from *hepC*, was shown to block heterocyst maturation and aerobic N_2 fixation. The gene mutated in a non-septate mutant of a unicellular cyanobacterium, *Synechococcus*, and showed homology to an *Arabidopsis* gene, suggesting that the latter may be involved in chloroplast division. Recent completion of sequencing of the PCC 7120 genome, work in which we collaborated, is facilitating analysis of *Anabaena* biology.

130. 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

$255,769

The plant hormone abscisic acid is derived from the oxidative cleavage of the 11,12 double bond of a carotenoid precursor. Enzymes that catalyze this carotenoid cleavage reaction, nine-*cis*-epoxy-carotenoid dioxygenases (NCEDs), have been identified in several plant species. Similar proteins are present in a variety of organisms. While some of these proteins are known to be involved in ABA biosynthesis, the function of most is unknown. Therefore, a general designation has been adopted: carotenoid cleavage dioxygenases (CCDs). There are nine putative CCD proteins in the Arabidopsis genome sequence. Based upon sequence similarity and some preliminary data, five of these CCDs are thought to be involved in ABA biosynthesis. Efforts are currently underway to determine the biochemical reactions catalyzed by the remaining four CCDs and the biological functions of the apocarotenoid products. CCD1 contains several highly conserved motifs found in other carotenoid cleavage enzymes. To determine the biochemical function of this protein, it was expressed in *E. coli* and used for *in vitro* assays. The recombinant protein was able to cleave a variety of carotenoids at the 9, 10 and 9', 10' positions. In most instances, the enzyme

cleaves the substrate symmetrically to produce a C_{14}-dialdehyde and two C_{13}-products, which vary depending on the carotenoid substrate. Based upon sequence similarity, orthologs of this gene are present throughout the plant kingdom. The characterization of these activities offers the potential to synthesize a variety of interesting, natural products and is the first step in determining the function of this gene family in plants.

131. Michigan State University

East Lansing, MI 48824-1319

Regulation of the Biosynthesis of Non-Phosphorus Membrane Lipids in Plants
Christoph Benning, Department of Biochemistry
$118,000

The galactolipids mono_ and digalactosyldiacylglycerol, as well as the sulfolipid sulfoquinovosyldiacylglycerol account for up to 80% of all polar lipids in green plant tissues. These non-phosphorous lipids are the major constituents of photosynthetic membranes (thylakoids) essential to oxygenic photosynthesis. Phosphate deprivation is one of the most potent environmental stimuli affecting the overall glycerolipid composition of plant membranes. Plants can substitute phospholipids with non_phosphorous glycolipids, even in extraplastidic membranes, under phosphate-limited growth conditions. It is one objective to understand the molecular and biochemical basis for this phenomenon as well as its physiological implications. The assembly of thylakoid lipids in plants such as Arabidopsis is highly complex and involves at least two pathways, one in the plastid and the other in the endoplasmic reticulum. Gene families encoding the enzymes involved in glycolipid biosynthesis have been identified in Arabidopsis, and recombinant lipid enzymes produced in *E. coli* provide new opportunities to study the underlying reaction mechanisms. The genetic dissection of plant lipid assembly in Arabidopsis has begun to shed light on how each of the enzymes fits into the overall two pathway scheme of thylakoid lipid biosynthesis. Unlike Arabidopsis, many plants including grass and legume species exclusively utilize the endoplasmic reticulum pathway for thylakoid lipid biosynthesis. Therefore, extending this investigation to the important crop plant maize may reveal considerable differences in glycolipid biosynthesis in these plants compared to Arabidopsis.

132. Michigan State University

East Lansing, MI 48824-1312

Biosynthesis of Triacylglycerol in Developing Oilseeds
John B. Ohlrogge, Department of Botany and Plant Pathology
$105,000

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.

133. Michigan State University

East Lansing, MI 48824-1319

Structure-Function Relationships of ADP-Glucose Pyrophosphorylase and Branching Enzyme
Jack Preiss, Department of Biochemistry and Molecular Biology
$94,000

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.

134. Michigan State University

East Lansing, MI 48824-1101

Molecular Biology and Biochemistry of Basidiomycete Laccases
C. A. Reddy, Department of Microbiology
$92,997

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.

135. Michigan Technological University

Houghton, MI 49931

Regulation of Guaiacyl and Syringyl Monolignol Biosynthesis
Vincent L. Chiang and Laigeng Li, Plant Biotechnology Research Center, School of Forestry
$100,000

Many of society's fiber and energy demands are met through the plant-based biomass conversion, in which lignin must be removed at enormous chemical and environment expense. Thus, our long-term research goal is to develop a basic understanding of monolignol biosynthesis genes and the interactive functions of their products in regulating lignin biosynthesis in plants to overcome the barriers to such conversion. As an essential step towards achieving this goal, the proposed research focuses on syringyl monolignol biosynthesis that leads to the formation of syringyl lignin, a type can be easily removed during biomass conversion. Both gymnosperms and angiosperms accumulate guaiacyl lignin derived from guaiacyl monolignol, from which syringyl monolignol is evolved in angiosperms for syringyl lignin formation. However,

the biosynthesis of this evolutionarily significant and economically important syringyl lignin has remained ambiguous. But, we previously demonstrated that two enzymes coniferaldehyde 5-hydroxylase (CAld5H) and 5-hydroxyconiferaldehyde *O*-methyltransferase (AldOMT), mediating coniferaldehyde 5-hydroxylation and methylation into sinapaldehyde, regulate a major metabolic flux diverting the guaiacyl pathway at coniferaldehyde to initiate the biosynthesis of syringyl monolignol. We then hypothesized a new model in which a novel syringyl-specific alcohol dehydrogenase is involved together with CAld5H/AldOMT in controlling the biosynthesis and utilization of sinapaldehyde for biosynthesizing sinapyl alcohol, the syringyl monolignol. In this funding period we cloned this novel gene encoding sinapyl alcohol dehydrogenase (SAD) and revealed that syringyl monolignol biosynthesis branches out at guaiacyl pathway coniferaldehyde and the reactions are regulated in sequence by CAld5H, AldOMT and SAD. This discovery overruled the traditional belief that a common enzyme cinnamyl alcohol dehydrogenase (CAD) mediates the guaiacyl and syringyl monolignol biosynthesis from coniferaldehyde and sinapaldehyde. In fact, we further confirmed that CAD is guaiacyl- or coniferaldehyde-specific. Further enzyme reaction kinetics, protein immunolocalization and lignin histochemistry evidence demonstrated that the distinct CAD and SAD functions are linked spatiotemporally to the differential biosynthesis of guaiacyl and syringyl lignins in different cell types. This funded work was featured as the cover story of the July 2001 issue of Plant Cell. Currently, we are using transgenic plants to verify whether CAld5H/AldOMT/SAD functions constitute the major metabolic path for syringyl monolignol biosynthesis *in vivo*.

136. University of Michigan

Ann Arbor, MI 48109-1048
CLV Signaling in Meristem Development
Steven E. Clark, Department of Biology
$95,000

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.

137. 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 (FY00 funds – 20 months)

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.

138. University of Minnesota

St. Paul, MN 55108-1022

Biochemistry of Ammonia Monoxygenase of Nitrosomonas

Alan B. Hooper, Department of Biochemistry, Molecular Biology and Biophysics

$218,000 (two years)

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

We have recently isolated a novel small periplasmic, soluble, red copper protein called Nitrosocyanin from Nitrosomonas europaea. It is a member of the cupredoxin family of copper proteins all of which, thus far have had electron transfer functions. Determination of its redox potential and spectral properties as well as, by our collaborators, of its three-dimensional crystal structure reveal that it is in the cupredoxin family of proteins having a particular three-dimensional fold and amino acid sequence similarity but binds copper in a manner unique for that group. The manner of copper binding, the relative ease of oxidation and the fact that the copper is found in a pocket open to the environment suggests that it may be the first member of this family to have catalytic activity. If so it might be involved in the oxidation or reduction of N-intermediates. The function of Nitrosocyanin is being investigated. Its gene's position in the genome does not hint at function.

139. University of Minnesota

St. Paul, MN 55108

Mutants of the Legume Medicago truncatula Defective in Root Hair Development and Infection by Rhizobium

Kathryn A. VandenBosch, Department of Plant Biology; in collaboration with Douglas Cook (University of California)

$131,000 (two years)

Nodulation mutants of *Medicago truncatula* that are defective in infection by the nitrogen-fixing bacterium *Sinorhizobium meliloti* are the topic of this renewal proposal. We have investigated the inheritance of mutations at 3 loci, and the phenotypes of *dmi1* and *dmi2* have been extensively characterized. Both mutants respond briefly to rhizobia through loss of polar growth of root hairs, a phenotype that is mimicked by application of the actin depolymerization drug cytochalasin. Downstream responses to *S. meliloti* do not occur, including root hair curling and infection, cell cycle activation in the root cortex, and induction of nodulin gene expression. The mutant phenotypes indicate that the affected genes may function in signal transduction. Positional cloning of the *dmi1* locus is underway. We have identified two tightly linked markers and have begun chromosome walking to this gene. We will also initiate cloning of the other two loci by using the same map-based approaches. Secondly, we will identify genes whose expression is dependent upon *DMI1*, *DMI2*, and *NSP* by employing microarray analysis of cDNA clones identified through

an independent genomics project. Expression analysis will emphasize genes likely to function in signal transduction and cytoskeletal control of cell architecture. Genes whose action is inferred to reside downstream of the mutant genes will provide targets for more extensive analysis of function. Thus, the research is part of a larger, long term strategy for defining the molecular, genetic and cell biological control points for symbiotic interactions.

140. University of Minnesota

St. Paul, MN 55108-1095

Growth and development of maize that contains mutant 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.

141. University of Missouri

Columbia, MO 65211-7411

Cellulose and the control of growth anisotropy
Tobias I. Baskin, Division of Biological Sciences
$97,000

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.

142. 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.

143. University of Missouri

Columbia, MO 65211

Genetics and Molecular Biology of Hydrogen Metabolism in Sulfate-Reducing Bacteria
Judy Wall, Biochemistry Department
$96,999

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.

144. 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)

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.

145. Mount Sinai School of Medicine

New York, NY 10029

The Respiratory Chain of Alkalophilic Bacteria
Terry Ann Krulwich, Department of Biochemistry
$115,000 (FY 00 funds)

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.

146. 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).

147. National Renewable Energy Laboratory

Golden, CO 80401

The Water-Splitting Apparatus of Photosystem II

Michael Seibert, Photoconversion Research Branch

$133,000

Photosystem II (PSII) is the site of one of the two light reactions of plant photosynthesis and is directly associated with the water-splitting process that produces O_2. The goal of this research project is to understand basic structural and functional relationships in PSII with emphasis on energy transfer, primary charge separation, and water-oxidation processes that extract electrons from water for use in carbon fixation or H_2 production. Primary charge separation occurs in the PSII reaction center (RC). Hole-burning spectroscopic studies of isolated spinach CP43, a PSII proximal antenna complex, have identified different chlorophyll (Chl) *a* trap states located on opposite sides of the complex. One may play a photoregulatory role (State A), and the other (State B) is the pigment that passes excitation energy on to the RC. Fluorescence quenching, CD and femtosecond transient absorption studies with *Chlamydomonas reinhardtii* (a green alga) site-directed mutants targeted to the PSII RC, show that the D2-H117N mutation alters energy coupling between a peripheral RC Chl and P680 (involved in charge-separation). The D2-H117 residue seems to coordinate the Chl that can donate electrons to P680 under stress conditions, Chl_z, previously thought to coordinate to the D1 protein. Flash-probe fluorescence work has demonstrated that iron cations can block the high-affinity (HA_Z), manganese-donation site in the PSII RC, which is closely related to the catalytic site of the photosynthetic water-splitting process. These results suggest similarities between iron and manganese in the initial steps of the photoactivation process that assembles the water-splitting active site. This site is in part composed of a 4-manganese cluster.

148. 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

$164,000

The green alga, *Chlamydomonas reinhardtii*, synthesizes a reversible Fe-hydrogenase enzyme when exposed to anaerobic conditions, and subsequently photoproduces H_2 gas. The objective of this research is to develop fundamental understanding about O_2 regulation of photosynthetic reductant partitioning (at the level of ferredoxin) between the hydrogenase pathway and the Benson-Calvin cycle that fixes CO_2. Our approach has been to identify and analyze mutants deficient in H_2 production. A *C. reinhardtii* library of 6,000 colonies (provided by Prof. A. Melis, University of California, Berkeley), generated by random insertion of the *Arg7* gene, was screened for clones defective in H_2 production activity using sensitive chemochromic H_2-sensor films. Sixteen colonies were identified. Four mutants that evolved the least amount of H_2 under the above assay conditions were further tested in liquid medium, using Clark electrodes. All were found to have normal rates of photosynthesis and respiration but lower rates of H_2 production than the WT. Using plasmid rescue and PCR techniques, the DNA flanking the insertion site of *Arg7* in one of the mutants was sequenced. The corresponding gene has high homology to plant isoamylases. This enzyme plays a crucial role in starch metabolism and the mutant stores less than 5% of the starch found in WT strains. Starch breakdown has been proposed to contribute some reductant for the production of H_2 in *C. reinhardtii.* It is also responsible for O_2 consumption and thus for the establishment and maintenance of anaerobic conditions required for hydrogenase induction in the dark. The isoamylase knockout mutant will lead to a better understanding of the role that starch plays in algal energetics and the production of H_2. Similar analyses are being performed with the other mutants identified as above.

149. University of Nebraska

Lincoln, NE 68588-0665
Regulation of nuclear response to mitochondrial dysfunction
Sally A. Mackenzie, Professor, Plant Genetics, Department of Agronomy
$95,001

The plant mitochondrion represents the central cellular regulator of several essential functions associated with energy metabolism and biomass production in the plant. The genetic information of the plant mitochondrial genome is organized and expressed in a manner that is quite distinct from the better characterized fungal and mammalian mitochondrial forms. This dramatic dissimilarity in mitochondrial genome form is likely due to distinct evolutionary paths taken by the plant kingdom, and more in-depth study will likely reveal important information regarding strategies for manipulating plant mitochondrial function. We wish to understand the role of nuclear genes in regulating mitochondrial genome structure, its rapid changes in organization (stoichiometric shifting) and its transmission to progeny. In our initial proposal, we had identified only a few nuclear genes in Arabidopsis that might contribute to maintenance of the mitochondrial genome and we intended to conduct functional analysis of two. We have now identified several more. We currently work with a nuclear-encoded mitochondrial DNA polymerase, likely involved in DNA replication, a mismatch repair component (MSH1), likely involved in recombination/repair mechanisms, the CHM3 locus, directing the process of stoichiometric shifting, and a multitude (more than 30) of additional loci of yet unknown functions within the mitochondrion. Astonishingly, all of these nuclear genes are encoded within a 2-Mbp gene cluster in Arabidopsis. With this discovery, we have now broadened our study to include functional analysis of several of these loci, microarray-based analysis of their expression patterns during development and in response to environmental stimuli, and cross-species comparative analysis to learn whether such a mitochondrial gene “cluster” is present and conserved in other plant species.

150. University of Nebraska

Lincoln, NE 68588-0664
Plant Formate Dehydrogenase
John Markwell and John Osterman , Department of Biochemistry
$185,000 (FY 00 funds - two years)

Formate dehydrogenase (FDH) is an enzyme that occurs in plant mitochondria. FDH is increased in response to drought and other stresses that reduce yield. Increases in FDH can be produced by treating plants with methanol and other one-carbon molecules. Recently, we have found FDH 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 whether this observation applies to other plants. FDH activity in wild-type *Arabidopsis* plants is typically 1 mU/mg protein; the highest level of FDH expression in the transformed *Arabidopsis* is approximately 5 mU/mg protein. The FDH activity in wild-type tobacco plants is barely detectable but a number of transgenic tobacco lines with FDH activities over 50 mU/mg protein have been identified. These plants will be used in water stress experiments to investigate how the increased FDH affects water use efficiency. We have begun screening *Arabidopsis* plants with T-DNA insertions for a knockout mutant in which FDH is not expressed. The *Arabidopsis* FDH promoter has been fused to two reporter genes (GFP and GUS) to study how the FDH gene is regulated both developmentally and in response to various environmental stresses and one-carbon molecules. The overall goal of this study is to substantiate that FDH is functioning as part of the plant stress-response system and to determine whether the physiological role of the FDH in the chloroplast compartment is different from that of the FDH in the mitochondrial compartment.

151. University of Nebraska

Lincoln, NE 68588-0118

The Role of a Host Protein (TIP) in the Resistance Response of Arabidopsis to Turnip Crinkle Virus Infection

T. Jack Morris, School of Biological Sciences

$99,000

Our research on mechanisms of disease induction by plant viruses of Tombusviridae Family has revealed several specific interactions between viral gene products and certain host factors. Studies on turnip crinkle virus (TCV) have shown that the viral capsid protein (CP) is the elicitor of the resistance response to TCV in *Arabidopsis*. We identified a protein from *Arabidopsis* that specifically interacts with the viral CP using a yeast two-hybrid screen. This protein, designated TIP for TCV-Interacting Protein, was found to be a member of the NAC family. NAC proteins have been implicated in the regulation of development and senescence. We also have some evidence to suggest that TIP is a transcription factor. TIP appears to be a component of the signal transduction pathway that leads to genetically specified resistance to TCV. Analysis of the TIP protein has revealed separate domains involved in the interaction with viral CP and the ability to activate transcription. Most recently, we have demonstrated that the TIP protein may also function in post-transcriptional gene silencing (PTGS), an RNA-mediated defense response of the host. In similar studies on tomato bushy stunt virus (TBSV), we have shown that p19, the host-dependent pathogenicity determinant of TBSV, is also important in sustaining viral infection by suppressing PTGS. Together these results support the conclusion that suppression of gene silencing is an essential feature of this family of plant viruses.

152. University of Nebraska

Lincoln, NE 68588-0664

Enzymology of Methane Formation from Acetate

Stephen W. Ragsdale, Department of Biochemistry

$110,000

Methanogenesis is a microbial process that occurs in most oxygen-depleted environments, such as the digestive tract of many animals. This process generates ~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.

153. University of Nebraska

Lincoln, NE 68588-0664

Role of the Rubisco Small Subunit

Robert Spreitzer, Department of Biochemistry

$96,000

The chloroplast enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) captures atmospheric carbon dioxide, thereby serving as the primary point of entry for carbon into the biosphere. The enzyme is remarkably slow, and oxygen competes at the same active site, further reducing net carbon fixation. Because Rubisco catalyzes the rate-limiting step of photosynthesis, there is much interest in engineering the enzyme as a means for increasing agricultural productivity and renewable energy resources, as well as for decreasing the greenhouse gas carbon dioxide. The structure-function relationships of the enzyme's chloroplast-encoded, active-site large subunit have been studied extensively. Much less is known about the role of the nuclear-encoded small subunit. Because there is a family of small-subunit genes in plants, it is difficult to substitute engineered subunits into the Rubisco holoenzyme. However, a photosynthesis-deficient mutant of the green alga *Chlamydomonas reinhardtii* was recently recovered that lacks both

members of the small-subunit gene family. Directed mutagenesis and transformation can now be used to answer questions about small-subunit function. Amino-acid substitutions in the small subunit have been found to suppress deficiencies in catalysis and stability that arise from a substitution in the large subunit. When created alone, these small-subunit substitutions improve the thermal stability of wild-type Rubisco. Alanine-scanning mutagenesis and construction of hybrids in the region containing the suppressors have identified small-subunit residues that can directly influence carboxylation and carbon-dioxide/oxygen specificity. Coupled with a new, high-resolution atomic structure of the enzyme, this region of the small subunit may be a fruitful target for engineering an improved Rubisco.

154. New York University

New York, NY 10003-6688

Asparagine Synthetase Gene Expression and Plant Nitrogen Metabolism
Gloria M. Coruzzi, Department of Biology
$229,000 (two years)

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.

155. 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 (FY 00 funds - two years)

Plants use a variety of biological mechanisms to regulate chloroplast function. At the gene expression level, the chloroplast is particularly strongly affected by post-transcriptional regulatory mechanisms. One common feature of post-transcriptional regulation in both unicellular and land-plant chloroplasts is the formation of RNA-protein complexes in mRNA 5' or 3' untranslated regions (UTRs). Many of these complexes have been shown to affect translation (5'UTRs), and/or mRNA stability (both 5' and 3'UTRs). Several years ago, we detected specific RNA-protein complexes within the 5'UTRs of chloroplast ATP synthase mRNAs. The long-term goal of our research is to analyze the components of these complexes and determine their function in chloroplast gene expression.

The DOE-funded portion of this project began in October 2000. There were two principal achievements during the first year. The first involved *in vivo* experiments carried out in collaboration with Dr. Lori Allison at the University of Nebraska. With her help, we discovered that the RNA regions involved in our RNA-protein complex have a significant effect on stability of reporter RNAs *in vivo*. These results provided the first indications of the function of these complexes within a living chloroplast. A second set of experiments revealed that the proteins in these RNA-protein complexes associate with 5'UTRs from many chloroplast mRNAs of disparate function. This surprising result implies that the function of these complexes may be nearly universal within the chloroplast.

156. 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
$96,000

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 understanding of the metabolic pathways involved but also the ability to predict the impact of genetic alterations on lipid and protein biosynthesis, 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 seeds of these mutants have increases in metabolic processes related to phospholipids and membrane synthesis. We have used a genomics approach to show that these mutants, but not all seed mutants, show coordinate regulation of a number of novel genes. 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 knowledge of the regulation of crucial metabolic pathways of seeds and should provide a framework for increasing and improving renewable energy resources.

157. North Carolina State University

Raleigh, NC 27695-7905

Proteolysis in Hyperthermophilic Microorganisms
Robert M. Kelly, Department of Chemical Engineering
$98,621

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. Direct purification of these from native biomass is also being pursued. Probes based on these genes have been included in targeted cDNA 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., thermal stress) on proteolysis patterns.

158. University of North Carolina

Chapel Hill, NC 27599-3280

Functions of Pseudomonas syringae avirulence genes in plant disease and disease resistance
Jeffery L. Dangl, Department of Biology
$292,000 (two years)

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 that 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, based on what we found last year regarding localization of the virulence proteins inside the plant cell, we were able to define Arabidopsis mutants that no longer respond to this virulence factor. We are nearly finished with isolation of this gene. Second, we identified one plant protein that interacts physically with the bacterial proteins, and we showed that this protein also interacts with the relevant plant disease resistance protein. Third, we finished sequencing a novel 39,500 base pair pathogenicity island that carries at least two virulence factors. We studied how this genomic island influences virulence of P. syringae. These advances will help us build a molecular model of how these pathogens cause disease.

159. 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 (FY 00 funds)

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.

160. University of North Carolina

Chapel Hill, NC 27599-3280

The role of the celC gene product in cellulose synthesis by *A. tumefaciens*

Ann G. Matthysse, Department of Biology; in collaboration with Alan R. White (North Dakota State University)

$61,000

Five genes, organised into three adjacent operons, have been shown to be required for cellulose biosynthesis in *A. tumefaciens* and have been designated *cel*A through *cel*E (Matthysse *et al*., 1995). In these experiments, the *A. tumefaciens* strain A1045 was used as a ‘wild type’ for comparison with the *cel*C mutant. This is a chvB mutant of the C58 strain and does not contain a β-galactosidase gene. In order to make the cellulose-minus mutant *cel*C, the transposon insertion used does have an active β-galactosidase gene.

The nature and role of the *cel*C gene product during cellulose biosynthesis in *A. tumefaciens* was investigated using a combination of spectroscopic, enzymatic and chemical analyses: Both arabinose and xylose were not detected during analysis of the *cel*C mutant. Furthermore, there was a marked reduction of glucose in the *cel*C mutant, relative to the ‘wild type’. FT-IR analysis suggests the presence of cellulose in both the ‘wild type’ and *cel*C mutant strains. Capillary electrophoresis indicates the presence of novel short chain sugar moieties in the *cel*C mutant strain. The *in vitro* synthesis product of the *cel*C mutant is β-(1,4)-linked, as shown by it’s susceptibility to digestion with endoglucanase-II. Bacteria deficient in the product of

the *cel*C gene can not incorporate small β-(1,4)-linked glucose units into cellulose. One hypothesis for the increased retention time of the sugar units in the *cel*C mutant seen by C.E. may be the presence of a build up of linked glucose units attached to the lipid-linked intermediate, first suggested by Matthysse *et al*. (1995).

161. North Dakota State University

Fargo, ND 58105-5517

The role of the celC gene product in cellulose synthesis by *A. tumefaciens*
Alan R. White, Department of Biological Sciences; in collaboration with Ann G. Matthysse (University of North Carolina)
$61,000

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162. Ohio State University

Columbus, OH 43210

The Role of Multiple TBP and TFB in Archaeal Gene Expression
Charles J. Daniels, Department of Microbiology
$107,000

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.

163. Ohio State University

Columbus, OH 43210

Transmethylation Reactions During Methylotropic Methanogenesis in *Methanosarcina barkeri*
Joseph A. Krzycki, Department of Microbiology
$105,000

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.

164. Ohio State University

Columbus, OH 43210

Regulation of Methane Genes and Genome Expression
John N. Reeve, Department of Microbiology
$124,000

The goal of this project is to determine how gene and genome expression are regulated in the methane-producing archaeaon Methanothermobacter thermautotrophicus (M.t.). Our studies are focused at both the level of global gene expression, and specifically on the environmental regulation of expression of methane genes, the genes that directly encode the enzymes that catalyze each step in methane biosynthesis. 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 hybridization to M.t. genomic DNA-microarrays. To reproduce and dissect the methane gene regulation documented in these in vivo studies, we have established and in vitro transcription system using only purified M.t. RNA polymerase and three M.t. general archaeal transcription factors, TATA-binding protein, transcription factor B, and transcription factor TFE. We have been able to reproduce transcription in vitro from some, but not all, M.t. promoters whose function we have characterized in vivo and therefore we are currently trying to identify and isolate the missing the methane gene transcription activators. We have also established that M.t. genomic DNA is compacted in vivo by archaeal histones but shown that archaeal histone binding to the template DNA in vitro blocks transcription. The step in transcription inhibited by histone binding in vitro is currently therefore being established, together with the identification and isolation of the M.t. factors from cell lysates that must relieve this inhibition in vivo.

165. 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 (FY 00 funds - two years)

This project addresses two key aspects of CO_2 fixation. First of all, the biochemistry, enzymology, and molecular control of a key CO_2 fixation route, the reductive tricarboxylic acid (RTCA) pathway, was

considered. 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. For these studies, we have developed the rapid-growing and genetically tractable green sulfur photosynthetic bacterium *Chlorobium tepidum* as a model system. All the key enzymes have been purified and characterized, including pyruvate synthase (PS), α-ketoglutarate synthase (KGS), and phosphoenolpyruvate carboxylase and we have isolated and determined the role of several ancillary proteins required for regulating the redox potential of PS and KGS. Indeed, two unique ferredoxins were isolated that appear to be chimeras of previously characterized low potential proteins of this class. Facilitating studies of the regulation of CO_2 fixation in *C. tepidum* was the recent completion of the genomic sequence of this organism by The Institute for Genomic Studies. Unexpectedly, a putative ribulose bisphosphate carboxylase/ oxygenase (RubisCO) sequence was found that resembles sequences previously found in archaea, however this RubisCO-like protein (RLP) was shown not to possess bonafide RubisCO activity. The second major thrust of our work was devoted to characterizing the RLP. Specific inactivation of the RLP gene in *C. tepidum* yielded an interesting phenotype; specifically it was shown that RLP plays a role in sulfur (thiosulfate) metabolism. A major consequence of the RLP mutant's diminished capacity to provide needed reducing equivalents via thiosulfate oxidation was related to the fact that RLP was shown to be part of a signal transduction mechanism that influences the ability of this organism to respond to oxidative stress. Several proteins were shown to be up-and down-regulated in the RLP mutant strain and two oxidative stress-related proteins were particularly affected. RLP thus appears to be a key player in the ability of this organism to respond to environmental stresses.

166. Ohio State University

Columbus, OH 43210-1292

The Rhodospeudomonas palustris Microbial Cell Project

F. Robert Tabita, Department of Microbiology; in collaboration with Drs. Janet L. Gibson & Thomas E. Hanson (Ohio State University), Caroline S. Harwood (University of Iowa), James C. Liao (UCLA), J. Thomas Beatty (University of British Columbia), Frank W. Larimer, Joe (Jizhong) Zhou and Dorothea Thompson (Oak Ridge National Laboratory), and Timothy D. Veenstra (Pacific Northwest National Laboratory)

$130,000

The nonsulfur purple photosynthetic (NSP PS) bacterium, *Rhodopseudomonas palustris*, is able to catalyze more processes in a single cell than any other organism thus far described. Thus, at this writing, this organism probably catalyzes more fundamentally and environmentally significant metabolic processes than any organism on this planet including anaerobic photosynthetic energy generation, aerobic and anaerobic lithoautotrophic CO_2 fixation (via the Calvin-Benson-Bassham (CBB) pathway, hydrogen evolution/uptake, sulfur oxidation, and nitrogen fixation. In addition, *R. palustris* can grow both aerobically and anaerobically on diverse one-carbon compounds, and it can also reduce various metals and oxyanions. Its abundance is most probably related to one of its unique characteristics; i.e., unlike other NSP PS bacteria, *R. palustris* can degrade and recycle components of the woody tissues of plants (wood is the most abundant polymer on earth). *R. palustris* can do this both aerobically in the dark and anaerobically in the light. Because of its intimate involvement in carbon management and recycling, *R. palustris* was selected by the DOE Carbon Management Program for genome sequencing by the JGI. Finally, *R. palustris* is capable of metabolizing a variety of complex organic substrates, again both aerobically and anaerobically, and it undergoes two defined developmental cycles related to photosynthetic energy generation or reproduction (by budding). The objective of this study is to examine how processes of global carbon sequestration (CO_2 fixation), energy generation from light, biofuel (H_2) production, plus organic carbon catabolism and metal reduction, operate and are coordinated in a single microbial cell. The recently sequenced *R. palustris* genome will serve as the raw material for these studies. We have assembled a team of investigators, from four academic and two DOE national laboratories, who share a common interest in bringing diverse approaches and types of expertise to bear on this important problem. Coordinated application of gene expression profiling, proteomics, carbon flux analysis and bioinformatics approaches will be combined with traditional studies of mutants and physiological/biochemical characterization of cells. Functional analysis of the *R. palustris* proteome and biochemical/physiological characterization will be addressed immediately; however, as the project develops, intracellular localization and modeling of the expression of the key cellular processes will also be studied.

167. Ohio State University

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

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.

168. Oklahoma State University

Stillwater, OK 74078-3035
The Structure of Pectins from Cotton Cell Walls
Andrew Mort, Department of Biochemistry and Molecular Biology
$107,000

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.

169. University of Oklahoma

Norman, OK 73019-0245
Enzymology and Energetics of Syntrophic Benzoate Metabolism
Michael J. McInerney, Department of Botany and Microbiology
$97,000

Benzoate and its coenzyme A derivative are key intermediates in the anaerobic degradation of aromatic compounds. In methanogenic environments, benzoate degradation requires the syntrophic interaction between two bacteria. One bacterium degrades benzoate to hydrogen, acetate and carbon dioxide only when the hydrogen concentration is maintained at a very low level by a second, hydrogen-using bacterium. Syntrophic benzoate metabolism represents a paradox. These associations gain energy for growth from the benzoate metabolism, yet it is not apparent how net ATP production occurs. We found that *Syntrophus*

aciditrophicus transiently produced large amounts of cyclohexane carboxylate along with several other compounds during growth on benzoate. Our data suggest that syntrophic benzoate degradation represents a third variation on how anaerobic bacteria activate and degrade benzoate. We also found that *S. aciditrophicus* ferments benzoate to acetate and cyclohexane carboxylate. Benzoate fermentation proceeds at values very close to thermodynamic equilibrium.

The discovery that syntrophic metabolism can occur at values close to thermodynamic equilibrium (□G' ~ 0 kJ/mol) rather than ceasing at the value postulated to be the smallest quantum of metabolically convertible energy (□G' ~ - 20 kJ/mol) is particularly relevant to oligotrophic environments such as deep-sea sediments and crustal rocks. If a "biological energy quantum" exists, then a significant amount of chemical energy found in these environments cannot be exploited by life. Our results indicate that bacterial metabolism can proceed near thermodynamic equilibrium and the large reservoir of chemical energy present in oligotrophic environments can be exploited by biological systems.

170. Oregon Health and Science University

Beaverton, OR 97006-8921

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.*

171. Oregon Health and Science University

Beaverton, OR 97006-8921

Cloning and Expression of Cellobiose Dehydrogenase
Michael H. Gold, Department of Biochemistry and Molecular Biology.
$94,000

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.

172. Oregon State University

Corvallis, OR 97331-2902

Regulation of the Genes Involved in Nitrification
Daniel J. Arp, Department of Botany and Plant Pathology
$97,000

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).

173. University of Oregon

Eugene, OR 97403-1229

Genetic Analysis of Chloroplast Translation in Maize
Alice Barkan, Institute of Molecular Biology
$101,000

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*, required for the translation of two chloroplast mRNAs. CRP1 is related to fungal proteins involved in the translation of mitochondrial mRNAs, and is the founding member of a large gene family in plants. Members of the CRP1 family are defined by a repeated 35 amino acid motif called a "PPR" motif. The PPR motif is closely related to the TPR motif, which mediates protein-protein interactions in a wide variety of processes and organisms. We speculate that PPR proteins likewise serve as scaffolding proteins for the assembly of protein complexes. In fact, 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 are also seeking mutations in genes encoding other members of the PPR family. Mutations in the maize "PPR-2" gene, encoding a second chloroplast member of the family, leads to the complete absence of chloroplast ribosomes. The basis for this deficiency is being explored, and molecules that interact with PPR2 are being sought.

174. Pacific Northwest National Laboratory

Richland, WA

The Rhodospeudomonas palustris Microbial Cell Project

Timothy D. Veenstra (Pacific Northwest National Laboratory); in collaboration with F. Robert Tabita, Janet L. Gibson & Thomas E. Hanson (The Ohio State University), Caroline S. Harwood (University of Iowa), James C. Liao (UCLA), J. Thomas Beatty (University of British Columbia), Frank W. Larimer, Joe (Jizhong) Zhou and Dorothea Thompson (Oak Ridge National Laboratory)

$125,001

The nonsulfur purple photosynthetic (NSP PS) bacterium, *Rhodopseudomonas palustris*, is able to catalyze more processes in a single cell than any other organism thus far described. Thus, at this writing, this organism probably catalyzes more fundamentally and environmentally significant metabolic processes than any organism on this planet including anaerobic photosynthetic energy generation, aerobic and anaerobic lithoautotrophic CO_2 fixation (via the Calvin-Benson-Bassham (CBB) pathway, hydrogen evolution/uptake, sulfur oxidation, and nitrogen fixation. In addition, *R. palustris* can grow both aerobically and anaerobically on diverse one-carbon compounds, and it can also reduce various metals and oxyanions. Its abundance is most probably related to one of its unique characteristics; i.e., unlike other NSP PS bacteria, *R. palustris* can degrade and recycle components of the woody tissues of plants (wood is the most abundant polymer on earth). *R. palustris* can do this both aerobically in the dark and anaerobically in the light. Because of its intimate involvement in carbon management and recycling, *R. palustris* was selected by the DOE Carbon Management Program for genome sequencing by the JGI. Finally, *R. palustris* is capable of metabolizing a variety of complex organic substrates, again both aerobically and anaerobically, and it undergoes two defined developmental cycles related to photosynthetic energy generation or reproduction (by budding). The objective of this study is to examine how processes of global carbon sequestration (CO_2 fixation), energy generation from light, biofuel (H_2) production, plus organic carbon catabolism and metal reduction, operate and are coordinated in a single microbial cell. The recently sequenced *R. palustris* genome will serve as the raw material for these studies. We have assembled a team of investigators, from four academic and two DOE national laboratories, who share a common interest in bringing diverse approaches and types of expertise to bear on this important problem. Coordinated application of gene expression profiling, proteomics, carbon flux analysis and bioinformatics approaches will be combined with traditional studies of mutants and physiological/biochemical characterization of cells. Functional analysis of the *R. palustris* proteome and biochemical/physiological characterization will be addressed immediately; however, as the project develops, intracellular localization and modeling of the expression of the key cellular processes will also be studied.

175. 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

$110,000

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, many representing new species or genera. Of special interest is one Antarctic isolate that only grows below 21°C and has unusual protuberances in the cells. We have used DNA from selected isolates to clone genes encoding over 20 glycosidases which, based on the gene sequence and hydrophobicity cluster analyses, belong to five different families. Our research is 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.

176. Pennsylvania State University

University Park, PA 16802-4500

Light-Energy Transduction in Green Sulfur Bacteria

Donald A. Bryant, Department of Biochemistry and Molecular Biology

$135,000

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 (GSB). Secondary goals are to develop methods for genetic analyses in GSB, to characterize novel biosynthetic pathways in GSB, and to understand the phylogenetic relationships among GSB and other eubacteria and archaea. GSB have novel Type-1 reaction centers (homodimers) and light-harvesting structures known as chlorosomes. Chlorosomes confer the ability to grow at extremely low light intensities. In collaboration with The Institute for Genomic Research, the complete 2.15 Mb genomic sequence of the moderately thermophilic, model GSB *Chlorobium tepidum* has been determined and annotated. Analysis of the sequence data has yielded the proposed biosynthetic pathways for quinones, tetrapyrroles and chlorophylls, carotenoids, and cobalamin. A rapid, highly reliable method for natural transformation of this same organism has been developed and optimized; this procedure has been used to construct knock-out mutations in more than 20 genes. Ten chlorosome proteins have been overproduced in *Escherichia coli*, and each protein was used to raise polyclonal antibodies. The resulting antisera were used to demonstrate that all chlorosome proteins are localized in the chlorosome envelope and that these proteins assemble even when BChl biosynthesis is severely inhibited. A mutant completely lacking BChl *c* has been constructed by insertional inactivation of the *bchK* gene encoding BChl *c* synthase. Vestigial chlorosomes, or “carotenosomes,” that contain carotenoids and BChl *a* but no BChl *c* have been isolated from the *bchK* mutant.

177. Pennsylvania State University

University Park, PA 16802

The control of lignin synthesis

John E. Carlson, School of Forest Resources

$91,000

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”): ρ-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. We have determined that a coniferin-specific glucosidase is expressed in lignifying (wood-forming) tissues and have cloned the complete gene transcript from one pine species. We are now cloning and sequencing monolignol-specific glucosidase genes from several tree species to determine the features that are important for activity of the enzyme, how many members exist in the gene family, and how this family of enzymes has evolved. This information will increase our understanding of how trees make lignin and wood, and may provide opportunities to create new tree genotypes for more efficient and environmentally friendly pulp and paper production.

178. 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
$111,000

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.

179. Pennsylvania State University

University Park, PA 16802-5301

Elongation Factor 1Alpha and the Plant Cytoskeleton
Richard J. Cyr, Department of Biology
$193,000 (FY 00 funds - 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.

180. Pennsylvania State University

University Park, PA 16802-4500

Biochemistry and Genetics of Acetate Conversion to Methane in Methanosarcina thermophila
James G. Ferry, Department of Biochemistry and Molecular Biology
$268,000 (two years)

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.

181. Pennsylvania State University

University Park, PA 16802

Electron Transfer Cofactors in Type I Reaction Centers of Anoxygenic Bacteria
John H. Golbeck, co P.I. Ilya Vassiliev, Department of Biochemistry and Molecular Biology
$202,000 (two years)

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*.

182. 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
$95,999

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.

183. 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
$120,000

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.

184. University of Pennsylvania

Philadelphia, PA 19104-6018

Light Responses and Photoperiodism in Arabidopsis thaliana
Anthony R. Cashmore, Plant Science Institute, Department of Biology
$164,000

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.

185. University of Pennsylvania

Philadelphia, PA 19104-6018

Membrane-Attached Electron Carriers in Photosynthesis and Respiration
Fevzi Daldal, Department of Biology
$118,000

The long-term goal of our project is to understand the structure, function, regulation and biogenesis of electron carrier cytochromes (cyt), using the facultative phototrophic bacteria of *Rhodobacter* species as model organisms. During the past year, our structure-function studies have been focused on the membrane-anchored electron carrier cyt c_y that convey electrons from the bc_1 complex to the reaction center (RC) in photosynthesis (Ps), and to the cbb_3-type cyt *c* oxidase in respiration (Res). It is now clear that *R. capsulatus* cyt c_y^{Rc} has homologues in many bacterial genomes, but whether these homologues play similar roles is unknown. We therefore initiated in-depth analyses of the closely related *R. sphaeroides* cyt c_y^{Rs}, and found that the physiological functions of cyt c_y^{Rc} and cyt c_y^{Rs} are different. While cyt c_y^{Rc} is an efficient electron carrier in both Ps and Res, cyt c_y^{Rs} only functions in Res. Molecular features underlying this difference are being pursued. We have also shown for the first time that cyt c_y^{Rs} is an electron donor to both the aa_3- and the cbb_3-type cyt *c* oxidases in *R. sphaeroides.* In our studies on the biogenesis of *c*-type cyts we have pursued analyses of the molecular usher CycH/CcmH that is required for efficient delivery of apocyts from the protein translocation to the heme incorporation pathways. We have also identified CycJ/CcmE and CcdA/DsbD as two novel components of cyt *c* biogenesis in *R. capsulatus*. CycJ is a heme chaperone routing heme from its transporter to the ligation site, and CcdA is a redox conveyer for reducing equivalents from the cytoplasm to the periplasm. Our ongoing work deals with the revertants of mutants lacking these components, and is geared to ultimately uncover all *R. capsulatus* components involved in cyt biogenesis, which is an important biological process essential for cellular energy transduction.

186. University of Pennsylvania

Philadelphia, PA 19104-6018

The function of the EARLY TRICHOMES gene in Arabidopsis in maize
Scott Poethig, Department of Biology
$102,000

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.

187. University of Pennsylvania

Philadelphia, PA 19104-6018

Genetic and Biochemical Analyses of an Archael Protein Translocation System
Mechthild Pohlschroder, Department of Biology
$98,000

Protein translocation plays a key role in all living organisms. Many secreted proteins, including an ever increasing number of extracellular archaeal enzymes, are useful for biotechnology. Currently, these proteins are expressed in bacteria and eukaryotes in order to obtain high yields of biologically active products. However, production of archaeal proteins in heterologous hosts from different domains of life can

be significantly decreased due to inefficient expression and incorrect post-translational modification, as well as inefficient secretion of the proteins. Biochemical and genetic analyses of the bacterial and eukaryotic systems have shown that proteins cross membranes via an evolutionarily conserved pore, while the mechanisms and energetics seem to be different. Archaea contain a combination of homologs of the bacterial and eukaryotic translocation components. Furthermore, they lack homologs of the essential bacterial and eukaryotic translocation ATPases, suggesting that some aspects of translocation in archaea are unique. Thus, understanding the mechanism by which proteins cross the cytoplasmic membrane of archaea is vital for the design of archaeal expression vectors. We are studying protein translocation in *Haloferax volcanii*, a halophilic archaeon amenable to biochemical and genetic analyses. Complementing our current analysis of *H. volcanii* homologs of bacterial and eukaryotic translocation components, this proposal focuses on the identification and characterization of archaeal protein translocation components that cannot be readily identified by homology searches and co-purification studies. In addition we will attempt to determine the mechanism of protein translocation across archaeal membranes.

The specific aims of the research are: 1) to use multiple genetic screens and selections based on approaches that have been successful in bacterial and eukaryotic systems in order to identify components of the archaeal translocation machinery; 2) to characterize the translocation components using molecular biological and biochemical approaches; and, 3) to distinguish between co- and post-translational translocation using in vivo and in vitro approaches. Understanding the archaeal protein translocation may also help to further the protein translocation studies in all organisms and aid in the optimization of protein translocation in already existing bacterial and eukaryotic expression hosts.

188. University of Pennsylvania

Philadelphia, PA 19104-6018

AVP1-type and AVP2-type Pyrophosphate-energized Proton Pumps

Philip A. Rea, Department of Biology

$110,000

This program of research is concerned with elucidating the bioenergetic and physiological impact of vacuolar pyrophosphate- (PPi-) energized proton pumps (V-PPases). These pumps were originally thought to be restricted to plants and some phototrophic bacteria and comprise a category of structurally and functionally uniform membrane proteins. Recent studies, however, have established that V-PPases are not only ancient, widespread and pertinent to the metabolism of many organisms including archaebacteria, proteobacteria and parasitic protists but also fall into two structurally and functionally distinct subcategories, termed type I and type II. With the objective of defining for the first time the impact these novel energy transducers have on the intact organism we have, using *Arabidopsis thaliana* as a model plant system, recently succeeded in isolating T-DNA insertion mutants for all of the key genes. Specifically, we have three types of null mutant, designated *avp1-1*, *avp2-1* and *avp3-1* respectively, for the sole gene encoding the type I V-PPase AVP1 (*Arabidopsis* V-PPase 1) and both of the genes encoding the type II V-PPases, AVP2 and AVP3. All three single mutants exhibit a phenotype, but those of the *avp2-1* and *avp3-1* mutants are the most pronounced. Thus, *avp1-1* homozygotes are less robust than wild-type and show indications of silique sterility, *avp2-1* mutants are inviable as homozygotes and dwarfed as heterozygotes, and *avp3-1* mutants are dwarfed as homozygotes. Having isolated and molecularly characterized these mutants, our efforts are now directed at determining the membranes and tissues with which these three AVPs are most closely associated and characterizing each class of mutant with respect to vacuolar energization, cellular PPi regulation, sucrose mobilization, salt tolerance and susceptibility to anaerobiosis. Our working hypothesis is that both type I and type II V-PPases maximize energy use-efficiency by deploying PPi, a by-product of a broad range of biosynthetic processes, for chemiosmotic work but in different membrane systems.

189. Purdue University

West Lafayette, IN 47907-1155

Mechanisms of bioynthesis of cereal mixed- linkage β-glucans
Nicholas C. Carpita, Department of Botany and Plant Pathology
$102,000

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

190. Purdue University

West Lafayette, IN 47907-1153

Ferulate-5-hydroxylase: requirements for expression and activity
Clinton C. S. Chapple, Department of Biochemistry
$200,000 (FY00 funds - 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.

191. Purdue University

West Lafayette, IN 47907-1165
Engineering Plant One-Carbon Metabolism
David Rhodes, Department of Horticulture and Landscape Architecture
$38,364

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.

192. Purdue University

West Lafayette, IN 47907-1392
The Impact of Environmental Stress on the Regulation of Photosynthesis
Louis A. Sherman, Department of Biological Sciences
$166,550

We have developed a full genome microarray for the unicellular, transformable cyanobacterium, Synechocytis sp. PCC 6803. We utilized a two-stage amplification strategy that minimizes cost and the long-term effort in array construction, with additional advantages for producing knock-out mutants or overexpressing proteins. The first-round of PCR amplification for each gene was carried out with a primer consisting of two components: an adaptamer sequence common to all 3,168 genes and a sequence specific for each gene (with a maximum length up to 2.0 kb). This two-stage amplification strategy proceeded virtually perfectly, and we used this DNA to print over 200 full-genome Synechocystis microarrays on glass slides.

Cyanobacteria are photosynthetic organisms that respond to a multitude of environmental stimuli, including light and nutritional stresses. The Synechocystis microarray are currently being used to analyze differential gene expression when cultures are exposed to different perturbations such as lack of iron, nitrogen or specific oxidative stresses. Gene expression patterns will also be compared among numerous mutants we have constructed in genes involving regulation and assembly of the photosynthetic apparatus. A key objective is to identify regulatory genes involved with redox control of photosystem I and II stoichiometry and the genes of the thioredoxin superfamily and their targets. To this end, we have made mutants in genes that encode many of the thioredoxin and disulfide-bond forming genes. We are working to determine the

differential gene expression patterns of cells grown in iron-sufficient and iron-deficient conditions, as well as of numerous mutations.

193. Purdue University

West Lafayette, IN 47907-1392
Identification of Actin-Binding Proteins from Maize Pollen
Christopher J. Staiger, Department of Biological Sciences
$98,000

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 F-actin affinity chromatography (FAAC) to isolate and characterize actin-binding proteins from maize pollen. Several putative actin-binding proteins have been isolated and are being identified by MALDI-TOF and tandem mass spectrometry. 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 are also using affinity chromatography to isolate a ubiquitous regulator of actin polymerization, the Arp2/3 complex. This complex of 7 polypeptides is likely to be the major nucleator of actin filaments, and understanding its biochemical properties and subcellular localization will yield further insight to the role of actin during tip growth.

194. Rensselaer Polytechnic Institute

Troy, NY 12180-3590
A Novel, Photosynthesis-Associated Thioredoxin-Like Gene
Jackie L. Collier, Department of Biology
87,000

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.

195. Rice University

Houston, TX 77005-1892
Complex Regulatory Controls of TCH Gene Expression
Janet Braam, Department of Biochemistry and Cell Biology
$101,999

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.

196. 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 (FY 00 funds - 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.

197. 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 (FY 00 funds - 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 further understand the dockerin-cohesin interaction, we undertook a solution NMR study to determine the structure of the dockerin domain from the *C. thermocellum* cellobiohydrolase CelS, the most abundant catalytic subunit. We previously reported that the dockerin domain secondary structure consists of a pair of Ca^{2+}-binding loop-helix motifs, in contrast to the helix-loop-

helix EF-hand motif. We also demonstrated that Ca^{2+} is required by the dockerin domain to fold into a stable tertiary structure. Here we report the three-dimensional structure of this extracellular, prokaryotic Ca^{2+}-binding domain. The structure consists of two Ca^{2+}-binding loop-helix motifs connected by a linker; the E helices entering each loop of the classical EF-hand motif are absent from the dockerin domain. Each dockerin Ca^{2+}-binding subdomain is stabilized by a cluster of buried hydrophobic side chains. Structural comparisons reveal that, in its non-complexed state, the dockerin fold displays a dramatic departure from that of Ca^{2+}-bound EF-hand domains. A putative cohesin-binding surface, comprised of conserved hydrophobic and basic residues, is proposed, providing new insight into cellulosome assembly.

198. The Rockefeller University

New York, NY 10021-6399

Function of Rac GTPases in Plants

Nam-Hai Chua, Lab of Plant Molecular Biology

$102,000

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.

199. 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

$92,552

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.

200. Rutgers University

Piscataway, NJ 08854-8020

Corn Storage Protein - A Molecular Genetic Model
Joachim Messing, Waksman Institute
$118,000

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.

201. The Salk Institute for Biological Studies

La Jolla, CA 92037

Signal Transduction Pathways that Regulate CAB Gene Expression
Joanne Chory, Plant Biology Laboratory
$120,000

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 most recent studies indicate that the signal is the accumulation of Mg-Protoporphyrin IX, a key intermediate in the pathway. We are continuing genetic studies to better understand the sensor for Mg-ProtoIX, as well as the components of the signaling pathway. 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.

202. 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 (FY00 funds - 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 have revealed hundreds of genes that are regulated by HLS1 acetyltransferase. Identification and characterization of the substrates responsible for *hls1* pheno-types 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. Recent cloning of *HSS* confirms the interaction of ethylene and auxin in regulating plant growth.. Biological functions for three homologs of *HLS1* (*HLH1, HLH2 and HLH3*) can now be investigated as we have identified mutations in each of 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.

203. The Salk Institute for Biological Studies

San Diego, CA 92186-5800
Regulation of the floral homeotic gene AGAMOUS
Detlef Weigel, Plant Biology Laboratory
$112,000

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.

204. The Scripps Research Institute

La Jolla, CA 92037
Membrane Targeting of P-type ATPases in Plant Cells
Jeffrey F. Harper, Department of Cell Biology
$122,000

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; *A*rabidopsis *t*haliana 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.

205. The Scripps Research Institute

La Jolla, CA 92037

Nuclear Genes Regulating Translation of Organelles mRNAs

Stephen P. Mayfield, Department of Cell Biology

$101,313

Our research is focused on understanding the basic tenets of translation in the chloroplast. Chloroplast gene expression is regulated primarily during translation, so an understanding of this process is essential to understanding photosynthetic function and plant development. Understanding translation in plants also has practical applications, as chloroplasts can be used for the production of recombinant proteins. The expression of recombinant proteins in plants has the potential to become a major source of proteins for use as human therapeutics. We are developing an efficient system for the production of recombinant proteins in the chloroplast of micro algae. This expression system is based on RNA elements and the corresponding translation factors characterized from our studies on *C. reinhardtii* chloroplast translation. For expression of recombinant proteins we have concentrated on monoclonal antibody expression. Monoclonal antibodies have enormous potential for pharmaceutical use, and these proteins can be expressed as simple single chain binding domains, or as multi-protein complexes, thus allowing us to examine both expression and assembly of a class of well defined proteins. We have succeeded in expressing a single chain antibody that recognizes the herpes simplex virus. This antibody accumulates in chloroplast and appears to fold into a fully functional antigen binding protein. We are presently examining synthesis and accumulation of more complex antibodies in chloroplasts.

206. University of South Carolina

Columbia, SC 29208

Regulatory role of ANT in organ initiation and growth

Beth A. Krizek, Department of Biological Sciences

$100,000

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.

207. Southern Illinois University

Carbondale, IL 62901-6508

Regulation of Alcohol Fermentation by Escherichia coli

David P. Clark, Department of Microbiology

$107,000

We are studying the regulation of fermentation in the bacterium *Escherichia coli*. The synthesis of alcohol is due to the AdhE protein, which is inactivated in air. The *adhE* gene is expressed in response to the build-up of NADH that occurs in the absence of oxygen. The mechanism of induction of *adhE* has been analyzed by using gene fusions between the regulatory region of *adhE* and the structural gene for beta-galactosidase (*lacZ*). Induction is due to the AdhR activator protein and appears to be modulated by the Cra carbohydrate

regulator. Both proteins bind to the DNA in the *adhE* regulatory region, but AdhR only binds in the presence of NADH. Inactivation of *adhR* by insertion of a kanamycin resistance cassette decreases expression of *adhE* and lowers levels of alcohol dehydrogenase so preventing growth by alcohol fermentation. Several other genes regulated by AdhR, which are only expressed anaerobically in rich medium, have been isolated and their role in fermentation is being investigated. Lactate is synthesized by lactate dehydrogenase, encoded by the *ldhA* gene. This occurs 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 the ArcAB system as well as several regulatory genes involved in sugar metabolism. The *ldhA* upstream region is presently being dissected by PCR to locate the promoter and regulatory sites.

208. Stanford University

Stanford, CA 94305-5020

R. meliloti-Medicago nodulation genes and signals: genetic and genomic approaches
Sharon R. Long, Department of Biological Sciences
$276,000

Economic, political and ecological factors all point to the desirability of decreasing U.S. dependence on foreign sources of oil. Chemical production of nitrogen-based fertilizers is an energy costly process that consumes vast quantities of fossil fuel. Nitrogen fixing symbioses provide an approach to more efficient and sustainable use of resources. Our lab employs molecular genetic approaches to unravel the interactions between the nitrogen-fixing bacterium *Sinorhizobium meliloti* and its legume hosts including alfalfa. As a result of this interaction, plants are able to grow in nitrogen poor soils without added fertilizer, thus reducing energy input required for agricultural production.

This year the results of our bacterial genome sequencing efforts were published in *Science* and *Proceedings of the National Academy of Sciences*: we determined the complete DNA sequence of the 1.35 Mb megaplasmid that encodes most of the required symbiosis genes. We are preparing to mine the information contained in that DNA sequence by analyzing global gene expression. We are particularly interested in determining the regulatory circuitry that dictates how the bacterium recognizes and responds to its plant hosts.

We also have further characterized early plant responses to its symbiotic partner by genetic and cell biology approaches. Our analyses of plant genes and factors required for nodulation and for root hair calcium response were published in *P.N.A.S.*, *The Plant Cell*, and *Plant Physiology*. Better understanding of the mechanistic details of the symbiosis should lead to improvements in efficiency and freedom from dependence on chemical fertilizer.

209. Stanford University

Stanford, CA 94305-5020

Global Characterization of Genetic Regulatory Circuitry Controlling Adaptive Metabolic Pathways
Harley H. McAdams
$255,000 (15 months)

An interdisciplinary team of scientists from Stanford, Harvard, and SRI International is collaborating to identify and characterize genetic regulatory circuitry and metabolic pathways of the aquatic bacterium, Caulobacter crescentus. The team will measure the global physiological responses of C. crescentus cells and cultures during starvation, during adaptation to exposure to toxic chemicals, during exposure to alternative, environmentally-relevant catabolic substrates, and in biofilms. The initial focus will be to identify the overall regulatory and metabolic networks in C. crescentus, largely through gene expression microarray assays of the wild type strain and bioinformatics analysis. For example, gene expression of wild type cells will be assayed for a selected set of time courses when subjected to diverse environmental conditions, such as different nutrient levels, transition into and out of stationary state, sudden exposure to several stresses and to changed nutrients in the environment, and growth as biofilms. Sophisticated statistical techniques will be used to analyze the timing patterns in these datasets to predict sets of genes that are regulated as cascades or cassettes. Engineering microbial cells to be effective bioremediation agents requires detailed

understanding of the regulation and metabolism of the bacterial cell, including how the cells process environmental stimuli and turn that information into a coordinated, adaptable metabolic response. This project will provide detailed insight into C. crescentus regulation and behavior in conditions more similar to the "wild" environment than normally present in laboratory experiments. This will provide a powerful base for eventually engineering situation-specific "cassettes" into C. crescentus cells for targeted remediation applications.

210. 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)

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.

211. University of Tennessee

Knoxville, TN 37996-0845

Plant recognition of rhizobial Nod factors
Gary Stacey, Department of Microbiology
$91,000

The nitrogen-fixing bacterium, *B. japonicum,* infects soybean roots and induces the formation of a nodule, a new organ, in which the bacteria reside. Substituted lipo-chitin molecules synthesized by the symbiont induce organogenesis. The potency of these molecules, as well as their high specificity, suggests the presence of plant receptors. We isolated cDNA clones encoding for Nod factor binding proteins (i.e., apyrases) from soybean and the model legume, *Medicago truncatula*. These genes are rapidly induced upon bacterial inoculation of either soybean or *Medicago*. Our work suggests that legumes possess more than one receptor for Nod factors and that one of these receptors may have evolved from a general chitin recognition pathway that exists in all plants. Biochemical studies identified an 85-kDa protein in soybean membranes that bound chitin with high affinity. The lipo-chitin Nod signal likely binds to this receptor but with low affinity. This chitin receptor is involved in activation of a plant defense response upon chitin elicitation. Indeed, both chitin oligomers and lipo-chitin Nod signal elicit defense responses in soybean. The latter results suggest that a plant defense response could be important during normal soybean nodulation. We are currently investigating this possibility using a variety of approaches, including transgenic plants and DNA microarray studies. 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.

212. University of Tennessee

Knoxville, TN 37996-1100

Mechanism of Regulated Protein Transport between Nucleus and Cytoplasm
Albrecht G. von Arnim, Department of Botany
$102,000

Subcellular protein localization is a fundamental regulatory mechanism, which plays an important role in guiding the activity of many proteins that regulate nuclear gene expression. 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 physically separable nuclear and cytoplasmic localization signals, which appear to mediate the nuclear import of COP1 in darkness and its apparent nuclear exclusion under light conditions. In addition, COP1 possesses a subnuclear localization signal, which targets COP1 to nuclear speckles resembling those associated with nuclear photoreceptors. The cytoplasmic localization signal contains motifs resembling nuclear export signals. Site directed mutagenesis is consistent with this notion and is now being employed to address the physiological significance of the striking nuclear exclusion of COP1. It is plausible that COP1 localization is regulated by dimerization or by interactions with other cellular proteins. In part to test this hypothesis, we have begun to develop a novel tool to investigate protein-protein interactions, termed BRET for Bioluminescence Resonance Energy Transfer. Supported initially by DOE funds, this method allows us to document protein-interactions in live plant cells and in real time.

213. 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 □-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 □-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, the subunit structure of the related leukocidin pore has been determined. By contrast with □-hemolysin, the leukocidin pore contains four each of two different subunits. Thus, interactions at interfaces between the subunits control their number and organization, and manipulation of the subunit interactions will allow further control in the engineering of these pores. We have also begun to examine single-chain variants of □-hemolysin and 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.

214. Texas A&M University

College Station, TX 77843-3258

Regulation of Development and Nitrogen Fixation in Anabaena
James W. Golden, Biology Department
$197,000 (two years)

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 suspected antiquity of heterocyst development, and the contribution of fixed nitrogen to the environment. 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. Our research, initially focused on transcriptional regulation by group 2 sigma factors, is being expanded to group 3 sigma factors and their regulators. We are also exploiting extragenic suppressors and epistatic analysis to elucidate genetic interactions between key regulatory genes such as patS, hetR, and hetC in signaling and developmental pathways.

215. 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 (FY00 funds - 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. We are developing a green fluorescent protein tag 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. We anticipate that this *in vivo* imaging tool will enable many additional questions to be addressed as well. We are also determining whether proteins that bind to the *psbA* gene(s) and the *psbAI* mRNA are important for the complex regulatory events known to control D1 synthesis.

216. Texas A&M University

College Station, TX 77843-3255

Genetic Probes of Acetyl-CoA Synthase Cluster Assembly Mechanisms
Paul A. Lindahl, Department of Chemistry
$211,000 (two years)

Some enzymes contain complex metal clusters, which often serve as active sites or electron transfer agents. Due to their complexity, metallochaperone or accessory proteins are required to assemble and insert such clusters into the host enzyme. Large gaps remain in understanding of how such processes occur. Such knowledge would be important in biotechnology applications involving recombinant metalloenzymes induced in heterologous host organisms. The enzyme investigated in this project, acetyl-Coenzyme A synthase (ACS) contains two novel Nickel-Iron-Sulfur active sites called the A- and C-clusters. ACS is naturally found in homoacetogenic bacteria but the acsAB genes encoding it can be overexpressed in *Escherichia coli* to yield active recombinant ACS. How this occurs is uncertain, as *E. coli* does not contain specific accessory proteins required for cluster assembly or insertion. The objective of this project is to elucidate the mechanism by which these centers assemble. Towards this end we have cloned and overexpressed a gene (acsF) that appears to encode an enzyme that inserts Ni into ACS. Sequence alignment shows that 1 histidine and 2 cysteine residues are conserved in AcsF, as well as a “P-loop” region used to bind nucleotide triphosphates and catalyze their hydrolysis. One homolog called CooC, is an ATPase/GTPase that inserts Ni into a precursor form of the C-cluster of the ACS-homolog from *Rhodospirillum rubrum*. Purified AcsF lacked Ni and Fe, and slowly catalyzed ATP hydrolysis. Such similarities to CooC suggest that AcsF inserts Ni into a Ni-deficient form of ACS using the free energy of ATP hydrolysis.

217. 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 (FY 00 funds)

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.

218. 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
$102,000

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.

219. Texas Tech University

Lubbock, TX 79409-1061

Ferredoxin-Dependent Plant Metabolic Pathways
David B. Knaff, Department of Chemistry and Biochemistry
$95,000

The active sites of oxidized thioredoxins contain a single disulfide. In plants, thioredoxins f and m are located in the chloroplasts, where the reduced forms regulate the activity of several enzymes. The redox

properties of the three regulatory disulfides of sorghum malate dehydrogenase (MDH) have been characterized in detail. The effects of mutations that alter the substrate specificity of MDH 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 mutants and their ability to bind to FBPase have been characterized, to provide information about the binding domains of chloroplast thioredoxins for the enzymes that they regulate. The ability of thioredoxin to regulate sulfur assimilation by plants, at the level of APS reductase, has been demonstrated and the role of this response in plant defense against oxidative stress has been characterized.

The gene encoding the chloroplast form of glutamate synthase has been sequenced and a possible pathway for the evolution of this enzyme has been proposed. X-ray diffraction studies on crystals of spinach chloroplast glutamate synthase have produced data of sufficient quality to determine the structure of the protein.

220. 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
$102,000

We have produced an excellent antibody (ab) to callose synthase. Using this antibody along with the CesA ab to cellulose synthase, we have investigated the role of proteases in the activation of callose synthase upon wounding. We found that 5 min after wounding, cellulose synthase ab's no longer labeled tissue near the wound site. Instead, callose synthase labeled these tissues. If tissues were wounded in the presence of protease inhibitors, cellulose synthase ab's continued to label the wound regions up to 30 min. This work is being prepared for publication. We also prepared membrane sheets from cotton cytoplasts and successfully labeled the cellulose synthases with ab's. In addition, we achieved *in vitro* cellulose synthesis from these sheets which was monitored by staining with Tinopal. These results are being prepared for publication. We expressed a chimeric protein containing the central globular region of cotton fiber cellulose synthase and the N-terminal and C-terminal regions of the *Acetobacter xylinum* cellulose synthase in *Acetobacter xylinum.* Unfortunately, the membrane fractions containing the chimeric protein showed no cellulose synthase activity based on the formation of a crystalline product. We are presently investigating the possibility of a non-crystalline glucan product being produced. We attempted to clone the entire CesA sequence of cotton cellulose synthase into *E. coli*, but this did not work; however, we used a new vector and were able to obtain a full length clone of CesA. We are now investigating the activity of this preparation. Purification and sequencing of cellulose and callose synthase-associated proteins continues, and these will be tested for function in cellulose and callose biosynthesis.

221. University of Texas

Austin, TX 78712-1167

Phosphorylation of Plant Protein Synthesis Initiation Factors
Karen S. Browning, Department of Chemistry and Biochemistry
$97,000

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.

222. University of Texas

Austin, TX 78712

Function of the Ubiquitin Protein Ligase SCF-TIR1 During Auxin Response
Mark Estelle, Institute for Cellular and Molecular Biology
$115,000

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.

223. 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)

As part of our DOE-funded project "Sequencing of Chromosome 10 of Rice and Validation of Annotation Methods for Rice", we have submitted ~29 Mb of rice genomic DNA for high throughput sequencing to our sequencing core facility. We have deposited over 24 Mb of sequence to Genbank, the public DNA database that provides access to the entire scientific community. 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 species and we have developed several tools and resources to allow the greater plant community to leverage our data to other organisms. First, we have used *in silico* techniques to anchor the physical map to the genetic map. Second, we have annotated finished and unfinished rice sequences and provided this annotation to the public through releases to Genbank (finished only) and through the TIGR Rice Genome Project web page (finished and unfinished; http://www.tigr.org/tdb/e2k1/osa1/annotation.shtml). This annotation provides biological interpretation of the DNA sequence thereby allowing for more rapid interpretation of the significance of our rice sequence data. We also provide a low level of annotation by searching all the rice sequences against the 13 TIGR Plant Gene Indices which are clusters of Expressed Sequence Tags. We have generated a Rice Repeat Database that is available to the public for either BLAST searching or as a multi-fasta file via anonymous FTP. 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.

224. The Institute for Genomic Research

Rockville, MD 20850-3319

Structural and Functional Analysis of a Minimum Plant Centromere
C. Robin Buell; in collaboration with Jiming Jiang (University of Wisconsin)
$310,436

The centromere, a chromosomal landmark governing the proper segregation of chromosomes during mitosis and meiosis, is the site of sister chromatid attachment and kinetochore assembly. Characterization of centromeric DNA is not only essential to understanding the structure and organization of plant genomes, but it is also a critical step in the development of plant artificial chromosomes. The centromeres of several model eukaryotic species, including human and *Arabidopsis thaliana*, consist predominantly of long arrays of satellite DNA. To date, technical limitations have prevented the construction of a DNA contig that spans the entire centromeres in these species. Thus, the centromeric regions have been left as "gaps" in the sequencing data for all five *A. thaliana* chromosomes. We have found that the DNA structure of rice centromeres differs from the "single repeat-dominated characteristics" of *A. thaliana* centromeres. We have found that the centromere of rice chromosome 8 contains only about 50 kb of RCS2 repeat, a centromere-specific satellite repeat that is the counterpart of the pAL1 satellite in *A. thaliana* and the alpha satellite in humans. In our DOE-funded project "Structural and Functional Analysis of a Minimum Plant Centromere", we have proposed to sequence the centromere of rice chromosome 8 using a combination of cytogenomics with high throughput sequencing. Once we have the entire sequence that spans this centromere, we will characterize the components of a minimal plant centromere.

225. 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 (FY00 funds - 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.

226. Virginia Polytechnic Institute & State University

Blacksburg, VA 24061-0308

Enzymology of Acetone-Butanol-Isopropanol Formation
Jiann-Shin Chen, Department of Biochemistry
$106,000

A bacterial process known as solvent fermentation produces acetone, butanol, and isopropanol. These chemicals are traditionally used as industrial solvents and feedstocks, but they are also ingredients of healthcare and consumer products and are useful additives in gasoline-ethanol blends. Butanol and isopropanol are now manufactured from the petrochemical propylene, and their production from biomass should be useful. Acetone is now a byproduct of the phenol industry and does not need another source. In

some bacteria, acetone is converted to isopropanol to become more useful. For over half a century, solvent fermentation was an important industrial process for the production of butanol and acetone. *Clostridium acetobutylicum* was initially used, but it was replaced by other species after molasses replaced corn as the raw material. *Clostridium beijerinckii*, some of which produce isopropanol, remained in use until the late 1970s. Although solvent fermentation was a successful commercial operation, several problems can hinder the revival of this industry. These problems relate to a lack of knowledge to prevent the bacteria from losing productivity, to extend the solvent-producing mode of metabolism, and to control the product ratio to add value. To help alleviate these problems, we study the enzymes and genes that are crucial to solvent production. Our current focuses are on the function of alcohol dehydrogenases, the enzymes that are responsible for the formation of butanol and isopropanol, and the regulation of genes for solvent-production and nitrogen-fixation. We have identified a connection between nitrogen metabolism and solvent production, and we will pursue this lead.

227. University of Virginia

Charlottesville, VA 22903-2477

Protein Structure in Catalytic Function of NADPH: Protochlorophyllide Oxidoreductases
Michael P. Timko, Department of Biology
$210,001 (two years)

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, form a stable ternary complex that accumulates to high levels in the prolamellar bodies (PLB) and prothylakoids 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. There is an increasing amount of evidence indicating that PChlide formation and reduction play an important role in the regulation of plastid development and overall photomorphogenesis. However, the basis for the light-dependency of the POR catalyzed reaction and the details of the reaction mechanism are unknown. Using bacterially (*E. coli*) expressed recombinant wild-type and mutant POR proteins detailed kinetic analysis, chemical modification studies, X-ray diffraction analysis of the protein is underway in an attempt to determine the residues/domains within POR required for PChlide and NADPH binding and structural constituents of the protein, pigment, and cofactor that control the assembly of photoactive POR-PChlide-NADPH ternary complexes. In our studies we hope to determine the active site residues necessary to stabilize substrate and cofactor in a conformation that permits their specific interaction upon illumination, and to elucidate the reaction mechanism leading to Chlide formation. Within this framework, experiments are being carried out to clarify the role of Tyr-275, Lys-279, and Cys-281 in the reaction and to identify additional residues within POR necessary for the formation of the enzyme photoactive state and required in photoreduction. We also are investigating the contributions made by the unique 35-residue loop region (termed URL; amino acids 231 to 265) and carboxy-terminus of protein to POR function with particular emphasis on their possible role in pigment binding or in modulating intermolecular interactions. Finally, we are analyzing 2D crystals of the enzyme grown on lipid monolayers and defining conditions for the growth of crystals in solution suitable for X-ray diffraction analysis. When completed, our studies should provide significant new information on structure-function relationships in an enzyme mediating one of the most crucial biosynthetic steps in the development of photosynthetic organisms.

228. Washington State University

Pullman, WA 99164-6340

Lipid Signaling and Membrane Function in Mutants of Arabidopsis
John A. Browse, Institute of Biological Chemistry
$113,000

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. In collaboration with

scientists at Monsanto, we have also identified a novel pathway of polyunsaturated fatty acid synthesis catalyzed by a polyketide synthase in the microalga *Schizochytrium*. 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 oaf 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 though not by OPDA. Cloning of the mutant locus indicated that it encodes an isozyme of OPDA reductase, designated OPR3. Characterization of the *opr3* mutant demonstrated that JA itself is required for fertility. However, the JA-precursor OPDA is able to activate defense genes and *opr3* plats are resistant to insect attack and to fungal pathogens.

229. Washington State University

Pullman, WA 99164-6340

Regulation of Terpene Metabolism
Rodney Croteau, Institute of Biological Chemistry
$116,000

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 and expressed, and the encoded enzymes have been characterized and employed for antibody preparation. Studies using these tools have led to an understanding of the cellular location, structure and function of central regulatory enzymes, and to successful genetic engineering of mint to increase production yield and improve quality of the essential oil. 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.

230. 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 (FY 00 funds - 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.

231. Washington State University

Pullman, WA 99164-6340

Carbon Metabolism and Dinitrogen Reduction in Symbiotic Nitrogen Fixation
Michael L. Kahn, Institute of Biological Chemistry
$100,000

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 contribute to soil acidity. Some plants are able to obtain nitrogen through symbiotic associations with nitrogen-fixing bacteria by exchanging carbon compounds made through photosynthesis 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 *Sinorhizobium meliloti*. Specifically, the 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) and isocitrate dehydrogenase (ICDH) we are probing the relationship between the TCA cycle and nodule development and metabolism. Bacteria with temperature-sensitive alleles of CS were shown to form effective nodules at low temperature but they are degraded rapidly when the temperature is raised. This indicates a role for CS in maintaining bacteria within 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.* Efforts are underway to integrate knowledge of these catabolic processes into the larger picture of *S. meliloti* metabolism potentially available from the bacterial genome sequence.

232. 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
$99,436

Photosynthesis is the central process in which energy is taken up into the biosphere and only major pathway for uptake of CO_2 from the atmosphere. It is thus critical to understand the processes that limit and regulate the productivity of photosynthesis. The photosynthetic apparatus must balance and regulate energy flow to meet biochemical demands while preventing over-excitation of the reaction centers, which in turn can lead to photoinhibition. The central intermediate in both ATP synthesis and regulation of the photosynthetic apparatus if the transthylakoid proton motive force (*pmf*). Proton motive force established across the thylakoid membrane by photosynthetic electron transfer functions both to drive the synthesis of ATP and initiate processes that down-regulate photosynthesis. The focus of our research is on understanding the relationships among electron transfer, proton pumping and the regulation and control of photosynthesis. New instrumentation and techniques developed in our laboratory have allowed us to address key issues about the overall energy budget of photosynthesis, its regulation and response to environmental changes: 1) The effects of storage of *pmf* as $\Delta\Delta$ and ΔpH; 2) The importance (or lack thereof) of alteration in the stoichiometry of proton pumping to electron transfer at key steps; 3) the influence of changes in the conductivity for protons of the ATP synthase; 4) the mechanisms of steps of the electron transfer process that pump protons; and 5) the mechanisms by which reactive O_2 is generated as a side reaction to photosynthesis, and how these processes are minimized.

233. 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 an/or degenerative in biological systems, resulting in, for example, degradation of lignocellulosic materials 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.

234. Washington State University

Pullman, WA 99164-6340

Enhancement of Photoassimilate Utilization by Manipulation of ADPGlucose Pyrophosphorylase

Thomas Okita, Institute of Biological Chemistry

$99,000

ADP-glucose pyrophosphorylase catalyzes a dominant control step in starch biosynthesis in both photosynthetic tissues and reserve organs. The objective of this project is to understand the structure-function of this enzyme at both the biochemical and physiological levels and to apply this knowledge to enhance plant productivity and yields. Using a powerful *glgC* complementation system generated in this laboratory, information on the role of the large and small subunits, which together form the heterotetrameric enzyme, in enzyme catalysis and allosteric regulation has been obtained. Residues that confer allosteric regulatory properties of the large subunit have been mapped by the identification of dominant up-regulatory mutations. Molecular shuffling approaches have generated small subunits capable of efficient assembly of a homotetrameric structure and high catalytic turnover. Some of these small subunit enzyme variants display altered effector specificity. Overall, these results suggest that evolution of this enzyme with regard to quaternary structure, allosteric effector selectivity, and effector sensitivity, occurred through the introduction of a few point mutations with low-level recombination hastening the process. Ongoing studies are being conducted to determine the role of this enzyme in carbon partitioning and its effect on photosynthetic capacity in both *Arabidopsis* and rice.

235. Washington State University

Pullman, WA 99164-6340

Targeting and Processing of the Thiol Protease Aleurain
John C. Rogers, Institute of Biological Chemistry
$100,000

Plant cells store a diverse variety of molecules in vacuoles. Storage is the central reason why plants are so important to humankind. For example, the wars against cocaine and heroin result from the harvest of products in storage vacuoles, and the nutrition of domestic animals and humans ultimately depends upon proteins stored in plant vacuoles. Many molecules that are targets in plant biotechnology are stored in vacuoles. Only by understanding the different compartments, their internal contents and environment, and how proteins and membrane are directed to each will we be able to program a cell to make and accumulate a desired product. We postulate that the complexity of the plant vacuolar system results from the need to separate a digestive compartment similar to the yeast vacuole or mammalian lysosome from the plant-specific storage compartments. Our studies have defined the pathways by which membrane proteins move after synthesis to separate lytic and protein storage vacuoles in many plant cells. Recent studies, however, have defined a remarkable interaction between lytic and storage compartments. In seeds, and in certain other cells, protein storage vacuoles contain an internal vacuole that represents a lytic compartment. This vacuole within a vacuole arrangement may ensure that storage products are kept separate from harmful digestive enzymes during seed development, but may facilitate rapid digestion of the stored products during germination. Our current focus is to understand how such a complex organelle is generated.

236. University of Washington

Seattle, WA 98195-1750

Global Regulation in the Methane-Producing Archaeon Methanococcus maripaludis
John A. Leigh, Department of Microbiology; in collaboration with William B. Whitman (University of Georgia) and Dieter Söll (Yale University)
$623,723

In the microbial fermentation of organic material to methane gas, about 95 % of the combustion energy of complex substrates is retained in the methane formed. This process has great potential in converting biomass to a fuel because it is CO_2 neutral and does not contribute to the net evolution of radiatively important trace gases. It can also be applied to a wide variety of organic wastes. The hydrogenotrophic methane-producing archaea play critical roles in this process. They produce about one-third of the methane formed. By maintaining a low partial pressure of H_2 gas, they also prevent the accumulation of toxic intermediates that would otherwise poison the fermentation. *Methanococcus maripaludis* is typical of many hydrogenotrophic methanogens. Because of its rapid growth and the availability of a well developed genetic system, this organism is especially suitable for detailed studies of this type of physiology. The proposed research will delineate the global regulatory systems and the molecular mechanisms that govern them using *M. maripaludis* as a model organism. Special emphasis will be given to regulatory systems that are key to the methanogenic physiology or are universally important but poorly understood in the Archaea. For example, the regulatory response to hydrogen levels and to nutrient-limited variations in growth rate will be investigated. A combination of experimental approaches will be utilized based upon the genome sequence of *M. maripaludis*, the proven genetic methodology, and a practical understanding of *M. maripaludis* physiology. Expression array analysis will be complemented by proteomic and metabolic analyses.

237. 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 C1 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 genes involved in the regulatory cascade (*mxcQET* and *mxbDM)* are also transcribed from low-level methanol-inducible promoters as operons. We have analyzed alternative sigma factors in this bacterium, and they are not involved in transcription from methanol-inducible promoters. This work is providing the foundation for development of methylotrophic strains to convert methanol into higher value added products.

238. Washington University

St. Louis, MO 63130-4899

Biogenesis of Photosystems in Synechocystis 6803, a cyanobacterium
Himadri B. Pakrasi, Department of Biology
$94,000

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.

239. Wisconsin, Medical College of - Milwaukee

Milwaukee, WI 53226

Enzyme Regulation and Catalysis in Carbon Fixation Metabolism
Henry M. Miziorko, Department of Biochemistry
$198,000 (FY00 funds - 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 (PRK) is the focal point of the proposed studies.

This project combines chemical, physical, and molecular biology approaches in an enzymological investigation of purified R. sphaeroides phosphoribulokinase (PRK). During the current reporting period, we employed electron spin resonance (ESR), nuclear magnetic resonance (NMR), and steady state kinetic techniques to evaluate the function of several active site residues in PRK. These experiments were

performed on mutant PRKs in which we eliminated the alcohol functionality of serine-14, threonine-18, serine-19, and threonine-20 (which map in a consensus ATP binding motif). While phosphorus NMR indicates that bound ATP is sensitive to changes at residues 18, 19, and 20, it does not suggest which residue functions most directly in interacting with ATP. Steady state kinetic approaches indicate that the major contribution of a side chain alcohol with ATP involves serine-19. Elimination of this alcohol reduces catalytic rate by 500-fold and catalytic efficiency by 15,000 fold.

240. University of Wisconsin

Madison, WI 53706-1569

Identification and characterization of Arabidopsis thaliana cell-plate proteins
Sebastian Y. Bednarek, Department of Biochemistry
$78,999

Plant cell division is highly critical for plant growth and development and is dependent upon membrane trafficking and fusion. Our studies have suggested that two molecular chaperones, Cdc48p/p97 and Sec18p/NSF are required for the various complex and dynamic membrane fusion events that occur at the division plane during plant cytokinesis. In yeast and mammalian cells, Cdc48p/p97 and Sec18p/NSF have been shown to regulate secretory membrane fusion through their interaction with membrane-associated fusion proteins known as SNAREs. We have shown that the cell plate SNARE, KNOLLE, which is required for cell plate membrane fusion, interacts with Sec18p/NSF and have identified several plant-specific SNAREs that bind to KNOLLE and localize to the cell plate. Work in progress is aimed at the further characterization of these factors to determine their role in cytokinesis and other aspects of plant secretory membrane trafficking and fusion. In contrast to Sec18p/NSF, AtCdc48p was found to interact with another SNARE, AtSed5p, which has been suggested previously to function in ER to Golgi membrane trafficking, and several novel soluble proteins that may functions as adapters between AtSed5p and AtCdc48p. Interestingly AtCdc48p and AtSed5p are targeted to the division plane in dividing plant cells. Our results suggest that in addition to the cell plate other membranes are targeted to and fuse within the division plane during plant cytokinesis. The significance of AtCdc48p and AtSed5p localization at the division plane and the role of the putative adapter proteins are being analyzed using a multidisciplinary approach involving microscopy, biochemistry and reverse genetics.

241. University of Wisconsin

Madison, WI 53706

Genetic Analysis of Ethylene Perception and Signal Transduction in Arabidopsis
Anthony B. Bleecker, Department of Botany
$103,000

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.

242. University of Wisconsin

Madison, WI 53706-1567
Molecular Genetics of Ligninase Expression
Daniel Cullen, Department of Bacteriology
$112,000

In addition to playing a key role in the carbon cycle, lignin-degrading fungi have considerable potential in several 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 ligninolytic fungi such as *Phanerochaete chrysosporium*, *Ceriporiopsis subvermispora*, and *Trametes versicolor*. Our long term objective is the development of environmentally friendly bioprocesses. Significant advancements were made on the molecular biology of *C. subvermispora*, the most promising species used in biomechanical pulping processes. The results provide a framework for strain improvement through genetic manipulations. Separate investigations on *T. versicolor* characterized the enzyme systems believed involved in the degradation of lignin and organopollutants. In collaboration with other DOE-funded laboratories, our studies continue to advance our understanding of gene organization and regulation in the model experimental system, *P. chrysosporium*. New and unusual enzyme systems have been discovered. Among these, several extracellular oxidases and cellulolytic enzymes have considerable potential in fiber treatment and modification.

243. University of Wisconsin

Madison, WI 53706
Microbial Formaldehyde Oxidation
Timothy J. Donohue, Department of Bacteriology
$98,000

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.

244. University of Wisconsin

Madison, WI 53706
The Molecular Basis for Metabolic and Energetic Diversity
Timothy J. Donohue, Department of Bacteriology
$125,000 (15 months)

Our long-term goal is to engineer microbial cells with enhanced metabolic capabilities. As a first step, this grant supports research to acquire a thorough understanding of metabolic pathways, energy-generating processes, and genetic regulatory networks of the metabolically versatile microbe, *Rhodobacter sphaeroides*, strain 2.4.1. No organism has the number and variety of different metabolic pathways that are known or predicted to be present in this well-studied bacterium. This relatively new area of research was made possible by the recent completion of the *R. sphaeroides* genome sequence with DOE support. This team of scientists and engineers is using this and other information to learn more about important activities

of this bacterium, to identify new pathways of agricultural, environmental and medical importance, and to design microbial machines that can efficiently degrade toxic compounds, remove greenhouse gases, or synthesize biodegradable polymers.

245. University of Wisconsin

Madison, WI 53706-1567

One-electron oxidative mechanisms for lignocellulose decay by fungi
Kenneth E. Hammel, Department of Bacteriology
$170,000 (FY00 funds - two years)

Most terrestrial biomass consists of or is derived from dead plants. These recalcitrant lignocellulosic residues must be recycled to keep the earth's carbon cycle in operation. Certain fungi are the organisms chiefly responsible for this essential step, yet we lack an understanding of how they degrade lignocellulose. One hitherto unproven hypothesis is that decay fungi employ extracellular, reactive oxygen radicals for this purpose. Results from this project show that a wide variety of wood decay fungi produce an extracellular oxidant with properties similar to those of the hydroxyl radical, which is the most reactive biological oxidant known. Work on one of these fungi, *Gloeophyllum trabeum*, show 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 and the gene that encodes it have been characterized. The quinone reductase has a very high catalytic efficiency for *G. trabeum* quinones. It is related to a group of flavoprotein quinone reductases that occur in many fungi, plants, and bacteria.

246. University of Wisconsin

Madison, WI 53706-1567

Structural and Functional Analysis of a Complete Plant Centromere
Jiming Jiang, Department of Horticulture; in collaboration with C. Robin Buell (The Institute for Genomic Research)
$273,836 (two years)

The centromere is the most characteristic landmark of eukaryotic chromosomes. Centromeres function as the site for kinetochore assembly and spindle attachment, allowing for the faithful pairing and segregation of sister chromatids during cell division. Characterization of centromeric DNA is not only essential to understand the structure and organization of plant genomes, but it is also a critical step in the development of plant artificial chromosomes. The centromeres of several model eukaryotic species, including human and *Arabidopsis thaliana*, consist predominantly of long arrays of a single class of satellite DNA. To date, technical limitations have prevented the construction of a DNA contig that spans the entire centromere from any higher eukaryotic species. Thus, the centromeric regions have been left as “gaps” in the sequencing data for all *A. thaliana* and human chromosomes. We have demonstrated that the DNA structure of rice centromeres differs from the “single repeat-dominated characteristics” of human and *A. thaliana* centromeres. We have found that the centromere of rice chromosome 8 contains only about 50 kb of RCS2 repeat, a centromere-specific satellite repeat that is the counterpart of the pAL1 satellite in *A. thaliana* centromeres and the alpha satellite in human centromeres. In our DOE-funded project “Structural and Functional Analysis of A Complete Plant Centromere”, we have proposed to sequence the centromere of rice chromosome 8 using a combination of cytogenomics with high throughput sequencing. This project will reveal the complete DNA sequences of the first intact higher eukaryotic centromere.

247. University of Wisconsin

Madison, WI 53706-1574

Epigenetic Silencing of the Maize r Gene
Jerry L. Kermicle, Laboratory of Genetics
$90,000 (FY 00 funds)

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.

248. 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
$105,000

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.

249. University of Wisconsin

Madison, WI 53706-1381

Starch Conversion to Sucrose in Plant Leaves
Thomas Sharkey, Department of Botany
$110,000

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 mobilization pathways of transitory starch in leaves. The nighttime pathway of sugar export from chloroplasts differs from the daytime pathway. Primarily maltose, but also some glucose, are the primary export sugars from chloroplasts at night. Maltose export could lead to increased energy efficiency at night but the reactions involved in converting maltose to sucrose are not yet known. Metabolite analyses and nonaqueous fractionation of plant leaves has shown that maltose is distributed equally between chloroplasts and the cytosol but is not present in the vacuole. *Arabidopsis* knockout lines have been found that lack either cytosolic starch phosphorylase or cytosolic

disproportionating (D) enzyme. The need for these enzymes in the cytosol is unclear but the D enzyme deficient plants grow much more slowly than wild type and these plants will be used to determine if this enzyme has a role in maltose to sucrose conversion.

250. University of Wisconsin

Madison, WI 53706-1580
Molecular Mechanism of Energy Transduction By Plant Membrane Proteins
Michael R. Sussman, Director, Biotechnology Center
$115,000

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. The enzyme is encoded by a family of a doze genes (collectively called AHA's, for **A**rabidopsis **H**+-**A**TPase) 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.

251. University of Wisconsin

Madison, WI 53706-1590
Post-Translational Regulation of Phytochrome Action
Richard Vierstra, Department of Horticulture
$113,000

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.

252. 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
$92,015

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. Additionally, metal reducing bacteria have obvious potential as agents of bioremediation in anaerobic environments. 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. This unusual location is thought to be an advantage for organisms that use highly insoluble iron oxides for respiration. This 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. We have identified menaquinones as essential components of the electron transport chain that leads to iron reduction. We are currently characterizing a formate dehydrogenase that is involved in metal reduction, and are in the process of generating an expression vector that would allow the overexpress of electron transport proteins for biochemical analyses.

253. 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 (FY 00 funds - two years)

Cell division is carefully regulated during normal development of all organisms. Plants, however, have a unique ability to withstand defects in cell division or cell expansion. For example, mutations that alter the pattern or timing of cell division can have surprisingly little impact on the overall growth pattern of leaves and other organs. To understand this developmental process, we are identifying and characterizing genes that are important during cell division and expansion. Mutations in the Warty1 (Wty1) gene cause localized sites of excessive cell expansion, or warts, suggestive of a dysfunctional cell cycle. We cloned Wty1 by transposon-tagging, identified the full genomic and cDNA sequence, and have confirmed that Wty1 encodes a small GTP binding protein in the RAB family, based on molecular analysis of three additional insertion alleles. We have also identified at least one additional gene that fails to complement but is not allelic to Wty1. Currently we are mapping and analyzing five other non-complementing potential alleles of Wty1. Our goals are to understand the molecular and developmental basis for how cell size is regulated during normal leaf development in maize.

254. Xavier University of Louisiana

New Orleans, LA 70125

Molecular Characterization of Bacterial Respiration on Minerals
Robert Blake II, College of Pharmacy
$108,568

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. One long-term goal is to elucidate the molecular principles whereby these bacteria recognize and adhere to their insoluble mineral substrates. The specific, high affinity adhesion of *Acidithiobacillus 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 long-term 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 a novel acid-stable red cytochrome produced in large amounts by *Leptospirillum. ferrooxidans* as it respires aerobically on ferrous ions. Stopped flow spectrophotometric studies indicate that the Fe(II)-dependent reduction of the cytochrome is sufficiently rapid to be physiologically significant. The aim of these studies is to identify and characterize the unique electron transfer proteins in each iron respiratory chain. It is anticipated that this project will provide useful information toward manipulating *A. ferrooxidans* and related organisms for commercial use.

255. Yale University

New Haven, CT 06520-8114

Asparagine and Cysteine Metabolism in Bacteria and Archaea
Dieter Söll, Department of Molecular Biophysics and Biochemistry
$234,600 (two years)

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 a well-understood biosynthetic pathway in which of free aspartate is converted to asparagine by two different asparagine synthetase enzymes encoded by *asnA* and *asnB* or related genes. While this is the case in some bacteria *(e.g., Escherichia coli* and *Bacillus subtilis*) this amidation of aspartate is achieved by a tRNA-dependent process. Our recent genetic and biochemical analyses with *Deinococcus radiodurans* combined with an analysis of all known bacterial and archaeal genomes suggest that most bacteria and some archaea lack the asparagine synthetase pathway. Instead they use exclusively a transfer RNA-dependent route, the conversion of Asp-tRNA^{Asn} to Asn-tRNA^{Asn} by a novel hetero-trimeric enzyme, aspartyl-tRNA^{Asn} amidotransferase. This enzyme is essential in many bacteria as it 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.

256. Yale University

New Haven, CT 06520-8114

Global Regulation in the Methane-Producing Archaeon Methanococcus maripaludis
Dieter Söll, Department of Molecular Biophysics and Biochemistry; in collaboration with William B. Whitman (University of Georgia) and John Leigh (University of Washington)
$178,194 (two years)

In the microbial fermentation of organic material to methane gas, about 95 % of the combustion energy of complex substrates is retained in the methane formed. This process has great potential in converting biomass to a fuel because it is CO_2 neutral and does not contribute to the net evolution of radiatively important trace gases. It can also be applied to a wide variety of organic wastes. The hydrogenotrophic methane-producing archaea play critical roles in this process. They produce about one-third of the methane formed. By maintaining a low partial pressure of H_2 gas, they also prevent the accumulation of toxic intermediates that would otherwise poison the fermentation. *Methanococcus maripaludis* is typical of many hydrogenotrophic methanogens. Because of its rapid growth and the availability of a well developed genetic system, this organism is especially suitable for detailed studies of this type of physiology. The proposed research will delineate the global regulatory systems and the molecular mechanisms that govern them using *M. maripaludis* as a model organism. Special emphasis will be given to regulatory systems that are key to the methanogenic physiology or are universally important but poorly understood in the Archaea. For example, the regulatory response to hydrogen levels and to nutrient-limited variations in growth rate will be investigated. A combination of experimental approaches will be utilized based upon the genome sequence of *M. maripaludis*, the proven genetic methodology, and a practical understanding of *M. maripaludis* physiology. Expression array analysis will be complemented by proteomic and metabolic analyses.

CONFERENCES

Gordon Research Conference – Applied and Environmental Microbiology, July 22-27, 2001, New London, Connecticut

Gordon Research Conferences – 2001 Archaea: Ecology, Metabolism & Molecular Biology, August 5-10, 2001, Andover, New Hampshire

Marine Biological Laboratory – Investigations into the Metabolic Diversity of Microorganisms as Part of Microbial Diversity, Summary 2001, Woods Hole, Massachusetts

Michigan State University – Minority Summer Research Program in the Plant Sciences, Summer 2001, East Lansing, MI

University of Wisconsin – 12th International Workshop on Plant Membrane Biology, August 11-16, 2001, Madison, WI

INVESTIGATOR INDEX