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**Division of Energy Biosciences
Annual Report and Summaries of
FY 1997 Activities**

October 1998



U.S. Department of Energy
Office of Energy Research
Office of Basic Energy Sciences
Division of Energy Biosciences

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Cover Picture: Natural biodegradation of lignocellulosic material. Photo taken on the Department of Energy's Germantown, Maryland grounds.



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Washington, DC 20585

Program Overview of the Division of Energy Biosciences

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

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

The program's efforts in supporting interdisciplinary activities at the boundaries of biology and other disciplines represented within Basic Energy Sciences include: biocatalytic mechanisms, biomaterials and materials biosynthesis, interactions between plant/microbes with mineral particles, interface of photobiology and photochemistry, and bioengineering. The program also interacts extensively with other Federal agencies to coordinate and cooperate in areas of joint interest. The most prominent of these efforts has been the three-agency plant science activities in partnership with the National Science Foundation (NSF) and the U.S. Department of Agriculture (USDA). This partnership, initiated in 1987 under

the auspices of the Office of Science and Technology Policy, focuses on multi-institutional research coordinating group awards and interdisciplinary research training group awards. The three agency programs have done much to stimulate plant science research nationwide on university campuses.

The Division of Energy Biosciences has also joined with NSF and USDA to fund three groups to sequence, at a large scale, the genome of the plant *Arabidopsis thaliana*. These U.S. groups will coordinate with activities in Europe and Japan to decipher the entire genome of this model plant. The three U.S. groups, along with their international partners, are making excellent progress in providing this valuable resource to the plant research community.

Recent Accomplishments

Silicon is an element that is a principal component of glass, computer chips, coatings and numerous consumer products (including, alas, silicone implants). There are only a few biological systems that metabolize this element. Silicon is metabolized by some simple animals, by algae to make the equivalent of glass houses, and by some higher plants (the rough feel of corn leaves comes from chards of silicates in the leaves). Recently a gene was identified that encodes a protein that is involved in binding and transporting silicon into a cell. This discovery will extend our understanding of how silicon is taken up and processed by biological systems which may lead to applications such as the mining of silicon from seawater and the manufacture of silicon-containing products, all energy requiring processes.

The enormous variety of chemical reactions constantly occurring in live biological systems demands that each reaction be precisely controlled. Enzymes are proteins that act to catalyze particular reactions such that normally only one chemical is converted into another. No byproducts are formed. A new type of protein was discovered during studies on the biosynthesis of lignin, a major component of wood and other biomass resources. This protein acts by specifically orienting the chemical substrate of an enzyme permitting only one product to be formed. This discovery is important to studies on lignin formation and may be relevant to numerous other biological processes. This biological strategy may also be applicable to the development of chemical catalysts leading to industrial processes that do not produce harmful byproducts.

Cellulose is the most prevalent biological compound on earth. It is the principal component of all plants, wood, paper and cotton. When considered globally, cellulose constitutes an enormous supply of chemical energy, all of it renewable. Recently, several plants have been manipulated to make significantly less cellulose. This is important because it may now permit identification of the factors that control the synthesis and deposition of cellulose and related compounds. This development may permit the genetic engineering of plants to produce either more cellulose, or plants that produce larger amounts of other chemicals such as liquid fuels and plastics.

The few microorganisms that possess the ability to produce methane (natural gas) have been studied for a number of years in the hope of using these organisms to produce a renewable energy source. These studies always showed that these bacteria were very unusual. Recently the genome of a methane producing bacterium was sequenced and

again showed the uniqueness of these organisms. It is now thought that they are unusual because they are among the first life forms ever developed on earth. Recently procedures have been developed which will permit the genes of methane-producing bacteria to be manipulated. This development will allow scientists to determine the nature and properties of these organisms and their unusual metabolism.

The Division continues to participate in the Life Sciences Research Foundation post-doctoral fellowship program, with three-year fellowship support for the following individuals in Fiscal Year 1997:

Dr. David Weiss (Dept. Microbiology & Molecular Genetics, Harvard Medical School)
Dr. S.P. Dinesh-Kumar (University of California, Albany)
Dr. Jacqueline Heard (Harvard Medical School)
Dr. Andrew Kloek (Washington University)
Dr. Jennifer Kuzma (The Rockefeller University)
Dr. Ramin Yadegari (University of California, Berkeley)
Dr. Karen Century (San Francisco State University)
Dr. Jay Gulledge (Harvard University)
Dr. Kristin LeVier (Massachusetts Institute of Technology)
Dr. Allen Sessions (Salk Institute for Biological Studies)

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

During Fiscal Year 1997 the Division provided partial support for the following conferences, workshops or training activities:

1. Steenbock Symposium on The Biosynthesis and Function of Metal Clusters for Enzymes, University of Wisconsin, Madison, WI, June 10-13, 1997
2. Eighth International Conference on Arabidopsis Research, University of Wisconsin, Madison, WI, June 25-29, 1997
3. An Advanced Course in Plant Biochemistry, Washington State University, Pullman, WA, Summer 1997
4. Conference on Microbial Structural Biology: Novel Enzymes from Diverse Microbes, Pennsylvania State University, University Park, PA, August 7-9, 1997
5. Gordon Research Conference on Plant Cell Walls, Tilton School, Tilton, NH, August 17-22, 1997
6. Minority Summer Research Program in the Plant Sciences, Michigan State University, East Lansing, MI, Summer 1997
7. Investigations into the Metabolic Diversity of Microorganisms as Part of Microbial Diversity, Marine Biological Laboratory, Woods Hole, MA, Summer 1997

The Division of Energy Biosciences was provided approximately twenty-eight million dollars in fiscal year 1997. The breakdown of how the resources were distributed is indicated in the following table.

	Number of Projects	FY 97 funding (in thousands)	Percent of total funds
University and Non-Profit Institutions	191	20,088	70
Michigan State University Plant Research Laboratory	15	2,750	10
National Laboratories Brookhaven National Lab Lawrence Berkeley Lab Los Alamos National Lab National Renewable Energy Lab.	11	2,531	9
Small Business Innovation Research (SBIR) contribution, and Miscellaneous		2,764	10
Conferences, Educational Activities	9	452	<1
	<hr/> 226	<hr/> \$28,585	

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

The staff members of the Energy Biosciences program are:

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Dr. James E. Tavares

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Abstracts of Projects Supported in FY 1997

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

- 1. Molecular Organization in the Native State of Wood Cell Walls: Studies of Tertiary Structure and its Development Using the Raman Microprobe, Solid State ^{13}C NMR, Fluorescence Spectroscopy and Photoconductivity**
R.H. Atalla, Forest Products Laboratory \$240,000 (FY 95 funds/2 years)

A new-generation Raman microprobe that allows acquisition of spectra in seconds, rather than minutes or hours, enables us to monitor the progress of lignification in secondary walls of woody tissue and in the secondary thickenings of cultured cells of *Zinnia elegans*. Our model of biogenesis of the secondary walls includes consideration of the coupling of the processes of biogenesis between cells of similar type in the same tissue. Together these two developments have provided the basis for investigating the degrees of synchrony between processes of biogenesis at different levels of structure and at different stages of development in woody tissue, and under different conditions of culture for the *Zinnia* system. Our studies include examination of adjacent cells in annual rings as well as contemporaneous cells further removed from each other. We make comparisons between tissues from different annual rings, and from juvenile wood and mature wood. From a developmental perspective, our greatest interest is in monitoring the progress of secondary cell wall deposition and lignification in the cambial zone. In a similar way, we are investigating the progress of lignification in secondary thickenings in a number of populations of *Zinnia* cells grown under different conditions of culture and possessing different patterns of wall thickenings.

In addition to the studies of biogenesis we continue to explore the capacity of the cell wall polysaccharides to induce coherence of order, at the 1 to 5 nanometer level, among molecules adsorbed on their surfaces or associated with them. We believe this is the key to the coherence of order in lignin that accounts for the pathways for electron transport that we and others have detected. The methods we use for characterizing woody tissues include, in addition to Raman and Raman microprobe spectroscopy, solid state ^{13}C NMR, fluorescence spectroscopy and photoconductivity measurements. We complement these with theoretical modeling of intermolecular interactions and other traditional analytical procedures.

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

- 2. Metabolic Regulation of the Plant Hormone Indole-3-acetic Acid**
J.D. Cohen, Beltsville Agricultural Research Center \$90,000

The phytohormone indole-3-acetic acid (IAA, auxin) is important for many aspects of plant growth, development and responses to the environment. Our understanding of the biosynthesis, conjugation and degradation of auxin in plants, and the mechanisms by which plants regulate auxin levels has changed remarkably within the last few years. We showed that mutant plants that

cannot make the amino acid tryptophan still make IAA, and in very high amounts. We also showed that both the traditional tryptophan pathway and a non-tryptophan pathway to IAA occur in plants, and sometimes both are used by the same plant at different times of development. An additional source of IAA in plants is the relatively large pool of IAA stored within plant cells in conjugated form. We are working on three fundamental problems related to how plants get their IAA: 1) Characterizing the non-tryptophan pathway to auxin using an *in vitro* system from maize seedlings; 2) Determination of how a plant decides to use one or the other pathway, or both, during various developmental stages or in response to environmental stresses and stimuli; 3) Isolation and characterization of bacterial and plant enzymes that hydrolyze conjugates that could be useful for altering IAA metabolism in specific plant tissues. 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.

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

3. Regulation of Sucrose-Phosphate Synthase and Other Cytosolic Proteins by Reversible Protein Phosphorylation

S.C. Huber, USDAARS and Departments of Crop Science and Botany, NCSU

\$83,216

We have continued our studies to elucidate the control by phosphorylation of sucrose-phosphate synthase (SPS) and sucrose synthase (SuSy) - important enzymes of sucrose (Suc) synthesis and degradation, respectively. With SPS, we have focused on the phosphorylation of the enzyme's two regulatory sites: Ser158, responsible for dark-inactivation of the enzyme, and Ser424, which appears to be responsible for "osmotic-stress activation" of the enzyme in darkened leaves. The amino acid residues surrounding Ser158 and Ser424 are similar and both conform to the motif targeted by SPS-kinase(s): Basic-Hydrophobic-X-Basic-X(2)-Ser-. However, the two sites are phosphorylated by distinct kinases. Ser158 is phosphorylated by PK_{III}, a likely member of the sucrose non-fermenting (SNF1) family of kinases that is itself controlled by phosphorylation. In contrast, Ser424 is phosphorylated by PK_{IV}, a unique stress-stimulated kinase. Understanding those processes may uncover new information about how plants cope with stress, and could provide new avenues to increase stress tolerance of crop plants. With SuSy, our efforts have focused on identifying the physiological significance of Ser158 phosphorylation. Phosphorylation results in a small activation of the cleavage reaction that is probably not significant *in vivo*. However, phosphorylation appears to be part of the mechanism that controls the membrane association of SuSy. Our current working model is that phosphorylation causes a conformation change that reduces exposure of surface hydrophobic residues, thereby causing release of SuSy from the membrane. The localization of SuSy may directly control whether imported assimilates are utilized in cell wall biosynthesis or other biosynthetic pathways.

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

4. Consequences of Altering Rubisco Regulation

A.R. Portis, Jr., USDA/ARS and Department of Crop Sciences, University of Illinois
 \$50,100

Rubisco initiates photosynthetic carbon acquisition and its activity can be limiting for photosynthesis. Even when Rubisco activity is not limiting (e.g. low light, high CO₂, limited sinks), the activation state in the enzyme is often reduced such that its activity is no longer explicitly limited by the steady state level of the RuBP substrate. Several hypothesis have been proposed which attempt to account for this response. 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, generated by alternative splicing of the pre-mRNA, and of unknown significance. Activase activity requires ATP hydrolysis which responds to the ATP/ADP ratio and appears to be influenced by light intensity via the thylakoid membranes by an unknown mechanism.

The goal of this project is to examine the consequences of altering Rubisco regulation by examining the properties of *Arabidopsis* plants expressing different forms of Rubisco activase and achieved via transformation of either the wild type or the *rca*⁻ mutant (containing no Rubisco activase). Plants containing only one of the two isoforms and plants which have mutant forms of Rubisco activase less inhibited by ADP are currently being characterized. Plants overexpressing each form of activase or forms with no catalytic activity will also be created. The information gained by this research will directly address the significance of Rubisco regulation as a determinant of plant productivity and in the response of plants to their environment.

Arizona State University
Tempe, AZ 85287-1604

5. Structure, Function and Regulation of Antenna Complexes of Green Photosynthetic Bacteria

R.E. Blankenship, Department of Chemistry and Biochemistry \$116,000

All photosynthetic organisms contain chlorophyll pigments that function as an antenna, absorbing light and transferring excitations to a photochemical reaction center where energy storage takes place by a series of chemical reactions. The green photosynthetic bacteria are characterized by large antenna complexes known as chlorosomes. The overall objective of this project is to determine the molecular organization of pigments in chlorosome antennas, as well as the mechanisms of excitation transfer and regulation of this unique antenna system. The chlorosome pigments are organized *in vivo* into pigment oligomers in which direct pigment-pigment interactions are of dominant importance. 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. Ultrafast absorbance measurements have indicated that the pigments are very strongly coupled, leading to subpicosecond energy transfer. Green sulfur bacteria contain a redox-activated quenching mechanism for control of energy transfer efficiency. The quenching effect may be a

control mechanism that protects the cell from damage during conditions where light and oxygen are present simultaneously. We have recently determined the X-ray structure (PDB ID code 1KSA) of the bacteriochlorophyll *a* antenna protein from the green sulfur bacterium *Chlorobium tepidum*. This protein is an intermediate in the energy transfer pathway from chlorosome to reaction center. Efforts to develop a system for site-directed mutagenesis of this protein are underway.

Arizona State University
Tempe, AZ 85287-1601

6. Chlorophyll Binding and Biogenesis of Photosystem II

W. Vermaas, Department of Plant Biology and Center for the Study of Early Events in Photosynthesis \$101,000

Chlorophyll synthesis is coupled tightly to biogenesis of chlorophyll-binding proteins in photosynthetic systems. This tight coupling is important to avoid accumulation of free chlorophyll, which is toxic in aerobic environments in the light. One way to address the *in vivo* mechanisms of this coupling is by genetic modification of the rate of chlorophyll synthesis and the conditions under which it occurs, followed by analysis of the effects of these modifications on synthesis of chlorophyll-binding proteins. For these studies the cyanobacterium *Synechocystis* sp. PCC 6803 is used because this organism is very suitable for gene replacement studies and because its genome has been sequenced. By deletion of the light-independent pathway of chlorophyll synthesis in this organism, a mutant has been generated that does not make chlorophyll in darkness and that starts synthesizing chlorophyll when exposed to light. Interestingly, the synthesis rate of a chlorophyll-binding photosystem II protein, D1, is very low at early times of illumination, and increases as chlorophyll accumulates. Transcript levels for this protein are high at all times, implying a translational control involving chlorophyll. Indeed, in another mutant with a lower capacity for chlorophyll synthesis, D1 translation is even more impaired. This observation has further increased our interest in identifying potential chlorophyll-binding proteins, and determining their role in synthesis, assembly, and function of the photosynthetic apparatus. Several potential chlorophyll-binding proteins in *Synechocystis* sp. PCC 6803 have been identified on the basis of either sequence homologies or biochemical evidence, and analysis of the role of several of these proteins is in progress.

University of Arizona
Tucson, AZ 85721-0088

7. Polyol Functions in Stress Protection of Photosynthesis

H.J. Bohnert and R.G. Jensen, Department of Biochemistry \$100,000

The long-term goal of this project is the engineering increased whole-plant tolerance to drought and salinity stresses by the modification of metabolic pathways in carbon allocation. We explore the roles of accumulating substances, polyols in particular, and also proline, trehalose, ectoine, and glycine betaine. Several laboratories reported that the transgenic overexpression of enzymes that lead to the accumulation of these compounds provided limited stress protection, but mechanisms of action are still largely unknown. Effects of accumulating osmolytes have been measured in

transgenic tobacco. Extending these studies we can now pinpoint a specific function for mannitol, at approximately 100 mM in the stromal space, in the protection against hydroxyl radicals. We observe that mannitol protects enzymes of the Calvin cycle, while the water-splitting machinery and photosystems are not inhibited under the conditions used. The protective effect of mannitol has been shown *in vivo*, in isolated cells, chloroplasts and *in vitro* systems. We expect that other polyols may have a similar function. Protection is exerted in a narrow range of accumulating polyols; tobacco plants with high accumulation of, e.g., sorbitol are apparently osmotically challenged and are stunted. We suspect that either osmosensing pathways are affected or that high amounts of polyols are interfering with sugar sensing in these high accumulator lines. We are now targeting the expression of different enzymes of osmolyte production to different compartments and to different tissues by the use of tissue- and cell-specific promoters. We expect multiple osmolytes to exert different protective effects.

University of Arizona
Tucson, AZ 85721

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

M.C. Hawes, Department of Plant Pathology

\$96,000

We proposed a model to describe border cell separation, based on published results describing cell wall degradation by microbial pathogens. We first tested the hypothesis that pectinesterase (PME) activity is a driving force that sets in motion steps leading to cell separation. If correct, then inhibiting PME activity in the root cap should inhibit border cell separation. Our results are consistent with that hypothesis: Transgenic hairy roots of pea expressing antisense mRNA to a root cap specific PME encoding gene (*rc-pme1*) makes border cells but the cells do not separate into suspension like normal border cells. Cell elongation also is inhibited. To our knowledge, this is the first evidence that inhibiting expression of a plant cell wall degrading enzyme can significantly alter plant growth and development. In transgenic root tips with reduced *rc-pme1* expression the pH at the cell surface is measurably higher than in normal roots, as predicted by our model. Removing border cells constitutes a signal to induce not only PME gene expression but also mitosis in the root cap meristem, and a global switch in gene expression throughout the root cap leading to border cell production and separation. An extracellular chemical, 'Factor B,' appears to act as a repressor to regulate border cell development, but the effects of this signal can be overridden by increased atmospheric CO₂ levels.

University of Arizona
Tucson, AZ 85721

9. Regulation of DNA Endoreduplication in Zea mays Endosperm

B.A. Larkins, Department of Plant Sciences

\$100,001

Nuclear DNA endoreduplication occurs widely in the metabolically active tissues of plants and animals. While a normal cell cycle has S, G₂, M and G₁ phases, the endoreduplication cycle has only DNA synthesis and gap phases. Maize endosperm cells engage in multiple rounds of

endoreduplication from 10 to 25 DAP. Though the biological significance of this process is not understood, one suggestion is that an increase in gene copy number can increase mRNA transcript levels in a developmentally regulated manner. Investigations in our laboratory suggest that endoreduplication in maize endosperm is associated with the inhibition of mitotic cyclin-dependent protein kinases (CDKs) and enhancement of S-phase CDKs. Inhibition of mitotic CDKs is achieved via the production of an inhibitor present throughout endoreduplication. Unlike regulators of CDKs in other eukaryotes, neither covalent modification nor stable binding is required for the inhibition. The inhibitor is associated with the microsomal fraction and its activity increases significantly between 9 to 13 days after pollination. To learn more about this inhibitor, we have established a purification protocol that allowed us to purify the protein component of this inhibitor to near homogeneity. Maize endosperm contains a high level of CDK-like kinase activity which can be detected as soon as the kinase is separated away from inhibitory factors. This kinase possesses characteristics expected for the S-phase CDK of higher plants. Partially purified activity from 16 DAP maize endosperm cofractionates with electrophoretically distinct polypeptides immunologically related to *Zea mays* p34^{cdc2}.

University of Arizona
Tucson, AZ 85721

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

B.A. Larkins, Department of Plant Sciences

\$100,000

Endosperm texture is an important quality trait in maize, as it influences the shipping and handling characteristics of the grain, its susceptibility to boring insects, the yield of grits from dry milling, energy costs during wet milling and baking properties of the flour. Factors contributing to texture, i.e. hardness and vitreousness, are poorly understood. However, there appears to be a causal relationship between kernel hardness and protein body formation in the endosperm, because mutations that affect protein body size, number and organization give rise to soft, starchy kernels. To better understand the relationship between protein body formation and the vitreous phenotype, we have conducted experiments to investigate the molecular mechanisms regulating protein body formation. Associations were found between protein bodies, zein polysomes, actin and EF-1 α *in vitro*. These observations led us to speculate that the cytoskeleton plays a role in the synthesis of storage proteins in maize endosperm. To approach this question, we have begun experiments to visualize the cytoskeleton in intact maize endosperm cells and determine its relationship to protein bodies, determine whether zein mRNAs are targeted to specific sites on the rough endoplasmic reticulum (RER), and determine whether zein mRNAs localize to the RER membrane in a cytoskeletal-dependent manner. We have been able to document dramatic changes in the distribution of actin filaments, microtubules and EF-1 α accompanying the accumulation of storage proteins and starch in endosperm cells. Microtubules become juxtaposed with protein bodies, which are enmeshed in EF-1 α and actin. The EF-1 α and actin appear to exist in a complex, as pretreatment of the tissue with cytochalasin D causes the redistribution of both proteins. Results from recent experiments in which we have localized zein mRNAs in fixed tissue and microinjected fluorescently labeled zein mRNAs into living endosperm cells, support the hypothesis that these mRNAs are associated with, and perhaps trafficked by, this cytoskeletal network.

University of Arizona
Tucson, AZ 85721

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

K.S. Schumaker, Department of Plant Sciences

\$90,000

During their development, plants convert the information from environmental and physiological cues into specific growth responses. Transduction of this information into the appropriate cellular compartment often involves changes in energy coupling across cellular membranes. In several species of moss, changes in cellular calcium levels have been implicated in the regulation of growth habit. During the progression from filamentous to meristematic growth, cells undergo a dramatic alteration in the pattern of development involving a reorientation in cellular polarity and subsequent hormone-induced changes in cell proliferation. Calcium influx has been implicated in both of these processes: in selection of the site of asymmetry and in hormone-induced changes in cell expansion, elongation, and division.

Using calcium channel modulators (agonists and antagonists), whole plant studies have implicated control of calcium regulation during moss development to a dihydropyridine-sensitive calcium channel. Our studies of calcium influx into moss protoplasts and binding of a calcium channel antagonist to moss plasma membranes have provided a biochemical and molecular characterization of this moss calcium channel. We have shown that cytokinin, changes in voltage, and heterotrimeric guanine nucleotide binding proteins can regulate channel activity in vitro. We have identified two proteins in the moss plasma membrane that bind specifically to the channel antagonist, and studies are underway to isolate these putative channel proteins. Our studies continue to focus on the expression and regulation of the channel during development. To localize the channel spatially and temporally during development, in vivo assays using a fluorescently-labeled channel antagonist are in progress. To extend our studies of channel regulation to stages of development, specific cell types, and specific regions of the cells involved, we are performing electrophysiological measurements to characterize channel activity. Understanding the properties, expression, and regulation of the channel will help us determine the molecular mechanisms underlying calcium regulation and calcium's role in plant development.

University of Arizona
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12. Phytoalexin Detoxification Genes and Gene Products: Implication for the Evolution of Host-specific Traits for Pathogenicity

H. VanEtten, Department of Plant Pathology \$192,000 (FY 96 funds/2 years)

Pea plants produce the antibiotic pisatin in response to infection by *Nectria haematococca*, a pathogenic fungus that uses pisatin demethylase (pda), a cytochrome P450 monooxygenase to detoxify pisatin. Several PDA genes have been identified that encode three different whole cell Pda phenotypes distinguishable by the induction lag period and resulting level of enzyme activity (PdaSH = short lag, high activity; PdaSM = short lag, moderate activity; PdaLL = long lag, low activity). Only the PdaSH and PdaSM phenotypes have been correlated with pathogenicity on pea.

We used heterologous expression of the *PDALL* gene *PDA6-1* and the *PDASH* gene *PDAT9* in *Aspergillus nidulans* to compare the biochemical properties (substrate specificity, Km and inhibitor sensitivity) of these gene products with the products of the *PDASH* genes *PDA5* and *PDA1* expressed in *N. haematococca*. Our results show that the whole cell phenotypes of *N. haematococca* are not due to significant differences in the biochemical properties of the gene products and are consistent with recent results that indicate the phenotypic differences are due to different levels of gene expression. These and other previous results that demonstrated rapid induction of *PDASH* genes *in situ* and that *PDASH* gene-specific mutants are reduced in virulence, substantiate the hypothesis that rapid production of a phytoalexin detoxifying enzyme by a pathogen is needed to counteract the host plant defense of rapid phytoalexin biosynthesis.

University of Arizona
Tucson, AZ 85721

13. Role of HSP100 Proteins in Plant Stress Tolerance

E. Vierling and S. Lindquist, Department of Biochemistry, University of Arizona and The Howard Hughes Medical Institute, The University of Chicago

\$150,009 (FY 95 funds/30 months)

During high temperature stress plants express heat shock proteins (HSPs) which are hypothesized to protect them from heat damage. We are investigating whether Arabidopsis HSP101, a homologue of yeast HSP104, can be manipulated to increase plant thermotolerance. HSP104 is required for thermotolerance in yeast, and we have previously shown that Arabidopsis HSP101 will complement the thermotolerance defect of yeast HSP104 mutants. Arabidopsis HSP101 is strongly induced during heat stress in the plant and it is also developmentally regulated, accumulating during seed maturation and stored in the dry seed. An Arabidopsis T-DNA insertion line, which is homozygous for an insertion within the AtHSP101 promoter, has been identified. Plants homozygous for the insertion show wildtype expression of AtHSP101 during heat stress and have wildtype thermotolerance. The T-DNA insertion site is 143 bp 5' of the cDNA start. Therefore this minimal promoter is sufficient for heat-regulated expression of the gene. We do not yet know if the insertion alters developmental regulation of the gene. We are screening additional T-DNA insertion lines for a mutation in AtHSP101 and are also working to generate a homologous gene disruption. Homozygous transgenic lines carrying AtHSP101 promoter-GUS fusions have been established and shown to exhibit heat-regulated expression of GUS. These lines will be used to investigate further the tissue and cell type specificity of HSP101 expression during various types of stress and during development. An AtHSP101 homolog, AtHSP92.7, that is 78% similar and 71% identical at the amino acid sequence level to AtHSP101, was sequenced by the Arabidopsis genome project. In wildtype plants, accumulation of AtHSP92.7 mRNA is also heat-regulated, but the transcript is much less abundant than that of AtHSP101. We identified a T-DNA insertion line in which the T-DNA is inserted in the second exon of this gene. This line will be used to determine if HSP92.7 also contributes to thermotolerance. We have demonstrated that thermotolerance of luciferase activity in transgenic Arabidopsis plants can be imaged *in vivo*. We will cross the luciferase plants to the HSP92.7 insertion mutant and any HSP101 mutants that we obtain in order to determine how absence of these genes affects luciferase thermotolerance. The luciferase plants can also be used for a non-invasive screen for mutants defective in thermotolerance.

Boston College
Chestnut Hill, MA 02167-3860

14. Osmoregulation in Methanogens

M.F. Roberts, Merkert Chemistry Center

\$95,999

This project is aimed at understanding how methanogens deal with osmotic stress and to use these insights for increasing the salt tolerance of other cells. Present studies are focused in four different areas: (1) *in vivo* ^{13}C -edited ^1H NMR studies of *Methanococcus thermolithotrophicus* and *Methanohalophilus portucalensis* using soluble ^{13}C -labeled substrates for methanogenesis to monitor organic solute production, uptake, or loss upon alteration of external NaCl, (2) ^{39}K NMR and atomic absorption studies of these organisms upon salt stress to characterize changes in intracellular K^+ , (3) defining biosynthetic pathways (e.g., identifying and characterizing key enzymatic activities) for several unique osmolytes including *N*-acetyl- β -lysine and DIP (di-*myo*-inositol-1,1'-phosphate), an unusual osmolyte that occurs in hyperthermophiles, and (4) quantifying how other external perturbations (e.g., high external pressure or moderate molecular weight PEGs) affect osmolyte accumulation in *M. thermolithotrophicus* and other bacteria known to adapt to high pressure. The first of these provides a continuous assay of how the cells alter their intracellular solutes in response to different medium conditions. The second has allowed us to explore how K^+ ion fluxes are related to organic osmolyte accumulation. The third area has provided us with several candidate proteins that may be regulated by changes in external NaCl. The fourth study will provide information on the role of Na^+ in osmolyte production and balance.

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

15. Differential Regulation of Plastid mRNA Stability

D.B. Stern

\$176,000 (FY 96 funds/2 years)

Chloroplast gene expression is often regulated at the level of mRNA processing and stability. This regulation is required for the coordinated synthesis and assembly of the electron-transport chain macromolecular complexes. We have focused on the chloroplast genes *petA*, *petB* and *petD*, which encode subunits of the cytochrome *b6/f* complex. Using biochemical methods, we have characterized a 41 kDa protein (CSP41) which recognizes a conserved sequence in the 3' untranslated region of *petD* mRNA. This protein also exhibits endoribonuclease activity. When the CSP41 binding site was altered in chimeric reporter genes introduced into tobacco chloroplasts by biolistic transformation, alterations in mRNA accumulation were seen consistent with a role of CSP41 in regulating *petD* 3' end maturation and/or RNA stability. The Arabidopsis *csp41* gene was isolated and used to construct antisense plants with no detectable CSP41. These plants are viable, but preliminary results suggest that the accumulation of several chloroplast mRNAs may be reduced. In a related project, we have used tobacco chloroplast transformation to create disruptions of the *petA*, *petB* and *petD* genes. As expected, these plants exhibit high chlorophyll fluorescence and require sucrose. RNA accumulation patterns and polysome loading are being studied to investigate possible crosstalk between the genes encoding cytochrome *b6/f* complex subunits. Our hypothesis, based on findings for other chloroplast and mitochondrial macromolecular complexes, is that one or more subunits of the cytochrome *b6/f* complex will be under translational autorepression in these disruption plants. If so, it will illustrate that higher plant chloroplasts, like

Chlamydomonas chloroplasts and yeast mitochondria, utilize this intricate mechanism to ensure stoichiometric accumulation of photosynthetic protein subunits.

Brookhaven National Laboratory Upton, NY 11973

16. Plant Molecular Genetics

B. Burr and F.A. Burr, Biology Department

\$352,000

Simple sequence repeats (SSRs) for the basis for a new generation of molecular markers for higher plants. By their nature, these co-dominant markers detect higher levels of polymorphism than other single locus markers. SSRs have the further advantage that they can be detected with a simple PCR-based assay. We have modified an enrichment protocol that has allowed us to describe and map 409 SSR loci for maize and 225 for cotton. The maize loci were mapped in two recombinant inbred populations we maintain that are used by the maize genetics community for rapid gene mapping. The database for these populations has over 2250 mapped loci and is available over the internet (<http://burr.bio.bnl.gov:80>).

New milling methods require enhanced properties of cotton fiber. We are sequencing cDNAs from developing cotton fiber with a view toward finding genes that control cotton fiber properties such as length strength and thickness.

We are interested in the regulation of carotenoid and anthocyanin pigmentation. We have characterized *in1*, a negative regulator of anthocyanin biosynthesis in maize and are now focusing on its interaction with *r1*, the positive regulatory element that is its apparent target.

Brookhaven National Laboratory Upton, NY 11973

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

P.G. Falkowski, Department of Applied Science

\$105,000

This project addresses the basic molecular mechanisms responsible for the acclimation of the photosynthetic apparatus to changes in irradiance. We have recently identified that the redox status of the plastoquinone pool is a sensor that affects nuclear gene transcription in a eucaryotic green alga, *Dunaliella tertiolecta* (Escoubas, et al., Proc. Nat. Acad. Sci. 92:10237-41). The research builds on that discovery by analyzing the signal transduction cascade and the cue/response functions. The effect of redox modulation in the photosynthetic electron transport chain on the expression of a variety of nuclear genes is under investigation. The research goals are to characterize the key DNA binding factors, follow the effects of redox control on the activation of the binding factors, and examine how redox poise is related to environmental cues such as irradiance, temperature and CO₂. The research has broad implication for understanding how environmental information is transduced to biochemical information with photosynthetic organisms, and how that information, in turn, affects nuclear gene expression.

Brookhaven National Laboratory
Upton, NY 11973

18. Regulation of Energy Conversion in Photosynthesis

G. Hind, Biology Department

\$315,000

The efficiency of photosystem II and the distribution of excitation energy from photosystem II to photosystem I are dynamically controlled by thylakoid-bound enzymes that modify pigments and pigment-protein complexes. Reversible protein phosphorylation on threonine residues is an important component in this regulation, and involves many photosystem II proteins. Evidence from mutant and inhibitor studies indicates that multiple protein kinases are involved.

We have described a 64-kDa enzyme, which can be solubilized with octylglucoside and partially characterized. However, enzyme activity is consistently detected in the unsolubilized fraction. Established membrane-fractionation protocols were used to explore the origin of this residual activity, which remained in tight association with core complexes containing photosystem II reaction centers. An entirely novel 58-kDa protein kinase was identified in core complexes by renaturing a Western blot and probing for autophosphorylation and histone-phosphorylation activities. This enzyme catalyzes phosphorylation of the intrinsic CP43 component of photosystem II cores, and light-harvesting chlorophyll a/b protein supplied exogenously. Its possible interaction with minor cytochrome components in the cores is under study. Future work will explore the substrate preferences of the 64- and 58-kDa kinases, and possible interaction between these enzymes. Bulk fractionation of core complexes toward obtaining 58-kDa kinase for sequencing and cloning is in progress.

Latent polyphenol oxidase (PPO) is released from thylakoids by octylglucoside. An active form of PPO forms a complex with Rubisco: the possible significance of this for Rubisco turnover is being assessed owing to the presence in PPO of a putative metalloproteinase functionality.

Brookhaven National Laboratory
Upton, NY 11973

19. Modification of Plant Lipids

J. Shanklin, Biology Department

\$345,000

Fatty acids perform essential roles in plants both as structural components of membranes and as a form of fixed carbon in triacylglycerols. Their properties are defined by the presence of double bonds and other substituents in the acyl chain. As a model to study how fatty acids are modified we are using the soluble acyl-ACP desaturase system. By integrating information from primary sequences with three-dimensional crystallographic structural information we have identified the molecular determinants of substrate and regiospecificity. This model of the structure-function relationships of residues within the desaturase has allowed us to rationally design new enzymes with unique properties. We are currently attempting to express these enzymes in the model system *Arabidopsis* to evaluate their utility in transgenic plants. If successful these enzymes will be introduced into crop plants which will accumulate oils that contain desired fatty acids for particular industrial applications.

In a separate line of investigation we are probing the structure-function relationships of members of the predominant class of lipid modification enzymes that are integral membrane proteins. The alkane ω -hydroxylase system from *Pseudomonas oleovorans* is being developed as a model system for these experiments because it shares biochemical and structural similarities with the entire class of lipid modification enzymes. Mössbauer spectroscopy of this enzyme suggests that it has a diiron active site with properties similar to the soluble acyl-ACP desaturase active site. Experiments are also in progress to identify the molecular determinants of reaction outcome for the integral membrane desaturases and hydroxylase class of enzymes.

Brown University
Providence, RI 02912

20. δ -Aminolevulinic Acid Biosynthesis in Oxygenic Prokaryotes

S. Beale, Division of Biology and Medicine

\$107,000

Tetrapyrrole pigments function as essential components in the processes of respiration and photosynthesis, and also exist as cofactors in other important enzyme reactions. The tetrapyrroles arise from a branched biosynthetic pathway having δ -aminolevulinic acid (ALA) as its first committed member. ALA is known to be formed by two distinct routes: by condensation of glycine and succinyl-CoA in animal, fungal, and some bacterial cells, and by transformation of the intact carbon skeleton of glutamate in plants, algae and other bacterial cells. It has become apparent that the five-carbon pathway from glutamate is more widely distributed than was previously thought, and it may be the usual route of ALA formation in phototrophic organisms in addition to being the universal mode in oxygenic species. The macromolecular components of the five-carbon ALA-forming system have been fractionated into three enzymes and a required tRNA component. Cell-free preparations have been obtained in our laboratory from several phototrophic prokaryotes, including oxygenic cyanobacteria and strict anaerobes, that catalyze ALA formation from glutamate by reactions similar to those occurring in plants and algae. We propose to continue the characterization of the enzymes and RNA reaction components derived from phototrophic prokaryotes and from the unicellular alga, *Chlamydomonas reinhardtii*, to compare them to their counterparts in higher plants, and to study the regulation of their activity in response to light and nutritional status. The potential of these organisms for molecular genetic studies will be exploited by cloning and sequencing the genes encoding enzymes that catalyze steps of tetrapyrrole biosynthesis. The cloned genes will be used to generate probes to study the regulation of their expression during adaptation of the cells to light and nutritional status. The probes will also be evaluated for use in measuring expression of homologous genes in other algae and higher plants.

California Institute of Technology
Pasadena, CA 91125

- 21. Molecular and Genetic Analysis of LEAFY, a Gene Controlling Floral Induction and Flower Development in *Arabidopsis thaliana***
E. Meyerowitz, Division of Biology \$140,100

We are continuing two separate lines of research. One is directed to understanding the signal transduction pathway by which the plant hormone ethylene is sensed, and its presence is transduced to cause alteration in plant gene activity. We have now identified five different members of the ethylene receptor gene family, all of which can be mutated to give dominant ethylene insensitivity. One model for the function of the ethylene system is that the receptors act in air to repress the ethylene response; mutating them to ethylene non-binding forms thus causes inability to respond to the hormone. To test this model we have reverted the dominant receptor mutants to obtain loss of function alleles. While single such alleles have only subtle phenotypes, double and triple mutants, in which multiple receptors are unable to signal, have strong constitutive ethylene response phenotypes; this supports the "active in air" model.

Our second area of research is the study of LEAFY, a gene with key regulatory roles in floral induction and flower development. We have found a strong LEAFY enhancer gene, named SPLAYED, that may code for a new partner that acts with LEAFY in floral development. Work is now underway to characterize and clone this gene. We are also purifying LEAFY protein from plants; purification under native conditions seems to indicate that LEAFY is found in a protein complex. Attempts to identify the other protein components of the complex are in progress.

University of California
Berkeley, CA 94720

- 22. The Molecular Genetics of Ligule Induction**
M. Freeling, Department of Plant and Microbial Biology \$102,999

The maize leaf is comprised of proximal sheath and distal blade, separated at their boundary by the ligular region. We are gaining further understanding of how the ligular region, consisting of a fringe of epidermal tissue, the ligule, and hinge-like auricles, is positioned, induced, and formed. Recessive mutations in either *lg1* or *lg2* result in an absence of ligule and auricle structures and a broadening of the normally distinct blade/sheath boundary. DNA sequence analysis of *lg1* and *lg2* suggests their protein products may act as DNA binding proteins. *lg1* shares homology with squamosa promoter binding proteins (Moreno et al., *Genes and Development*, 1997) and *lg2* with basic leucine zipper type transcription factors (Walsh et al., in preparation). SEM analysis of developing ligular regions combined with genetic evidence suggests that *LG1* and *LG2* act in the same pathway to establish the blade/sheath boundary and position the ligule (Walsh et al., in preparation). Interpretation and propagation of a signal to induce ligule and auricle requires *LG1* activity.

The ligule of *Vg1-R*, a spontaneous dominant mutant, is greatly reduced. We found that *Vg1-R* individuals initially form a normal-appearing ligule, but that cells within this structure die before

development is complete. Genetic evidence suggests ectopic expression or altered function of *vg1*⁺ results in cell death (Jesaitis, unpublished). An attractive hypothesis is that Vg1-R aberrantly activates programmed cell death and to test this idea, we obtained several transposon tagged Vg1-R alleles and are working to clone the gene (Jesaitis, unpublished).

University of California
Berkeley, CA 94720

23. Cellular Integration of MVA Synthesis and Protein Prenylation

W. Gruissem, Department of Plant Biology

\$116,000

The multi-branched isoprenoid pathway from mevalonic acid (MVA) is one of the most important pathways in plants because isoprenes are required for many cellular processes such as cell division and growth, defense, hormone signaling, and photosynthesis. Research in animals and yeast has established MVA synthesis and prenylation of growth-related signal transduction proteins as critical factors for cell cycle progression and normal cell growth. MVA synthesis is also critical during plant development, but it is unknown how isoprenoid production is coordinated with cell division and growth. Modification of select regulatory proteins by prenylation provides a potentially important link between the isoprenoid pathway and growth control. Protein prenyl transferases have been found in all eukaryotic cells, and we have shown that the conserved plant farnesyl transferase can restore the function of Ras signaling in yeast. This project uses a biochemical and genetic approach to identify targets of protein prenyl transferases in plants. We have discovered that two plant regulatory proteins, the transcription factor APETALA-1 and a new type of calmodulin, are modified by prenylation. Mutations in the prenylation recognition motif, or genetic interactions between mutations in AP-1 and farnesyl transferase, show that the isoprenoid modification is necessary for the function of the proteins. Future experiments will clarify the cellular network that integrates early steps in the isoprenoid biosynthesis pathway with the function of these regulatory proteins.

University of California
Berkeley, CA 94720

24. Determinants of Environmental Stress Tolerance by Bacteria on Leaves

S.E. Lindow, Department of Plant and Microbial Biology

\$77,010

Bacteria that live as epiphytes on the surface of healthy plants are important as plant pathogens, in causing plant frost injury by catalyzing ice formation, and in other processes. The objective of this study is to determine those genes in plant-associated bacteria that are expressed in cells while on leaves but not in culture media and to determine how these traits enable epiphytic bacteria to survive the stresses encountered on leaf surfaces. We used a random mutagenesis approach to identify several loci in *Pseudomonas syringae* that are required for the survival of desiccation stress on leaves. We are developing an *in vivo* selection assay for plant-inducible genes using the *metY* locus which is required for stress survival on plants. Plant-inducible genes are being identified by complementing a *metY*⁻ mutant of *P. syringae*, which our previous work has shown to be deficient in both methionine biosynthesis and environmental stress tolerance on plants. A *MetY*⁻ strain

harboring a plasmid library consisting of DNA segments fused to a promoterless *meY* locus survive on dry plants only if the cloned segments harbor a plant-inducible promoter. The plant-inducible loci are being sequenced to ascertain their putative functions and inactivated by insertional mutagenesis to enable their role in epiphytic fitness and stress tolerance to be determined. Current work is directed to determining the lowest level of transcriptional activity of gene fusions that is sufficient to complement epiphytic fitness in this system. The loci identified in this selection scheme will be further characterized following isolation using inverse PCR by end-sequencing. A cassette enabling production of transcriptional fusions to a highly efficient GFP reporter gene has been made to enable the site-specific expression of plant-inducible genes to be determined.

University of California
Berkeley, CA 94720

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

N.R. Pace, Department of Plant and Microbial Biology

\$81,000*

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

16S rRNA genes in DNA isolated from high-temperature environmental samples are isolated by cloning, directly or following amplification by polymerase chain reaction, for sequence and phylogenetic analysis. Hybridization probes prepared from recombinant 16S rRNA genes are used to evaluate the quantitative representation of particular sequence representatives and to identify the corresponding organisms in environmental samples. Environments include diverse Yellowstone settings and deep-sea hydrothermal vents. Many novel hyperthermophiles have already been discovered using this approach, including new and most deeply divergent lineages of Bacteria and Archaea. The results of these studies have substantially revised our understanding of the phylogenetic make-up of the domains Archaea and Bacteria.

The program uses existing methods and continues to develop new ones for rapid analysis of natural communities. The sequences also are used for comparative analyses of hyperthermophilic and related mesophilic 16S rRNAs, to identify primary and secondary structural elements that may contribute to extreme thermostability.

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

University of California
Berkeley, CA 94720

26. Phytochrome from Green Plants: Properties and Biological Function

P.H. Quail, Department of Plant and Microbial Biology

\$105,000

Phytochrome B (phyB) is one of a five-member family of informational photoreceptors (designated phyA to phyE) in Arabidopsis. In fully green plants, phyB has a dominant role in monitoring for the appearance of competing neighboring plants and the consequent induction of the shade avoidance response. A central focus of this laboratory is to identify molecular components responsible for transducing signals perceived by phyB to the cellular response system. We have used a genetic approach to this problem beginning with a transgenic line of Arabidopsis (designated ABO) that overexpresses phyB. Seedlings of this line display enhanced deetiolation specifically in red light. To identify genetic loci necessary for phytochrome signal transduction in red light, we mutagenized ABO seeds and screened for revertants of the enhanced deetiolation response. One recessive, red-light-specific, extragenic revertant, designated *red1*, was isolated. The mutant phenotype was expressed in the original ABO background as well as in the nontransgenic Nossen (No-O) progenitor background. *red1* was mapped to the bottom of chromosome 4 at a position distinct from all known photoreceptor loci. Together with complementation analysis, the data show that *red1* is a novel photomorphogenic mutant. The evidence suggests that *red1* may represent a phytochrome signal transduction mutant potentially specific to the phyB pathway.

University of California
Berkeley, CA 94720-3120

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

B.J. Staskawicz, Department of Plant and Microbial Biology

\$111,000

During the past year our research has focused on the molecular events involved in the delivery of the AvrRpt2 protein to the plant cell and the genetic dissection of the defense signal transduction pathway. The construction of epitope-tagged avrRpt2 avirulence genes and the production of polyclonal antisera capable of detecting the AvrRpt2 protein has allowed us to detect AvrRpt2 in induced bacteria, inoculated plants and stable transgenic plants. The results of these studies suggest that the AvrRpt2 protein is most likely processed either during the delivery to the plant cell or once it is inside the plant cell. In addition, we are beginning to study the molecular processes involved in the type III secretion process as it relates to the delivery of AvrRpt2. We are currently determining the location of the processing site and the sub-cellular location of the protein once it is inside the plant cell. The construction of transgenic Arabidopsis plants with a glucocorticoid inducible promoter has allowed us to use this system as a conditional lethal selection for mutations in the defense signal transduction pathway. Several putative mutants have been identified in this screen and are currently being further characterized. The development of this screen should allow us to perform saturation mutagenesis and allow us to detect rare mutations in this pathway.

University of California
Berkeley, CA 94720

28. Analysis of Genes Essential for Floral Development in Arabidopsis

P. Zambryski, Department of Plant and Microbial Biology

\$95,000

First, we continued studies on the TOUSLED protein kinase (PK) gene, with emphasis on its role in gynoecium development. Mutations in *tsl* cause reduced differentiation of apical gynoecial tissues and eliminate fusion of the style and septum. This morphological defect correlates with the highest expression of TSL mRNA in the developing style. Double mutant analyses suggest TSL interacts with ETTIN, and redundantly with PERIANTHIA and LEUNIG. Biochemical analyses demonstrate the TSL PK trans-autophosphorylates on serine and threonine residues. TSL oligomerizes via its N-terminal domain utilizing a region containing two alpha-helical segments predicted to form a coil-coiled structure. Database analyses reveal a highly conserved TOUSLED-like kinase (TLK) family in both plants and animals, implying these enzymes perform similar roles in the nuclei of all multicellular eukaryotes. Second, our continuing studies on the ETTIN gene revealed it is expressed throughout stage 1 floral meristems and subsequently resolves into a complex pattern within petal, stamen and carpel primordia. ETT likely imparts regional identity in floral meristems affecting perianth organ number spacing, stamen formation, and regional differentiation in stamens and gynoecia. During stage 5, ETT expression appears in a ring at the top of the floral meristem before morphological appearance of the gynoecium, supporting that ETT prepatterns apical and basal boundaries in the gynoecium. Double mutant analyses suggest ETT requires some meristem and organ identity genes (LEAFY, APETELA1, APETELA2, and AGAMOUS) but not others (APETELA3 and PISTILLATA); and ETT acts independently of CLAVATA loci but redundantly with PERIANTHIA.

University of California
Davis, CA 95616

29. Where Do Nectaries Fit in the Floral Architecture?

J.L. Bowman, Section of Plant Biology

\$83,169

Nectaries are secretory organs often involved in offering rewards for pollinators in flowering plants. Despite extensive literatures on the morphology, ultrastructure, phylogenetic distribution, and pollination biology of nectaries, little is known about their ontogeny or the genes directing their development. In the Brassicaceae, nectaries are positioned at the base of the stamens. The nectaries consist of glands that are supplied by phloem with nectar secretion likely occurring through the stomata located at their apex. Although 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 singly and multiply mutant strains that lack stamens. Genetic evidence to date suggests that the development of nectaries may be independent of the expression of the ABC genes that specify the identity of the other floral organs. The only Arabidopsis mutant that is known to alter nectary development is *crabs claw (crc)*, mutations which result in the loss of all visible signs of nectary development. In the nectary, CRC expression commences in a small number of cells (the nectary anlagen) abaxial to the stamen primordia. Expression continues in all cells of the nectary throughout floral development. Characterization of CRC and other genes known to be expressed in nectaries will provide a foundation for the molecular and genetic

description of nectary development in a model species and develop tools that may be utilized to examine whether we may be able to manipulate nectar quantity and composition.

University of California
Davis, CA 95616

30. Biosynthesis of Cellulose and Callose in Developing Cotton Fibers
D.P. Delmer, Section of Plant Biology \$105,000 (FY 96 funds/15 ½ months)

The ultimate goal of this project is to elucidate the mechanism and regulation of synthesis of the cell wall polymer cellulose (beta-1,4-glucan) and the wound polymer callose (beta-1,3-glucan). Two cotton cDNA clones (CelA-1 and CelA-2) that encode homologs of the catalytic subunit of bacterial cellulose synthases represent the first genes isolated from plants that may encode subunits of a cellulose synthase. Evidence for CelA function includes: a) presence of conserved motifs believed to be involved in binding of UDP-glc and catalysis; b) recombinant protein binds UDP-glc in a Mg²⁺-dependent manner; c) genes are highly-expressed in cotton fibers at onset of secondary wall cellulose synthesis; d) another laboratory has identified a close homolog of this gene that is mutated in Arabidopsis plants that leads to a defect in cellulose synthesis. Current goals include: 1. Catalytic activity and topology: CelA, engineered to encode a myc epitope in N-ter and two HA epitopes in a predicted extracellular domain, is being expressed in yeast and plants to study the topology of CelA in the plasma membrane, to test for functional activity in yeast, and to perform immunolocalization; 2. Structure of cellulose and callose synthase complexes: the N-ter of CelA-1 contains two zinc fingers that we find bind two moles of zinc per mole of protein and results suggest this region interacts with itself and may be important in stabilizing CelA-CelA subunits in synthase complexes. We also search for other proteins that interact with CelA or callose synthase using yeast two-hybrid as well as biochemical studies (current candidates include cytoskeletal proteins, annexins and/or sucrose synthase). 3. Cel-A-GFP fusions are being expressed in transgenic plants to see if we can study the movement process for synthase complexes in living cells using the fluorescent GFP tag as a marker for CelA.

University of California
Davis, CA 95616

31. Cellulose Binding Proteins of Clostridium cellulovorans Cellulase
R.H. Doi, Section of Molecular and Cellular Biology \$115,000

We are determining the structure, function, assembly and regulation of the *Clostridium cellulovorans* cellulosome (cellulase) and non-cellulosomal cellulases. By our studies we hope to engineer a more efficient cellulase and a more efficient bacterial strain that will produce large amounts of hyperactive cellulase. Our first goal has been to characterize the three major subunits of the cellulosome. We have cloned and sequenced the genes for two major subunits, cbpA, which codes for the scaffolding protein, and exgS, which codes for the exoglucanase, ExgS. We have cloned the gene for the third major subunit, P100, which codes for an endoglucanase and are in the process of sequencing this gene. Our goal is to demonstrate that mini-CbpA containing 2-4 enzyme binding domains (EBDs), P100 and ExgS are capable of forming an active mini-cellulosome. We are characterizing the EBDs to see whether they are specific for one enzyme

subunit or can bind all the enzyme subunits. In addition we have cloned a gene for a second non-cellulosomal cellulase EngF. EngF is produced in large quantities during growth on cellobiose and in much lesser amounts during growth on cellulose. EngF is an endoglucanase and appears to function primarily as an oligodextrinase, since EngF is active on pentoses, but is far less active on carboxymethylcellulose than EngD, another non-cellulosomal endoglucanase, and EngB, a cellulosomal endoglucanase. We are testing to see whether synergism exists between the cellulosome and non-cellulosomal enzymes by mixing experiments with the cellulosome and EngF and EngD. By growing the cells on cellobiose and cellulose, we have demonstrated a very different pattern of cellulase synthesis. We will examine the molecular regulation of cellulosomal and non-cellulosomal genes by analyzing the transcripts and the promoters that are expressed during different growth conditions.

University of California
Davis, CA 95616-8627

32. Plant Physiological Aspects of Silicon

*E. Epstein, T.W.-M. Fan, W.K. Silk, Department of Land, Air and Water Resources,
and R.M. Higashi, Crocker Nuclear Laboratory*

\$98,968 (FY96 funds/2years)

The element silicon is unique: it is second only to oxygen in its abundance in soil, is readily absorbed by plants and constitutes an appreciable percentage of their mineral constituents, but is nevertheless not counted among the "essential" chemical elements for plants. Therefore, formulations of nutrient solutions for growing plants do not include silicon. We have shown that wheat plants grown in conventional, minus-silicon nutrient solutions are abnormal in physical properties of the cell walls of leaves and awns. The cells of silicon-supplied plants are rougher and tougher than those of silicon-deprived ones. For awns, we have developed a quantitative test of this large difference in roughness. The importance of this finding is that plant cell walls are responsible for many physical properties such as erectness of habit and favorable positioning of leaves for photosynthetic energy capture. Thus, the omission of silicon from the formulation of experimental and hydroponic nutrient solutions may lead to abnormal growth of plants, especially cereals. Silicon needs to be considered in the formulation of plant growth media. Our second major finding deals with the absorption of silicon by plant roots and its transport to the shoots. The element is rapidly absorbed by the roots, which transport nearly all of it to the shoots. The rapidity and extent of this root/shoot "partitioning" of silicon have no parallel with any other element. Suggestions now being followed up are that the transport of silicon is inexpensive in terms of expenditure of metabolic energy by the plant.

University of California
Davis, CA 95616

33. Regulation of Embryonic Development in Higher Plants

J.J. Harada, Section of Plant Biology

\$95,000

Many genes have been identified that are required for the completion of embryonic development in higher plants, however, relatively few have been shown to play major regulatory roles. The

Arabidopsis LEAFY COTYLEDON (LEC) genes, LEC1, LEC2, and FUSCA3, play central roles in embryogenesis. These genes are required to suppress the embryonic potential of the suspensor early in embryogenesis, to specify cotyledon identity, to maintain the maturation phase, and to prevent developing seed from germinating prematurely. These numerous and varied roles suggest that LEC1 may act in coordinating diverse aspects of embryonic development. To begin to understand the function of these genes at a mechanistic level, we isolated the LEC1 gene and analyzed its expression pattern. Sequence comparisons showed that the deduced polypeptide is homologous to a conserved eukaryotic transcription factor, suggesting a direct regulatory role for LEC1. As predicted from the mutant phenotype, we showed that LEC1 mRNA is present throughout embryogenesis, including the earliest stages, and that the gene does not appear to be expressed at any other developmental stages. We also showed that LEC1 mRNA is present in specific regions of the embryo, in the suspensor, and in the endosperm. Based on these results, we hypothesize that LEC1 may play its integrative role by establishing and maintaining an embryonic state within the seed. Studies are in progress to test this hypothesis.

University of California
Davis, CA 95616

34. Cellular and Molecular Characterization of Vascular Plasmodesmata

W.J. Lucas, Section of Plant Biology

\$120,000

In angiosperms, the functional enucleate sieve tube system of the phloem appears to be maintained by the surrounding companion cells. Analysis of sap collected from the sieve tubes has established that over 100 proteins are present in the sieve tube lumen. Presumably these proteins play a central role in maintaining the functional state of the sieve tubes. Parallel experiments performed with phloem proteins obtained from squash plants, the cucumber mosaic virus movement protein and KNOTTED1, a plant homeobox protein, indicated that these proteins all display comparable capacities to interact with and move through plasmodesmata. Of equal importance, we found that these phloem proteins exhibit a high affinity for the plasmodesmal binding sites; concentrations required for cell-to-cell transport were in the 20 nM range. A number of squash phloem proteins were cloned and sequenced to allow us to further explore the molecular determinants for protein trafficking through phloem plasmodesmata. In situ and immunolocalization studies confirmed that the mRNA was restricted to the companion cell whereas protein was detected only in the sieve tubes. A search of the data base revealed homologous genes in tobacco, Arabidopsis, rice and maize. We also identified sequences in these novel phloem proteins that exhibit homology to a viral movement protein. These sequences are presently being analyzed to identify the presence of common motifs that may reflect plasmodesmal localization signals. Information of this nature will allow us to explore the evolution of the capacity of plants to function as supracellular organisms.

University of California
Davis, CA 95616

35. Protein Translocation and Assembly in Chloroplasts

S.M. Theg, Section of Plant Biology

\$98,000

This project seeks to elucidate the mechanism of transport and assembly of the nuclear-encoded subunits of the photosynthetic oxygen-evolving enzyme complex (OEC). This complex, which produces essentially all the oxygen in the atmosphere, consists of three nuclear-encoded subunits working in concert with chloroplast-encoded subunits of the photosystem II reaction center. Our experiments revealed that the homeostasis of the OEC, which is complicated by the rapid turnover of the reaction center in the light, is maintained in part by cycling the subunits between the complex bound to the inner surface of the thylakoid membrane and a pool of soluble subunits located in the thylakoid lumen. In addition, our experiments support a model of stepwise assembly of the OEC with the photosystem II reaction center, with the largest of the subunits binding to the reaction center in the unstacked regions of the thylakoid membrane. The partially assembled complex is then postulated to migrate to the stacked membrane regions, where the smaller two subunits are bound and enzyme activity is manifested.

We are also investigating the mechanism of transport of these OEC subunits across the thylakoid membrane. Our experiments have recently revealed a role for an azide-sensitive component involved in the transport of the smallest OEC subunit to the lumen, a process that was previously thought to occur without azide-sensitive components. Different experiments demonstrated that this translocation process occurs without the concomitant ion leakage that would be expected if the protein traversed the membrane through an aqueous pore.

University of California
Davis, CA 95616-8515

36. Vacuole Biogenesis in Differentiating Plant Cells

T.A. Wilkins, Department of Agronomy and Range Science

\$110,000

Our current working model contends that the assembly and transport of function-specific vacuolar H⁺-ATPase (V-ATPase) holoenzymes depends on the synthesis of V-ATPase subunit isoforms. Operating on this premise, we have established that dynamic changes in vacuolar function during rapid expansion of cotton fiber cells is contingent upon the developmental expression of two isoforms of the V-ATPase catalytic subunit (subunit A). The differential expression of two subunit A genes in developing cotton fibers is consistent with preliminary results indicating that the promoter regions of these closely related genes are quite distinct. At the protein level, the dramatic increase in V-ATPase activity per unit membrane of the vacuole in expanding fibers is directly correlated with the preferential accumulation of subunit A termed the "expansion" isoform. Moreover, the suppression of V-ATPase gene expression, particularly of the expansion isoform, in an expansion-defective fiber mutant provides further evidence in support for our model. To begin investigating how isoforms may contribute to the specialized needs of a subcellular compartment through the assembly of V-ATPase subtypes, the clones encoding the two cotton V-ATPase subunit A isoforms were expressed in the yeast *vma1* mutant. Although the plant homologs were able to functionally complement the yeast mutant, only 60% of enzyme activity was fully restored by the

yeast/plant hybrid V-ATPase holoenzyme. Efforts to further characterize subtle structural differences that may influence V-ATPase activity are currently under investigation.

University of California
Irvine, CA 92697

37. Membrane Bioenergetics of Salt Tolerant Microorganisms

J.K. Lanyi, Department of Physiology and Biophysics

\$225,000

Salt tolerance in the extremely halophilic bacteria requires generation of electro-chemical ion gradients across the cytoplasmic membrane that drive sodium extrusion and chloride accumulation. We study the molecular mechanisms of the proton and chloride transporting bacterial rhodopsins in these organisms. Studies of bacteriorhodopsin and halorhodopsin, respectively, explore the thermodynamics of the transport, the chromophore and protein changes that determine the changing connectivity of the active site to the two membrane surfaces during the transport cycle, and the mechanisms of ion conduction to and from the retinal Schiff base. Site-specific mutagenesis, time-resolved optical multi-channel and infrared (FTIR) spectroscopy, Raman spectroscopy, stopped-flow measurements, and through collaborations x-ray diffraction are the principal methods used in this work. With bacteriorhodopsin the main effort is to refine a detailed mechanistic model now available, and to describe the transport in structural terms to increasing degrees of resolution. With halorhodopsin we are only beginning to establish the outlines of a mechanistic model.

University of California
La Jolla, CA 92093-0116

38. Structure, Biosynthesis and Role of Complex Protein-Bound Glycans

M.J. Chrispeels, Department of Biology

\$99,000

The vacuoles in the cotyledons of leguminous seeds contain glycoproteins used for storage of nitrogen (storage proteins) and involved in plant defense against insects and mammals (lectins and enzyme inhibitors). We are studying the interaction of some of these plant defense proteins with their targets in animals. We focus on two classes of proteins, alpha amylase inhibitors (aAI) and lectins, in the common bean, *Phaseolus vulgaris*, and other leguminous plants. The bean aAI inhibits the amylase of certain insects but not of others. To understand this specificity we recently cloned the amylase of the bruchid *Zabrotes subfasciatus* and purified the expressed enzyme. We have now cloned the amylase of a second insect *Acanthoscelides obtectus*. We have found in the common bean two variants of aAI and the amino acid sequences of both inhibitors have been obtained. We are now in a good position to study the interaction of the enzyme and the inhibitors. aAI-2 inhibits the amylase of *Z. subfasciatus*, but not that of *A. obtectus*. Our goals are to crystallize aAI-2 with the amylase of *Z. subfasciatus*, and to use biopanning of a peptide library to find an inhibitor for the amylase of *A. obtectus*. Progress has allowed us to obtain a grant from a joint Industry- State of California program to clone the amylase of the western corn root worm. We are investigating a lectin from the legume *Dolichos lablab*. The cloned lectin has been expressed in *E. coli* and the isolated protein is toxic to aphids at levels that will make it feasible to use it for genetic engineering.

University of California
La Jolla, CA 92093-0116

39. Molecular Structure, Function and Physiology of K⁺ Uptake Channels in Plants
J.I. Schroeder, Department of Biology **\$93,000**

Potassium uptake by higher plant cells is of central importance to plant growth, stomatal regulation, cell expansion, nutrition, tropisms, osmoregulation, enzyme homeostasis and plant membrane potential control. Elucidation of the molecular mechanisms by which higher plant cells selectively transport K⁺ is important for manipulation of the above processes. Patch clamp studies on guard cells and many other plant cells have suggested that "inward-rectifying" K⁺ uptake channels provide a molecular pathway for membrane potential control and for proton pump-driven K⁺ uptake. Arabidopsis K⁺ channel cDNAs have recently been cloned, and we are functionally characterizing the structure and function of two of these cDNAs expressed in leaf cells and guard cells.

The long-term goal of this research project is to gain a quantitative understanding of the physiological functions of Arabidopsis K⁺ channels for important biological functions in plant leaves. We will focus on K⁺ channel functions during stomatal regulation, which is important for CO₂ exchange and water transpiration. Furthermore we will study the importance of a leaf K⁺ channel cDNA for leaf growth. These studies will contribute to a molecular physiological dissection K⁺ channel functions in higher plant leaves and their roles in growth, development and environmental responses of plants.

University of California
Los Angeles, CA 90095-1606

40. Suspensor Differentiation During Early Plant Embryogenesis
R.B. Goldberg, Department of Molecular, Cell & Developmental Biology **\$103,000**

The molecular processes responsible for directing cells of the early plant embryo to follow distinct differentiation pathways are not understood. In many plants, the zygote divides asymmetrically into a small apical cell and a large basal cell. The apical and basal cells are destined to become the embryo proper and suspensor regions of the embryo, respectively. It is not known what molecular events direct the apical and basal cells to follow different developmental pathways. Nor is it known what genes control apical and basal cell specification within the two-cell embryo. This project utilizes the giant suspensor of the Scarlett Runner Bean, *Phaseolus coccineus*, to gain entry into the differentiation events that occur at the earliest stages of plant embryogenesis. Experiments being carried out in my laboratory are testing the hypothesis that "morphogenetic factors" are asymmetrically distributed to the basal cell and set off a cascade of events leading to suspensor differentiation. Specific experiments include (1) identifying and sequencing suspensor-specific genes, (2) studying the spatial and temporal expression patterns of suspensor-specific genes, (3) using targeted cell ablation studies to determine whether interactions occur between suspensor cells and between the suspensor and embryo proper, and (4) identifying corresponding Arabidopsis suspensor-specific genes and searching for mutant lines that have defects in these genes. The significance of this project is that it should provide new information on the molecular processes that control cell differentiation at the earliest stages of plant development.

University of California
Los Angeles, CA 90095

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

R.P. Gunsalus, Department of Microbiology and Molecular Genetics

\$220,000 (FY 96 funds/2 years)

Methane biosynthesis by the *Methanosarcina* species occurs from the full range of methanogenic substrates, and is often the rate limiting step in anaerobic habitats of natural and man-made environments. The substrates include acetate, methanol, tri-methyl, di-methyl, and methyl-amines, and in most cases, H_2/CO_2 as precursors for methane formation. The *Methanosarcina* are also the most versatile among the methanogens in their ability to adapt to different habitats that vary in osmolarity. To further explore the physiology of these organisms using molecular and biochemical approaches, we are identifying and characterizing different classes of differentially expressed genes. We plan to further examine how N-acetyl- β -lysine, α -glutamate, betaine, and potassium levels are modulated in the cell to provide appropriate osmoregulation. We will also examine the sets of genes employed for changes in carbon substrate utilization. We will complete a high resolution physical map of the *M. thermophila* genome to aid in further exploiting the powerful differential display methods for identifying and characterizing gene families in Archaea. The high resolution physical map and the ordered BAC library generated in this study should facilitate related studies in the other *Methanosarcina* species by this and in other laboratories. Such libraries can also be exploited to aid in genome sequencing, and to exploit genome information as it becomes available. These studies will enhance our understanding of how methanogens sense their environment and adapt physiologically to varying anaerobic habitats.

University of California
Los Angeles, CA 90024-1606

42. Sensory Transduction of the CO₂ Response of Guard Cells

E. Zeiger, Department of Biology

\$99,000

Plants need to balance the rate of water vapor loss against the rate of photosynthetic CO₂ uptake. Stomata sense environmental parameters and optimize the exchange water and CO₂ in the leaf. We have identified a growth chamber environment which greatly enhances stomatal response to CO₂ and are using plants from this environment to investigate the cellular mechanism of CO₂ sensing. Zeaxanthin is a member of the carotenoid class of chloroplast pigments that has been implicated in the sensory transduction of blue light in guard cells. In mesophyll chloroplasts, zeaxanthin formation is known to be modulated by CO₂. In growth chamber-grown *Vicia faba* leaves kept under constant light (500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and temperature, guard cell zeaxanthin tracked ambient [CO₂] and stomatal apertures. Changes in zeaxanthin and aperture were reversed when [CO₂] was lowered. Guard cell zeaxanthin was linearly correlated with stomatal apertures. In the dark, the CO₂-induced changes in stomatal aperture were much smaller, and guard cell zeaxanthin did not change with chamber [CO₂]. Guard cell zeaxanthin also tracked [CO₂] and stomatal aperture in isolated stomata. Application of dithiothreitol (DTT), an inhibitor of zeaxanthin formation, to illuminated epidermal peels eliminated CO₂-induced zeaxanthin changes in guard cells and partially

inhibited the CO₂ response of stomata to the levels observed in the dark. Zeaxanthin could modulate CO₂-dependent stomatal apertures in the light, while a zeaxanthin-independent CO₂ sensing mechanism would modulate the CO₂ response in the dark.

University of California
Santa Cruz, CA 95064

43. Regulation of Vacuolar pH in Citrus limon

L. Taiz, Department of Biology

\$100,000

The pH of plant vacuoles is under developmental and environmental control, but little is known about how vacuolar pH is regulated. Lemon provides an excellent system for such studies because it contains two types of vacuoles: a typical vegetative-type vacuole with a lumenal pH of ~5.5, and a highly acidic juice sac-type vacuole in the fruit with a lumenal pH as low as 2.2. Our work to date has established that both tonoplasts contain V-type ATPases. However, we have found a number of differences as well: 1) fruit tonoplasts have a lower H⁺ permeability than epicotyl tonoplasts; 2) in native membranes, the epicotyl V-ATPase is strongly inhibited by nitrate and bafilomycin, while the fruit V-ATPase is relatively insensitive to these inhibitors; 3) after reconstitution into *E.coli* or soybean phospholipids, the purified fruit V-ATPase becomes sensitive to nitrate and bafilomycin; 4) reconstituting the fruit V-ATPase into fruit tonoplast lipids restores the insensitivity to nitrate and bafilomycin, hence membrane lipids protect the fruit V-ATPase from these inhibitors. However, reconstituting the epicotyl V-ATPase into fruit lipids does not make the epicotyl V-ATPase insensitive to nitrate and bafilomycin. 5) the epicotyl V-ATPase has a lower H⁺/ATP coupling ratio and, when purified and reconstituted, has a higher slip rate than the fruit V-ATPase; 6) the fruit V-ATPase contains a fruit-specific 33/34 kDa polypeptide and multiple copies of a 16 kDa polypeptide. It is proposed that these subunits may contribute to the tight coupling of the fruit V-ATPase.

Carnegie Institution of Washington
Stanford, CA 94305

44. Production of Lipophilic Materials from Plants

C.R. Somerville

\$484,940

The overall goal of the proposed research is to expand the range of biomaterials produced from higher plants and increase the efficiency of biomass production. One aspect of our work is focused on identifying plant genes that would permit production of economically significant levels of industrially useful hydroxylated and epoxidated fatty acids in transgenic plants. Toward this end we are attempting to define the structural properties of desaturases, hydroxylases and epoxidases that determine the outcome of the enzymatic reactions catalyzed by these types of enzymes. One factor that limits oil production in many plant species is that oil accumulation is accompanied by high levels of energetically expensive storage protein accumulation. To examine the feasibility of uncoupling oil and protein accumulation in plants we will identify genes encoding transcriptional factors that regulate expression of genes involved in storage lipid accumulation. We will explore the ability of these factors to induce expression of the genes involved in storage oil accumulation

in tissues with comparatively low levels of protein. We are also exploring the long-term potential of producing oils and related materials in roots or tubers. In this respect, we are investigating the function of a gene (designated *pk1*) that alters cell identity during early embryo development. Finally, we are investigating the biological function of a family of genes that exhibit sequence homology to components of bacterial cellulose synthases. The principal goal of this work is to identify the role of these genes in higher plants.

Carnegie Institution of Washington
Stanford, CA 94305

45. Powdery Mildew Disease Resistance

S.C. Somerville, Department of Plant Biology

\$160,000

Our long term objective is to characterize genes conferring resistance to powdery mildew diseases, which are caused by obligate fungal pathogens of the *Erysiphe* genus. Because of the extensive molecular genetic resources available for *Arabidopsis*, we chose to isolate powdery mildew resistance genes from this plant species. In previous work, seven distinct resistance loci were found in six accessions suggesting that powdery mildew resistance in *Arabidopsis* is as diverse and complex as in crop species like barley. In a large survey, 66 of 360 accessions were found to be resistant to *E. cichoracearum* isolate UCSC. A complementation test based on the 40 most resistant accessions has been initiated. From this test, new powdery mildew resistance genes and stronger alleles of known resistance genes will be identified.

We have focused on the powdery mildew resistance locus, RPW1. Based on a preliminary QTL (quantitative trait loci) analysis, two modifier genes, in addition to RPW1, control disease resistance in the accession Kas-1, suggesting that some pyramiding of resistance genes has occurred in the weedy plant, *Arabidopsis*. A map-based cloning strategy will be used to clone RPW1. To complement the map-based cloning, we have mapped 47 R-ESTs (i.e., ESTs with sequence similarity to known resistance genes) and compared their map positions with those of known disease resistance genes. No R-EST maps near RPW1; thus, the candidate gene approach has not been helpful in cloning RPW1. However, the information generated by this project should facilitate the cloning of *Arabidopsis* resistance genes by the plant pathology community.

University of Chicago
Chicago, IL 60637

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

R. Haselkorn, Department of Molecular Genetics and Cell Biology

\$100,000

This work has become the *Rhodobacter* genome project with three goals: to complete the genome sequence for the type strain, SB1003; to develop expression chips for the determination of transcript levels for all the genes at once, under different growth conditions and in different mutant strains; and to develop genetic methods for deletion strain construction to enable a complete functional analysis of the genome.

The sequencing has progressed as follows: About 200 kb have been sequenced, polished and fully annotated and published. The paper by Vlcek, Paces, Maltsev, Paces, Haselkorn and Fonstein is in press in PNAS. Another 600 kb are being annotated at Argonne National Lab by Natalia Maltsev, working in the lab of Ross Overbeek. Another 600 kb are being polished for annotation. Another 600 kb have been subcloned and sequenced. The next 600 kb are being subcloned.

Expression chips are being developed in collaboration with the group of A. Mirzabekov at ANL. The major technical problems are stability of the acrylamide matrix, reduction in the fluorescence background from the acrylamide, improving the yield of the crosslinking reactions for the oligo probes. All of these are being studied. We were awarded a small starter grant from the Argonne/U of Chicago consortium to pursue these studies.

Regarding the construction of deletions, the methods are in place for cosmid-sized deletions. For single gene deletions, the approach is based on PCR amplification of the deletion target and will be developed in collaboration with Fevzi Daldal at U. of Pennsylvania.

University of Chicago Chicago, IL 60637

47. Molecular Genetic Analysis of Biophotolytic Hydrogen Production in Green Algae

L. Mets, Department of Molecular Genetics and Cell Biology

\$100,000

Several species of green algae have the unique biochemical capability of producing gaseous hydrogen from water using solar energy harvested by the photosynthetic apparatus, a process called biophotolysis. An understanding of factors that promote as well as limit biophotolysis may lead to a highly efficient solar energy conversion system that directly yields hydrogen gas as the energy storage medium. We are exploiting the powerful array of genetic analysis and molecular genetic manipulation techniques that have been developed for the green alga *Chlamydomonas reinhardtii* to investigate the molecular mechanisms underlying biophotolysis.

Efficient hydrogen production occurs only under anaerobic or anoxic conditions. This feature limits the utility of biophotolysis, since oxygen is a necessary co-product with hydrogen. Hydrogenase, the key enzyme that couples energy harvested by the photosynthetic light reactions in the chloroplast to hydrogen evolution is not synthesized except in the absence of oxygen. The mechanism of this regulatory effect of oxygen is being investigated as part of this project. The activity of the hydrogenase in green algae is also directly destroyed by molecular oxygen, although the detail is unknown. The enzyme from *Chlamydomonas* has only one subunit, making it an ideal target for exhaustive mutagenesis to test the possibility that an oxygen-insensitive form can be developed. We have initiated an effort to generate genetically altered enzymes with different degrees of oxygen sensitivity. Analysis of these mutant enzymes will help to build a picture of the mechanism of oxygen inactivation and support directed efforts to develop fully oxygen-insensitive activity.

University of Chicago
Chicago, IL 60637

48. Analysis of Cell-Cell Interactions During Arabidopsis Reproduction

D. Preuss, Department of Molecular Genetics and Cell Biology

\$99,999

Plants have a remarkable ability to interact favorably with pollen of the appropriate species, while inhibiting foreign pollen. To clarify the molecular basis for this discrimination, we have identified genes that are critical for pollen communication with female pistil tissues. We have shown that mutations in these genes can affect the abundance of proteins on the surface of pollen and pistil cells. Our work focuses on understanding these surface molecules, and these studies will likely provide valuable tools for manipulating plant resources, both in agriculture and in natural ecosystems.

We are investigating interactions between pollen tubes and ovules by characterizing a mutant with altered pollen tube guidance. This mutation affects the ability of pollen tubes to adhere to ovule cells, and we are exploring the requirements for the adhesive interactions by characterizing the mutant defect and cloning the altered genes. Importantly, the mutant has allowed us to determine that gene expression in both male and female tissues is required for guidance. In parallel, we are exploring the initial interactions between pollen and the receptive stigma cells on the pistil surface by determining the function of a family of related pollen surface proteins. In particular, we are constructing alterations in these proteins to test their roles in binding to stigma cells, in mediating communication with the stigma, or in specifying pollen identity. These studies will expand our knowledge of the unique signaling mechanisms that enable plant cells to communicate, despite their thick extracellular walls.

Clemson University
Clemson, SC 29634-1903

49. The Magnesium Chelation Step in Chlorophyll Biosynthesis

J.D. Weinstein, Department of Biological Sciences

\$123,500

In photosynthetic organisms, the synthesis of new energy generating membranes requires the coordinate synthesis of proteins, their associated cofactors, and various lipids. The important cofactors, chlorophyll and heme, share a common biosynthetic pathway, diverging at the point of metal ion insertion. Mg-chelatase catalyzes the insertion of magnesium into protoporphyrin-IX, the first step unique to chlorophyll synthesis. Our work is focused on the mechanism and regulation of this enzyme as it is a key to understanding how plants produce the proper proportion of chlorophyll and heme from a common pathway. We have shown by fractionation of chloroplast extracts and by cloning of the chlD gene that the enzyme in higher plants consists of three subunits (two other genes chlI and H have already been identified). Sequencing of the chlD gene suggests that it encodes a protein of 82.9 kDa (including a putative chloroplast transit peptide). The protein has a nucleotide binding motif and an unusual stretch of prolines followed by a stretch of polyglutamate. It is 58% homologous to the cyanobacterial protein. We have expressed the C-terminal third of the protein in *E. coli*. Although the expressed protein cannot substitute for a fraction containing the authentic pea D subunit in a reconstitution assay, it stimulates (20-25%)

activity when added to a soluble extract that contains all three components. Work is continuing on the expression of a full-length D subunit for subsequent analysis of the role of this protein in the reaction.

Cold Spring Harbor Laboratory **Cold Spring Harbor, NY 11724**

50. The *lojap* Gene in Maize

R. Martienssen and M. Byrne

\$96,000

Chlorophyll variegation in higher plants is a well known but poorly understood phenomenon. Single recessive nuclear mutations are often responsible for the variegated phenotype although, for a few mutants, defective plastids are transmitted through to the next generation. For more than 50 years the variegated *lojap* mutation in maize has been the model system for such cytoplasmic inheritance. Specifically *lojap*-affected plastids transmitted through the female egg cell remain defective independent of the parental nuclear genotype. The striping pattern of *lojap* plants indicates the *lj* 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. Sectors showing nuclear reversion to fully green arise only in heteroplastidic cells and are not observed in white tissue.

We have shown that the protein encoded by *lj* is a soluble, chloroplast localized protein that associates with chloroplast ribosomes. Consistent with the proposal that *lj* plays an essential role in translation, hypothetical proteins with sequence similarities to *lj* have been identified in all sequenced bacterial genomes with the exception of mycoplasmas and archaeobacteria. It has long been postulated that chloroplasts are the evolutionary descendants of endosymbiotic bacteria. The combined use of plant and bacterial molecular genetic systems will serve to answer fundamental questions pertaining to *lojap* variegation and cytoplasmic inheritance.

University of Colorado **Boulder, CO 80309-0215**

51. Microbial Production of Isoprene

R. Fall, Department of Chemistry and Biochemistry

\$94,000

This research project addresses the microbial production of the volatile hydrocarbon isoprene (2-methyl-1,3-butadiene). Biological systems, especially forest plants, currently emit 100s of millions of tons of isoprene to the atmosphere each year. We argue that capture of this biogenic potential in a regulated system could lead to bioreactors that produce useful amounts of isoprene as a feedstock for the polyisoprene rubber and elastomer industry. While we have made progress in understanding the enzymology of plant isoprene formation, plant systems are less tractable for biochemical and molecular manipulations than a well defined bacterial system. We have discovered that most *Bacillus* species are especially active isoprene producers. We propose that molecular investigations of isoprene synthesis in *Bacillus subtilis* will lead to a basic understanding of how and why bacteria produce this volatile hydrocarbon, and reveal the regulation of its

production. We are working to determine by ^{13}C -labeling experiments whether isoprene formation in *Bacillus* occurs by the traditional mevalonate pathway, or by the glyceraldehyde-3-phosphate/pyruvate pathway which gives rise to isoprene in plant chloroplasts. In addition, we are pursuing the idea that isoprene is a signaling molecule produced in exponentially dividing cells, and serving to repress stationary phase genes. The results of these experiments should pave the way for biochemical and molecular investigations of the mechanism and regulation of bacterial isoprene biosynthesis.

University of Connecticut
Storrs, CT 06269

52. Sugar Transport and Metabolism in Thermotoga

K.M. Noll and A.H. Romano, Department of Molecular and Cell Biology \$87,000

We are studying the energetics and regulation of sugar transport and metabolism in the genus *Thermotoga*, hyperthermophilic strict anaerobes that represent the deepest phylogenetic branch of heterotrophic fermentative bacteria known, with the expectation that studies of the comparative physiology and biochemistry of these organisms will yield insights concerning the evolution of these processes. Using non-metabolizable sugar analogs as transport substrates, we have found distinct sodium-dependent active transport systems for D-glucose and beta-D-galactoside in *T. neapolitana* that are energized by ion gradients generated by ATP, derived from substrate-level phosphorylation. While the glucose system is constitutive, both the transport and hydrolysis of beta-galactoside are inducible by galactose and lactose, and subject to a cAMP-independent repression by glucose (catabolite repression). Glucose did not inhibit beta-galactoside uptake by induced cells, however, indicating the absence of inducer exclusion. This absence of cAMP-dependent catabolite repression and inducer exclusion correlate with the apparent absence of the PEP:sugar phosphotransferase system, a multicomponent system with specific proteins that play key roles in catabolite repression and inducer exclusion in certain modern bacteria. The mechanism of the sodium requirement is being investigated to gain insight into how hyperthermophilic organisms generate and maintain ion gradients under conditions of presumed increased membrane permeability at high temperatures. Using ion-specific electrodes, movements of sodium and hydrogen ions are being measured in whole cells during metabolic energy generation and active sugar transport. Also, isolated membrane vesicles obtained by lysozyme-EDTA treatment of cells are being used to study sugar transport energized by artificially imposed ion gradients.

University of Connecticut
Storrs, CT 06269-3125

53. Genetic Analysis of Sugar Nucleotide Interconversions in Arabidopsis

W.-D. Reiter, Department of Molecular and Cell Biology \$78,733

The synthesis of plant cell wall polysaccharides depends on the availability of nucleoside-diphospho sugars representing activated monosaccharides generated via nucleotide sugar interconversions. We have chosen the plant model system *Arabidopsis thaliana* to isolate and characterize genes involved in the *de novo* synthesis of the monosaccharides L-rhamnose, L-fucose and L-arabinose via these biosynthetic pathways. Genes encoding the two enzymatic steps in the synthesis of

GDP-L-fucose from GDP-D-mannose have been identified and characterized. The first enzyme in this pathway (GDP-D-mannose-4,6-dehydratase) is encoded by at least two genes (MUR1 and GMD1), while the second enzyme (an epimerase-reductase) appears to be encoded by a single-copy gene. The MUR1 gene is expressed throughout the plant whereas GMD1 gene expression is subject to spatial and temporal regulation within the root. Mutations at the MUR1 locus lead to decreased mechanical strength of elongating inflorescence stems indicating that alterations in precursor availability can change the biophysical properties of the wall. Several genes believed to encode enzymes in the de novo synthesis of L-arabinose and L-rhamnose are currently under investigation to define their role in cell wall synthesis, and to determine their relationship to mutations leading to reduced amounts of these monosaccharides. Our long-term goal is to determine the significance of nucleotide sugar interconversion pathways for the regulation of cell wall synthesis leading to opportunities to alter cell wall compositions by modifying precursor availability.

Cornell University
Ithaca, NY 14853

54. Cytoplasmic Male Sterility and Mitochondrial Function During Microsporogenesis
M.R. Hanson, Division of Biological Sciences \$102,000

Cytoplasmic male sterility (CMS), a mitochondrially-encoded disruption in pollen development, is a valuable agronomic trait that facilitates production of hybrid seed. Understanding its mechanism may facilitate the incorporation of this trait into crop species lacking naturally-occurring CMS-encoding genes. Male reproductive development is often disrupted before or during meiosis in cytoplasmic male sterility (CMS) plants. We are using cell biological techniques, including two-photon confocal microscopy and two-photon fluorescence microscopy, to compare cells in reproductive tissues in normal, CMS, and fertile plants carrying the CMS-encoding mitochondrial gene and the fertility-restoring nuclear Rf allele. Membrane-potential sensitive dyes are being used to probe respiratory activity in vivo in CMS and fertile plants. Transgenic plants containing the green fluorescent protein targeted to mitochondria and other organelles are being produced so that subcellular structures can be visualized. Molecular markers closely linked to the fertility restorer locus are being used to identify male sterile and fertile plants with recombination events near the restorer allele.

Cornell University
Ithaca, NY 14853

55. Signal Transduction in the Pollen-Stigma Interactions of Brassica
J.B. Nasrallah and M.E. Nasrallah, Section of Plant Biology \$103,000

Self-pollination in *Brassica* is prevented by the action of genes that reside at the highly polymorphic self-incompatibility (S) locus. In this system, pollen is inhibited at the surface of stigmatic papillar cells when pollen and stigma express identical S-locus haplotypes. Our work focuses on the S-locus Receptor Protein Kinase (SRK), an S-locus encoded receptor-like protein with serine/threonine kinase activity. SRK is required for the operation of self-incompatibility and is expressed specifically in the papillar cells of the stigma surface. To investigate the subcellular

localization, topology, and biochemical properties of SRK, we expressed the protein in transgenic tobacco tissue and in insect cells. Biochemical fractionation methods, protein immunoblotting, and immunocytochemistry have been used to analyze SRK in these heterologous systems as well as in *Brassica* stigmas. Our data demonstrate that SRK is an integral plasma membrane protein that is oriented with its N-terminal domain towards the exterior of the cell and its C-terminal kinase domain towards the cytoplasm, as predicted for a signaling receptor. Intermediates in SRK signaling are being sought by molecular and genetic approaches. A potential end-point of the SRK signaling pathway has been identified as a protein related to aquaporins, a class of membrane channels that facilitate water transport across biological membranes. It is anticipated that further studies of the SRK receptor and its putative downstream target will contribute to an elucidation of the general principles that underlie receptor-mediated signaling and cell-cell communication in higher plants.

Cornell University
Ithaca, NY 14853-8101

56. Regulation of Denitrification in *Rhodobacter sphaeroides*

J. Shapleigh, Section of Microbiology

\$92,000

Like humans, bacteria respire oxygen to generate energy required for growth. Unlike humans, however, bacteria can respire compounds other than oxygen. Nitrate is one of the many compounds that bacteria can use as an alternative respiratory substrate. Nitrate respiration which leads to the production of nitrogen gas is referred to as denitrification. Our laboratory is interested in identifying and understanding the role of genes whose products are required for denitrification. The bacterium we are using for our studies is a denitrifying variant of *Rhodobacter sphaeroides*. We have focused our effort on understanding the function and regulation of genes whose products are required for nitric oxide metabolism. Nitric oxide is an obligatory intermediate during denitrification and is also a biologically important molecule in higher organisms, including humans. In addition to the genes encoding nitrite reductase and nitric oxide reductase, which respectively produce and reduce nitric oxide, we have identified several other genes whose products are essential for nitric oxide respiration. Several of these encode membrane proteins of unknown function.

We have also recently determined that strains of *Rhodobacter sphaeroides* closely related to the strain we have been studying carry the genes for nitric oxide reductase genes as well as for the membrane proteins discussed above but lack nitrite reductase. This makes them unable to carry out complete denitrification. The reason for this truncated form of denitrification is unclear but such cells may be able to use nitric oxide generated by other bacteria as an alternate respiratory compound when oxygen is limited.

Cornell University
Ithaca, NY 14853

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

P.L. Steponkus, Department of Soil, Crop and Atmospheric Sciences \$110,000

The ultimate goal of this project is to provide a mechanistic understanding of the cellular and molecular aspects of freezing injury and cold acclimation to provide for rational strategies for the improvement of freezing tolerance of crop species. The current objectives are to determine the mechanism(s) by which cytosolic sugars alter the lyotropic phase behavior of phospholipids and increase the cryostability of cellular membranes. Studies in which the endogenous sugar content of *Arabidopsis thaliana* leaves was artificially manipulated by incubation on sucrose solutions have revealed that low concentrations of sucrose (10-35 mM) are required as a metabolic substrate necessary for low-temperature-induced alterations in the lipid composition of the plasma membrane during the initial stages of cold acclimation and which are necessary to preclude expansion-induced lysis during a freeze/thaw cycle. At higher concentrations, sucrose has a direct cryoprotective effect that results in a decreased incidence of freeze-induced formation of the hexagonal II phase (30-200 mM) and the fracture-jump lesion (100-400 mM). The cryoprotective effect of the sucrose appears to be additive rather than synergistic to the low-temperature-induced alterations in membrane lipid composition and the synthesis of COR polypeptides. Studies of artificial bilayers composed of either DOPE or POPE have revealed that sugars, such as sucrose and fructose, decrease the propensity for the dehydration-induced liquid crystalline-to-hexagonal II ($L_{\alpha} \rightarrow H_{II}$) phase transition at moderate levels of dehydration and preclude the liquid crystalline-to-lamellar crystalline ($L_{\alpha} \rightarrow L_c$) at extreme levels of dehydration.

Cornell University
Ithaca, NY 14853

58. Genetic Control of Nitrate Assimilation in *Klebsiella oxytoca* (pneumoniae)

V.J. Stewart, Section of Microbiology \$90,000

Klebsiella oxytoca (pneumoniae) is an enteric bacterium closely related to *Escherichia coli*. Nitrate and nitrite are important nitrogen sources not only for *K. oxytoca*, but also for many other microorganisms and most plants. In the absence of ammonium, nitrate (or nitrite) induces the synthesis of assimilatory nitrate reductase and assimilatory nitrite reductase, which act in sequence to convert nitrate to ammonium. One aspect of our work has been to identify and characterize the structural genes for nitrate assimilation, which are organized as the nasFEDCBA operon. The nasCA and nasB genes encode assimilatory nitrate and nitrite reductase, respectively. Sequence inspection indicates that the nasFED genes encode the components of a periplasmic binding protein dependent nitrate uptake system. We have constructed and characterized a series of in-frame deletions in the nasFED genes. Growth tests and uptake assays reveal that the mutants are unable to transport nitrate. However, the mutants retain some ability to grow with nitrite, even at elevated pH values where nitrous acid diffusion is negligible. It is possible that the organism expressed a second, nitrite-specific uptake system. Another aspect of our work has been to explore the molecular basis for nitrate and nitrite induction of nasF operon expression. Our

previous studies identified the NasR positive regulatory protein, and established the nasF transcribed leader region as a target for NasR-mediated transcription antitermination control. We have purified a maltose binding protein (MBP) fusion form of NasR, and have reconstituted nitrate- and nitrite-responsive transcription antitermination *in vitro*. The minimal transcription system includes linear DNA template, RNA polymerase, and nucleotide triphosphates. Transcription efficiently terminates at the factor-independent terminator in the nasF leader region. Addition of both MBP-NasR and nitrate (or nitrite) results in efficient transcription readthrough. Other anions, including chlorate and sulfite, are unable to substitute for nitrate (or nitrite). We previously identified leader region deletions that confer uninducible or constitutive phenotypes *in vivo*. Results of *in vitro* transcription with these deletion templates are fully consistent with conclusions drawn from *in vivo* analysis: uninducible deletions do not support antitermination, and constitutive deletions do not support termination. Current experiments are aimed at defining the regulatory roles played by different segments of the leader region.

Cornell University
Ithaca, NY 14853

- 59. Studies of the Genetic Regulation of the *Thermomonospora fusca* Cellulase Complex**
D.B. Wilson, Section of Biochemistry \$94,000

The long term goal of our research is to produce cellulases with higher activity in degrading biomass cellulose to sugar that will allow the economical production of ethanol from biomass. The current goals are: (1) to purify and characterize the activity of a *Thermomonospora fusca* regulatory protein that functions in the induction of six *T. fusca* cellulases and (2) to determine the molecular mechanism of *T. fusca* endoglucanase E2. We have completed a study of the amount of all six *T. fusca* cellulases produced in cells grown on six different carbon sources using quantitative Western blotting. In *T. fusca*, as in *T. reesei*, the two exocellulases made up more than 80% of the cellulase protein and they were coordinately regulated and present in nearly equal amounts. The other cellulases showed similar changes on the carbon sources, but they were not coordinately regulated either with each other or with the exocellulases. Studies of E2 active site mutants have shown that Asp117 clearly functions as the catalytic acid and it has a pK near 10. Asp156 is partially responsible for this high pK as an Asp156 mutant has a much lower pH optimum. Mutation of either Asp79 or Asp265 to Asn reduces the activity of E2, but not to the extent expected if either were the catalytic base. The Asp79/Asp265Asn double mutant retains 0.4% of the wild-type activity on CMC, which is much more than should be present if either or both of these residues functioned as the catalytic base. We have mutated Lys259 to Ala and to His. The Ala mutant has very low activity (0.2%), while the His mutant has higher activity below pH 5 and much lower activity above pH 8. Clearly, residue 259 has to have a positive charge for E2 to be active.

Cornell University
Ithaca, NY 14853-8101

60. Nitrogen Fixation and its Regulation in the Archaeon *Methanosarcina barkeri*
S.H. Zinder, Department of Microbiology \$110,000

Our laboratory's finding that the Archaeon *Methanosarcina barkeri* is capable of nitrogen fixation raises interesting evolutionary questions. Our previous studies have shown that the nitrogenase is a two-component enzyme complex with the greatest phylogenetic similarity to the Mo nitrogenase of the Gram-positive eubacterium *Clostridium pasteurianum*. We have also shown that neither nitrogenase proteins nor mRNA are found in ammonia-grown cells, indicating repression, and that there is a TATA-box element 35 bases upstream of the *nif* transcription start. Recent results include: 1) The demonstration, using the gel-shift assay, that extracts from N₂-grown cells contain proteins which bind to DNA possessing the *nif*-promoter region. Shifts were not obtained if the promoter region was deleted or if extracts from ammonia-grown cells were used. Ammonia-grown extracts contained a factor which inhibited promoter binding by N₂-grown extracts; 2) We have cloned and expressed the TATA-binding protein (TBP) from *M. barkeri*, and demonstrated that antibodies prepared against TBP caused a greater gel shift, indicating involvement of TBP in *nif* promoter binding; 3) We demonstrated that cells accumulate large amounts of alpha-glutamate as an osmolyte when grown in saline medium with either ammonia or N₂ as a nitrogen source. Glutamine was only detected in ammonia-grown cells and was transiently present in diazotrophic cells switched off by ammonia and is therefore a good candidate for an N-regulatory signal; 4) We have cloned and expressed ORF105, a *nif*-associated gene with marked homology to the eubacterial PII protein, which plays a critical role in N-regulation in eubacteria.

University of Delaware
Lewes, DE 19958

61. Metabolic Mechanisms of Plant Growth at Low Water Potentials
J.S. Boyer, College of Marine Studies \$87,000 (10 months)

In land plants, growth is more frequently limited by water than by any other environmental resource. The project objective is to identify the underlying molecular mechanisms causing growth limitations by focusing on the process of cell enlargement. Studies so far have shown that, in localized growing regions of germinating soybean seedlings, turgor in most of the cells was completely maintained when water potentials were low enough to inhibit growth. A few h later, the extensibility of the cell walls decreased and an extractable 28kD protein accumulated in the wall fraction. By cloning the cDNA, the protein was found to be an acid phosphatase. There was no change in phosphorylated intermediates likely to be targets for the enzyme. Moreover, the enzyme accumulation and biochemical events leading up to the accumulation were too slow to account for the early growth inhibition. Therefore, we have begun to explore the earliest events altering the water potential because these correlate with the early growth inhibition. We recently found that local water potential gradients change and cause at least part of the early inhibition. The changes are localized close to the vascular system in small undifferentiated cells that impede the movement of water and deprive the outlying cells of water necessary for enlargement. We have been able to reverse the inhibition by pressurizing the roots thus returning the gradient to a favorable shape next

to the xylem. The resumption of growth indicates that water movement resumes. Experiments are underway to investigate whether transport limited growth occurs in fully established plants.

Duke University Durham, NC 27708

62. Molecular, Genetic and Physiological Analysis of Photoinhibition and Photosynthetic Performance in the Green Alga *Chlamydomonas reinhardtii* *J.E. Boynton, N.W. Gillham and C.B. Osmond, DCMB Group, Departments of Botany and Zoology* \$114,000

Using *C. reinhardtii* as a model, we have examined the relationship between structure/function of the chloroplast encoded D1 protein of Photosystem II in relation to photoinhibitory damage by high light. Mutants with 12 of 20 possible amino acid substitutions at the Ala₂₅₁ residue in the QB/herbicide binding domain of D1 were characterized. Five Ala₂₅₁ substitutions were nonphotosynthetic (*J. Biol. Chem.* 272: 210-217, 1997) whereas seven displayed varied photoautotrophic (*Z. Naturforsch.* 52C, 1997 in press) and photosynthetic (*J. Biol. Chem.*, in preparation) competence. We have isolated 10 nuclear suppressors which reduce the sensitivity of the Leu₂₅₁ mutant to high light and 27 suppressors that enable wildtype to survive under near sunlight intensity (VHL). Nonphotochemical quenching (NPQ), indicative of engagement of photoprotective energy dissipation pathways, was strongly increased in the wildtype- but not the Leu₂₅₁-suppressors. In both types of suppressors, pigments of the Xanthophyll cycle (Violaxanthin, Antheraxanthin and Zeaxanthin) are upregulated in VHL while Neoxanthin decreases and Lutein strongly increases. The Z+A/Z+A+V ratio increases only in the Leu₂₅₁ suppressors, indicative of photoprotection, and decreases in the wildtype suppressors, suggesting that other photoprotective mechanisms than xanthophyll interconversion provide VHL tolerance. We previously reported a correlation between upregulation of D1 synthesis and transient downregulation in *Rubisco* LSU synthesis when wildtype cells grown at low light are transferred to high light (*Plant Mol. Biol.* 33: 1001-1022, 1997). A search for proteins that bind in a light specific manner to RNP complexes on the leaders of the *psbA* and *rbcl* mRNAs to regulate their translation is in progress. Understanding molecular mechanisms involved in photoregulation of the D1 protein in *Chlamydomonas* may facilitate manipulation of land plants to improve efficiency of photosynthesis under light stress.

Florida State University Tallahassee, FL 32306

63. Role of Sucrose in Modulating Stomatal Aperture *W.H. Outlaw, Jr., Department of Biological Science* \$60,000 (FY96 funds/2 years)

Gas exchange between a leaf and the atmosphere occurs through adjustable stomata, each of which is surrounded by a pair of guard cells. 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 deformation enlarges the pore. Stomal closure occurs when the guard-cell pair loses solutes. The aperture size is generally a compromise between the opposing priorities of permitting CO₂ uptake and avoiding H₂O-vapor loss. As CO₂ 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 a plant's physiology.

The prevailing paradigm is that fluctuations in guard-cell potassium concentrations power stomatal movements. Recently, however, an important osmotic role for sucrose in stomatal regulation has emerged. Under the conditions we have studied, sucrose accumulation in the guard-cell symplast is secondary, and we have focused on the accumulation of sucrose in the guard-cell apoplast, which we study by use of quantitative histochemical methods. Our most recent DOE-supported manuscript on this topic (*Plant Physiol* 114: 109-118) reported that this sucrose originates in the mesophyll. On this basis, we postulated that sucrose accumulation in the guard-cell apoplast, and consequent diminution of stomatal-aperture size, is one means by which plants measure the rate of transpiration. Our current work is directed toward testing this hypothesis.

University of Florida
Gainesville, FL 32611

64. Ethanol-Tolerant Biocatalysts for Fuel Ethanol Production

L.O. Ingram, Department of Microbiology and Cell Science

\$114,511

Over the past years, our laboratory has genetically engineered biocatalysts for fuel ethanol production from agricultural and woody waste materials by inserting genes (*pdc, adhB*) encoding the ethanol pathway from *Zymomonas mobilis* (naturally producer of ethanol from glucose) into a variety of Enteric and Gram+ bacteria. In these new recombinant organisms, the normal fermentation process for organic acids is completely replaced by the production of ethanol and carbon dioxide. Effective replacement of the native fermentation pathway results from high levels of the recombinant pyruvate decarboxylase and the high affinity (low Km) of this enzyme for pyruvate, a key intermediate in fermentation. Although our engineered strains have a lower ethanol tolerance than *Saccharomyces* yeasts used in commercial starch-based fermentations, our strains have a greatly expanded ability to ferment many different sugars including all which are constituents of biomass (hexoses and pentoses). We are currently attempting to improve the ethanol tolerance of our ethanol-producing recombinant bacteria and to identify genes which are important for this trait. New strains developed during the first year are capable of producing over 60 g ethanol/liter in 72 h, 7.5% ethanol by volume. Approximately 15 different fragments of chromosomal DNA have been isolated which alter ethanol tolerance. Further studies are underway to identify the specific genes which are responsible and to investigate the physiological/biochemical changes which cause an increased tolerance. Additional studies are directed at the genetic engineering of *Zymomonas mobilis*, a naturally tolerant bacterium which produces ethanol very efficiently from glucose. This organism is being improved by the insertion of genes from *Klebsiella oxytoca* to expand the range of sugars fermented to include soluble products from the enzymatic digestion of cellulose (cellobiose and cellotriase).

University of Florida
Gainesville, FL 32611

65. Gene-Enzyme Relationships of Aromatic Amino Acid Biosynthesis in Higher Plants

R.A. Jensen, Department of Microbiology and Cell Science \$180,000 (18 months)

The biosynthesis of aromatic amino acids in higher plants is of great significance, not only because of the role of these amino acids in protein synthesis, but because they are precursors of a vast array of compounds of biotechnological and medical interest. Some of the pathway enzymes also offer potential targets for design of new herbicides. Full-length cDNAs encoding five plastid-localized enzymes will be obtained from both *Nicotiana* and *Arabidopsis*. These correspond to four proteins: the bifunctional AroD•E (having catalytic domains for dehydroquinase and shikimate dehydrogenase) and the postprephenate enzymes: prephenate aminotransferase, arogenate dehydrogenase, and arogenate dehydrogenase. N-terminal amino acid sequencing will identify the putative transit peptides. The genomic DNAs will be isolated and sequenced. Intron-exon organization will be determined. Molecular studies will include determination of subcellular location using mono-specific antibody and immunogold electron microscopy, analysis of the organ-specific expression pattern of transcript abundance, determination of cell-specific expression in transgenic plants using GUS fusions, effect of antisense RNA and sense RNA manipulations, and demonstration of the import of unprocessed protein precursors into intact chloroplasts. Approaches that might lead to the possible identification of cDNAs specifying cytosolic enzymes of aromatic amino acid biosynthesis are developed.

University of Florida
Gainesville, FL 32611-0690

66. Genetic Analysis of Abscisic Acid Biosynthesis

D.R. McCarty, Horticultural Sciences Department \$91,000

The hormone abscisic acid (ABA) is synthesized from cleavage of carotenoids and regulates seed maturation and stress responses in plants. The viviparous seed mutants of maize identify genes involved in synthesis and perception of ABA. A new mutant blocked in ABA synthesis, viviparous14 (vp14), has been identified in maize and the gene cloned. Our analysis of the VP14 protein has shown that it is an enzyme responsible for the key regulatory step in ABA biosynthesis in plants. Thus, understanding how this enzyme is regulated in plant tissues is essential to understanding how ABA synthesis is regulated and used as a stress signal in plants. Our ongoing studies indicate that Vp14 belongs to a diverse family of related genes in maize. The Vp14 mRNA is expressed in embryos and roots and is strongly induced in leaves by water stress. A family of Vp14-related genes evidently controls the committed step of ABA biosynthesis and these genes are likely to play a key role in the developmental and environmental control of ABA synthesis in plants. This project is focused on identifying and determining the function of Vp14-related genes in plants. Key experimental approaches will be to 1) identify mutations in other members of the gene family, 2) characterize the proteins encoded by these genes, and 3) determine how these genes are regulated in normal and stressed plants.

University of Georgia
Athens, GA 30602-7229

- 67. The Metabolism of Hydrogen by Extremely Thermophilic Bacteria**
M.W.W. Adams, Department of Biochemistry and Molecular Biology, and Center for Metalloenzyme Studies \$104,000

Extremely thermophiles or "hyperthermophiles" are a unique group of microorganisms that grow optimally near 100° C. They have been isolated mainly from marine volcanic environments, including deep sea vents. The majority of them grow by fermenting peptides and carbohydrates and produce hydrogen (H₂) gas. To investigate the nature of the metabolic pathways and the enzymes involved, we are using the archaeon *Pyrococcus furiosus* (Pf, T_{max} 105°C) which is grown in 600 liter culture. From it we have purified an NADP-dependent hydrogenase, redox proteins such as ferredoxin and rubredoxin, a ferredoxin NADP oxidoreductase (FNOR), four different types (POR, KGOR, IOR and FOR) of 2-ketoacid ferredoxin oxidoreductases (KAORs), and two ATP-generating enzymes (acyl CoA synthetases I and II), together with three different aldehyde ferredoxin oxidoreductases, all of which contain tungsten, an element rarely used in biological systems. One of the tungstoenzymes (GAPOR) couples glyceraldehyde-3-phosphate oxidation to H₂ production in an unusual glycolytic pathway, while the other two (AOR and FOR) and the KAORs are part of an unusual pathway for peptide catabolism. The reductant that is produced is converted to H₂ via FNOR, NADP and hydrogenase. The genes for ferredoxin, rubredoxin, FOR, AOR, POR and VOR from Pf have been cloned and sequenced and some of them have been expressed in a mesophilic host. In collaborative studies with D. Rees (Caltech), the crystal structures of rubredoxin, AOR and FOR from have been determined. These studies provided the first structure for a hyperthermophilic, a tungsten-containing, or a pterin-containing enzyme. Since H₂ plays a central role in the commercial production of many chemicals, a long term objective of this research is to assess the utility of hyperthermophilic hydrogenases and oxidoreductases in industrial energy conversions.

University of Georgia
Athens, GA 30602-4712

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

The computerized Complex Carbohydrate Structure Database (CCSD) and database management system, CarbBank, were created by scientists at the Complex Carbohydrate Research Center (CCRC) in collaboration with scientists in Europe. Building the database and making the software available to researchers worldwide were the initial goals of the project. CarbBank/CCSD is designed to provide a searchable central repository of all carbohydrate structures published in the scientific literature. CCSD records encompass all types of carbohydrate structures that contain tri- and larger saccharide moieties, and include the structure of each molecule along with information on the biological or chemical source, biological activity, antigenicity, and association with particular developmental stages of the organism from which it was obtained or with a disease of the

organism. CarbBank is available in a Windows 95/NT program, and CarbBank and the CCSD now reside on the CCRC's Web site, where they can be used directly from all computer platforms by means of a Web browser. The CCRC CarbBank staff is emphasizing software development rather than exerting an all-out effort to keep up with data entry. As new programming tools designed to enhance user interaction with Web sites have become available, they are applied to CarbBank and the CCSD. Plans are proposed to streamline database building by initiating direct author submission of structures as part of the manuscript publication process; to enhance Web browser-compatible systems for author submission and searching the CCSD; to functionally integrate the CCSD with other biosequence and informatic databases including CCRC-Net; to create and coordinate "mirror" server sites in Europe, Japan, and Australia for CarbBank/CCSD so that all users enjoy optimum performance; and to integrate Web-based accounting/user access software. The CCSD now contains ~50,000 records.

*Funding provided by the National Library of Medicine.

University of Georgia
Athens, GA 30602-4712

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

\$930,000

The University of Georgia Complex Carbohydrate Research Center (CCRC) has a multidisciplinary faculty and staff who serve as a national resource for basic research in complex carbohydrate science. The CCRC faculty currently has nine tenured or tenure-track members, with two additional members joining the CCRC in 1997-98 (a full professor in nuclear magnetic resonance spectroscopy and an assistant professor in plant biochemistry). A twelfth tenure-track senior faculty member (in the area of synthetic carbohydrate chemistry) is being recruited, and the CCRC expects to grow to a total of 14 to 15 faculty members in the next three to four years. The grant supports research, analytical services, and training in plant and microbial complex carbohydrates. Six of the CCRC's faculty are active participants in the plant and microbial carbohydrate program supported by this grant. Educational activities involve the training of undergraduate and graduate students, postdoctoral research associates, and visiting scientists. Thirty-one undergraduate and 28 graduate students are currently pursuing research projects or graduate degrees (4 M.S. students, 24 Ph.D. students) in the CCRC; 16 undergraduate and 10 graduate students are working in plant or microbial carbohydrate science. Four week-long, hands-on laboratory training courses are held annually for scientists from institutions and industries located throughout the United States; 16 and 15 scientists attended these courses in 1996 and 1997, respectively, some attending more than one course. The plant and microbial carbohydrate program has provided service to 198 individuals by analyzing 974 samples in the time that the service program has been active. These analyses include determination of glycosyl-residue and glycosyl-linkage compositions, and acquisition and interpretation of one-dimensional NMR and mass spectra. The faculty and staff of the CCRC are currently involved in more than 120 internal or external collaborative research projects of which 58 are part of the plant and microbial carbohydrate program. The CCRC has, in one way or another, assisted more than 135 corporations during the same time period.

University of Georgia
Athens, GA 30602-4712

70. The Structures and Functions of Oligosaccharins

P. Albersheim, Complex Carbohydrate Research Center

\$175,000

The broad goal of this project is to determine the functions in plants of oligosaccharide signal molecules (oligosaccharins) and to learn in the process more about the interactions of plants and microbes and the regulation of plant growth and development. The importance of oligosaccharins to plants and their pathogens is made evident by the multiple mechanisms plants use to accumulate bioactive levels of these signal molecules and the corresponding mechanisms by which pathogens prevent the signal molecules from accumulating. Many of the biochemical battles that determine whether the plant overcomes or succumbs to the pathogen take place in the cell walls of plants and their microbial pathogens. Several of the subprojects study aspects of host-pathogen interactions, emphasizing the functions of molecules of cell wall origin. These subprojects concern: (I) plant cell wall-localized polygalacturonase-inhibitor proteins and the fungal extracellular enzymes they inhibit; (ii) fungi-secreted *endo*- β -1,3-glucanase-inhibitor proteins and the enzymes of the cell walls of plants that they inhibit; and (iii) fungi-secreted *endo*- β -1,4-xylanases and their role in activating defense responses in Gramineae. In addition subproject (iv) studies the bioactivities, in wild-type and mutant plants, of a plant cell wall-derived oligosaccharin that functions in regulating plant growth, and subproject (v) centers on the isolation from plants of endogenous lipo-oligosaccharide growth regulators.

University of Georgia
Athens, GA 30602-4712

71. Structural Studies of Complex Carbohydrates of Plant Cell Walls

A. Darvill, Complex Carbohydrate Research Center

\$400,000

Plant primary cell walls control several important properties of plant cells. They control the rate and direction of cell growth that determine ultimately the shapes of cells, tissues, and organs. They form a barrier to pathogens, are the source of oligosaccharins that elicit plant defense responses in host-pathogen interactions, and participate in controlling plant growth and development. Primary wall components are also used in many industrial and medical applications and thus constitute an important natural resource. The goal of this research project is to determine the structures and functions of the non-cellulosic matrix polysaccharides of primary walls that, with cellulose, account for more than 90% of the wall's dry weight. The structural studies emphasize developing analytical methods and using them to elucidate the detailed structures of hemicellulosic (xyloglucan) and pectic (rhamnogalacturonans I and II) wall matrix polysaccharides. We are also characterizing the interactions between some of the wall matrix polysaccharides by studying the structure of covalent wall cross-links. In addition, we are examining the cell-, tissue-, and species-dependent expression of cell wall epitopes using well-characterized monoclonal antibodies. This analysis may reveal developmentally and spatially regulated variations in the fine structure of the cell wall matrix polysaccharides. Finally, we are studying the structures and epitope localization of matrix polysaccharides in *Arabidopsis* mutants lacking or deficient in specific glycosyl residues. The long-range objective of this research is to better understand the diverse roles of the wall polysaccharides in cell wall function.

University of Georgia
Athens, GA 30602-7229

- 72. Mechanisms of Lignin Biosynthesis During Xylogenesis in *Zinnia elegans***
K-E.L. Eriksson and J.F.D. Dean, Department of Biochemistry and Molecular Biology, and School of Forest Resources \$91,527

This project continues to focus on the roles played by laccases in the development of vascular tissues. Several new laccase cDNAs representing the high-pI class of plant laccases have been cloned and characterized. Expression of these genes in suspension-cultured tobacco or yellow-poplar cells led to production of laccases that were retained within the cell rather than being secreted as was the case for the low-pI laccase cloned from sycamore maple cells. Heterologously expressed laccases are being analyzed by LC-MS and MALDI to map the locations and characterize the composition of the glycosyl chains attached to the laccase polypeptides. Alignment of genomic laccase sequences with the cDNAs shows that the location and size of intervening sequences has been conserved within species, and to at least some extent between species. These new sequences are being analyzed with respect to their evolution in plants. Experiments with promoter/reporter gene fusions are being performed to determine the temporal and spatial patterns of laccase gene expression during xylem development.

University of Georgia
Athens, GA 30602-7229

- 73. Fermentation of Cellulose and Hemicelluloses by Clostridia and Anaerobic Fungi**
L.G. Ljungdahl, Department of Biochemistry and Molecular Biology and the Center for Biological Resource Recovery \$166,000

Biomass consists mostly of cellulose, hemicelluloses, and lignin. Degradation of lignin does not occur by anaerobic microorganisms. It is, however, partly solubilized by the action of specific *p*-coumaroyl and feruloyl esterases produced by anaerobic fungi. On the other hand, cellulose and hemicelluloses are efficiently hydrolyzed by consortia of anaerobic microorganisms to simple components such as sugars, which are fermented to carboxylic acids, alcohols, CO₂, H₂, and methane. Our work deals with the anaerobic bacterium *Clostridium thermocellum* and the anaerobic fungus *Orpinomyces* PC-2, both of which effectively degrade plant tissue. The efficient hydrolysis of the plant tissue (biomass) is attributed to that the two microorganisms produce multiprotein cellulose/hemicellulases complexes (cellulosomes) containing endo- and exo-cellulases, and hemicellulases (xylanase). In addition, the cellulosome of *Orpinomyces* seems to contain esterases. The catalytic subunits of the cellulosomes are held by scaffolding proteins. This involves interactions between specific domains called dockerins of the catalytic subunits and cohesins of the scaffolding proteins. The dockerins and cohesins of the cellulosomes of the fungus and *C. thermocellum* are functionally similar but structurally distinct. Studies of the cellulosomes will greatly enrich the understanding of the biochemistry of enzymes for the degradation of plant biomass, the most abundant resource on earth, which can be used for the production of biofuels and chemicals. Research is also conducted with *C. thermoaceticum*, which ties into the cellulose degradation by cellulolytic consortia. This bacterium converts the degradation products by the

cellulolytic microorganisms to acetate, an important feedstock chemical. Studies with *C. thermoaceticum* involve carbon dioxide fixation by the acetyl-CoA pathway and generation of energy coupled to this pathway.

University of Georgia
Athens, GA 30602

74. Plant Arginine Decarboxylase, Regulation and Function

R.L. Malmberg, Botany Department

\$104,000

Polyamines are small positively charged compounds, hypothesized to be involved in a wide variety of plant physiological and developmental functions. These include stress response, floral and root development, and senescence. One feature of plant polyamine synthesis is the presence of two alternative pathways to putrescine, from arginine via arginine decarboxylase and from ornithine via ornithine decarboxylase. Ornithine decarboxylase is found in nearly all organisms, however arginine decarboxylase is not found in animals and some other eukaryotes. Arginine decarboxylase is regulated by post-transcriptional and post-translational mechanisms; we wish to determine the mechanisms of this regulation. We are performing molecular and genetic analysis of the polyamine synthesis pathway in the model system *Arabidopsis thaliana*. This builds upon our recent isolation of mutants in the pathway and generation of molecular probes for enzymes in the pathway. There are two structural genes encoding arginine decarboxylase, and we have also identified genes on other chromosomes that regulate this enzyme. A complex pattern of post-transcriptional regulation appears to determine arginine decarboxylase activity. We are continuing our analysis of the regulation and function of these genes using the mutants, clones, and antibodies. We also are isolating several classes of mutants that we do not currently have. The mutants we have isolated allow us to test for the roles of polyamines in processes such as plant stress response and plant senescence.

University of Georgia
Athens, GA 30602-7223

75. The Determinants of RNA Turnover in Higher Plants: The role of poly(A) binding proteins

R.B. Meagher, Department of Genetics

\$100,000

In the last decade it has become increasingly clear that post-transcriptional regulation is as important to gene regulation and organismal development as transcription. The operation of post-transcriptional regulation is mediated by the action of a post-transcriptional apparatus (PTA). Poly(A) binding proteins (PABPs) form an integral part of the PTA by binding mRNAs at both the 3' end on the poly (A) tail and to the 5' end on protein factors involved in translational initiation. The presence or absence of PABPs in the PTA effect translational initiation, polysome stability, and mRNA decay. We have shown that *Arabidopsis* and probably all higher plants have an ancient, diverse, and differentially expressed gene family encoding PABPs. *Arabidopsis* PAB2 and PAB5 complement cell viability and some molecular functions associated with yeast PABP. PAB2 is strongly expressed in young ovules and the transmittal tissue of flowers, in primordia and young

leaves, and in the stele of roots. The five specific aims of this grant are: 1) to localize the tissue and developmental stage-specific expression of PAB2 gene and protein; 2) to elucidate possible molecular mechanisms of PAB2 action in the PTA in yeast; 3) to identify specific transacting factors that bind to PAB2 in the PTA using the dihybrid system and suppressor genetics; 4) to determine if PAB2 binds distinct RNA sequences with specificity; and 5) to identify and characterize PABP mutants in Arabidopsis. Understanding the different biological functions of plant PABPs will help us to dissect important components of plant gene regulation.

University of Georgia
Athens, GA 30602-7271

76. Identification of Novel Cell Wall Components
M. Momany, Department of Botany

\$75,000

Fungi are superbly adapted to break down organic matter. They are able to scavenge nutrients in environments ranging from leaf litter on the forest floor to soap scum on bathroom tiles. Indeed, one of the defining characteristics of the fungal kingdom is the use of extracellular digestion before absorption of nutrients.

The cell wall is the main interface between the fungus and its environment. Information about nutrient availability, enzymes which break down organic matter, and digested nutrients must all transit the cell wall. In addition to its central position in fungal metabolism, the cell wall is the main determinant of morphogenesis and may contain developmental signals.

The major goals of this proposal are to identify novel cell wall components and clone the genes responsible for their synthesis in the filamentous fungus *Aspergillus nidulans*. Two parallel molecular genetic approaches will be exploited to meet these goals. In the first approach, a group of temperature-sensitive mutants with a swollen cell phenotype (*swo* mutants) will be characterized. The swollen cell phenotype is often associated with cell wall defects. In the second approach, mutants lacking specific cell wall epitopes (*cwe* mutants) will be identified through screening with phage display antibodies. Selected *swo* and *cwe* genes will be cloned by complementation of mutant phenotypes.

University of Georgia
Athens, GA 30606-7271

77. Nitrogen Control of Chloroplast Development and Differentiation
G.W. Schmidt, Department of Botany

\$98,000

Nitrogen deficiency is the most common limitation to plant growth. Our work concerns how nitrogen deficiency affects metabolism, biosynthetic pathways and development of the photosynthetic apparatus. The model system for our work is the green alga, *Chlamydomonas reinhardtii* grown in continuous cultures, in which steady-state levels of nitrogen can be precisely controlled and in which the classical symptoms of deficiency, chlorosis and slow growth rates, are perpetuated. Changes in gene expression and physiological activities in a uniform population of cells are

monitored in comparative analyses with nitrogen-sufficient cultures. Primary effects elicited by nitrogen provision, marked by rapid greening and dramatic chloroplast differentiation, are also measured. For example, reversal of chlorosis correlates with selective expression of nuclear genes encoding light-harvesting proteins, which we have shown to directly participate in synthesis of chlorophylls a and b, as well as enzymes involved in early steps in porphyrin synthesis. At another level, profound changes in respiration and carbon metabolism and the mitochondrial/chloroplast interrelationship accompany long-term adaptation to N-deficiency. Finally, we are employing mutant strains of *Chlamydomonas* for dissection of responses to nutrient limitation; defined disruptions of physiological and biochemical processes impact on the nitrogen-dependent signal transduction and intracellular communication pathways.

University of Georgia
Athens, GA 30602

78. Structure, Regulation and Evolution of the R Transcriptional Activators from Maize and Rice

S.R. Wessler, Departments of Botany and Genetics

\$104,000 (FY 96 funds/2 years)

The maize *R/B* gene family and their homologs in other plants regulate the anthocyanin biosynthetic pathway by activating the transcription of at least five of the structural genes. Our studies encompass three diverse but related projects. First, a collection of Ds-induced alleles of *R* is being used to identify structural domains required for function *in vivo*. Analyses of two alleles, *r-m9* and *r-m1*, have demonstrated the importance of three nuclear localization sequences (NLS-A,M,C) and the basic helix-loop-helix (bHLH) domain. These results differ dramatically from findings obtained using transient transformation assays. Understanding the mechanism by which an upstream open reading frame (uORF) represses expression of the *R* gene is the second goal. We have succeeded in demonstrating uORF-mediated repression both in a rabbit reticulocyte translation system and in bombarded kernels. Using these systems, we find that repression is not due to the sequence of the 38 codon uORF but, rather, is caused by the intercistronic DNA (ID) which leads to inefficient ribosome reinitiation. Characterization of the *R* gene family of rice and correlation with known genetic loci is the third goal. We have determined that *O. sativa* has at least four *R* genes, at least two of which map to the same two chromosomal regions previously shown to contain most of the genes that determine pigment pattern in rice. Further, the rice gene family has evolved recently and independently of the maize *R/B* family.

University of Georgia
Athens, GA 30602

79. Biochemistry and Genetics of Autotrophy in Methanococcus

W.B. Whitman, Department of Microbiology

\$86,000

The methanogenic archaea catalyze the terminal step in the anaerobic oxidation of organic matter to methane, a clean fuel already in wide use. The development of methods for the introduction of

genes into methanococci will be essential for the construction of more versatile microbial catalysts and further understanding of the molecular and biochemical basis of methanogenesis. Recent research has developed an efficient transformation system and shuttle vector for moving genes containing novel activities in *Methanococcus maripaludis*. The next step in the development of the shuttle vector is to introduce additional cloning sites next to a strong methanococcal promoter for efficient expression of heterologous genes, research which is in progress. The genetic system is also being utilized for construction of an integration library to mutagenize wild type cells. Screening these mutants has identified several auxotrophs for acetate and other compounds. This method is providing new insights into the pathways of carbon metabolism of these facultative autotrophs. Lastly, isotopic labeling was utilized to establish the pathway of ribose biosynthesis in the methanococci. In contrast to other methanogens, the methanococci utilize the nonoxidative pentose phosphate pathway. Surprisingly, these same experiments suggested that methanococci may utilize a novel pathway of aromatic amino acid biosynthesis. If confirmed, this will be the first report of an alternative to the pathway originating in 3-deoxy-D-arabino-heptulosonate 7-phosphate.

University of Georgia
Tifton, GA 31793

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

W.W. Hanna and G.W. Burton, Department of Agronomy

\$55,000

The objectives of this project are to: (1) establish the cytoplasmic diversity of germplasm in the weedy subspecies of the primary gene pool and demonstrate its value, (2) identify mechanism(s) for transfer of germplasm from the secondary gene pool to the cultivated species and evaluate the plant breeding potential of this germplasm, and (3) transfer gene(s) controlling apomixis from the tertiary gene pool to cultivated pearl millet for the purpose of producing true-breeding hybrids. Species within the genus *Pennisetum* are being used as test organisms. The approach uses plants of wild species with different genetic and cytoplasmic backgrounds, ploidy levels, and chromosome numbers crossed and backcrossed with different genotypes of diploid and tetraploid pearl millet. Research showed that seed loss in apomictic BC6 pearl millet plants was due to chromosome imbalance in the endosperm of the seed and possibly to an inbreeding effect of the backcrossing process. Germplasm from the secondary gene pool (Tift 93 and Tift 8593) used to produce commercial pearl millet forage hybrid, Tifleaf 3, was released and registered with Crop Science. Selection procedures in two exotic gene pools of the primary of the primary gene pool increased genetic rust resistance 20% per cycle while morphological variability was maintained.

University of Hawaii
Honolulu, HI 96822

81. Mechanisms Regulating psbD Transcription in Higher Plants

D.A. Christopher, Department of Plant Molecular Physiology

\$88,079

This project investigates the genetic mechanisms controlling the development of the photosystem II reaction center (PSII) in higher plants. Proper PSII development and function are necessary for maintaining photosynthetic efficiency, crop productivity and plant adaptation to high light environments. The *psbD* gene, which is localized on the chloroplast genome of plants and algae, encodes the D2 subunit of PSII. In higher plants, high-fluence blue light, but not red light, differentially activates *psbD* transcription from a blue light-responsive promoter (BLRP). The objective of this work is to understand how blue light regulates *psbD* transcription. To facilitate genetic investigations on the BLRP, we mapped and studied the expression of the BLRP in Arabidopsis chloroplasts. We will determine the nucleotide sequence of the Arabidopsis BLRP and determine which DNA elements are necessary for light-responsive transcription. Electrophoretic mobility shift and DNase I protection assays are being used to define the protein binding sites in each element from Arabidopsis. These binding sites will be used to clone cDNAs for the putative regulatory proteins. The cDNA sequence is expected to assist in identifying the functional domains involved in DNA-binding, protein-protein interactions and transcription. Studying the nuclear-encoded factors and their genes will provide insight into the regulation of *psbD* transcription by blue light, which will give clues into the fundamental mechanisms by which the nucleus controls chloroplast gene expression. Implementation of the Arabidopsis system will enable the detailed genetic dissection of the photosensory pathways regulating *psbD* expression.

University of Hawaii
Honolulu, HI 96822

82. Violaxanthin De-epoxidase: Structure, Regulation and Function

H.Y. Yamamoto, Department of Plant Molecular Physiology

\$95,000

The overall aim of this project is to understand the mechanism and role of violaxanthin de-epoxidase (vde) activity in protecting plants against the potentially damaging effects of high light intensities. Zeaxanthin and antheraxanthin formed by vde enhance the dissipation of excess light energy as heat, thus protecting photosynthetic apparatus from damage. As previously reported, we have cloned vde from lettuce, tobacco and Arabidopsis. The specific aims of the current project are twofold: (1) to determine the physiological significance of vde activity on growth and development by over expression or suppression of vde and (2) to gain insight into the mechanism of vde activity by site-directed mutagenesis studies. Analysis of tobacco transformed earlier with sense and antisense vde constructs were continued. Preliminary results suggest that maximum over expression was around twofold. Constructs of vde using other promoters for higher levels of over expression were initiated. A range of plants with vde activity inhibited up to 95 percent was obtained. Propagation of transformed plants for homozygous selection was initiated. Detailed analysis of wild-type (control) tobacco was completed. The results suggest that vde is developmentally regulated in contrast to xanthophyll-cycle pool size that depends on light environment.

University of Illinois
Chicago, IL 60612

83. Heavy Metal-lux Sensor Fusions and Gene Regulation

S. Silver, Department of Microbiology and Immunology

\$107,000

We are studying the molecular genetics of bacterial resistances to the toxic heavy metals, mercury, cadmium, arsenic and silver. Bacterial cells have highly specific genetic systems for each of these toxic minerals. Each system contains gene(s) for regulation (so that the system is functional only when needed) and gene(s) for the proteins that physiologically and biochemically confer resistance itself. The resistance systems offer promise for "bioremediation" of environmentally polluted settings, while the regulatory genes may be used as components of metal-specific "biosensors" (especially with luciferase gene fusions that respond to "bioavailable" metal ions with increasing light emission). Mercury and methylmercury-detoxifying *Bacillus* isolates from Minamata Bay, Japan are being analyzed in detailed laboratory studies. In addition, these same strains are being used for studies of the range of arsenic resistance determinants (and their genes) in *Bacilli*. The CadC transcriptional repressor of the cadmium-resistance cadmium-efflux ATPase has been overproduced and is being studied by protein-DNA interactions *in vitro* and metal cation-protein binding. The first silver-resistance system was cloned and the 14 kb required for its 7 genes sequenced. Now more in detail on the phenotypes and functions of each gene will continue. Additional environmental and clinical isolates having the silver resistance genes will be characterized by Southern blot analysis, RFLP analysis and PCR of selected genes.

University of Illinois
Urbana, IL 61801

84. Studies on the bo_3 -type Ubiquinol Oxidase from *Escherichia coli*

R.B. Gennis, Department of Biochemistry

\$125,000

The bo_3 -type ubiquinol oxidase is the predominant respiratory oxidase present in the bacterial membrane of *Escherichia coli* when the cells are grown with high aeration. This enzyme is a coupling site, which means that the redox reaction catalyzed by the enzyme, i.e., the 4-electron reduction of oxygen to water, is coupled to the generation of a proton and voltage gradient across the cytoplasmic membrane. This enzyme is a very efficient device to convert chemical energy into the form of a transmembrane ion gradient. Our primary interest is to learn how this is done.

Our studies have established this enzyme as a member of the superfamily of heme-copper respiratory oxidases. This superfamily includes the cytochrome c oxidases for which there is now detailed structural information recently available from X-ray diffraction studies. The focus of our work is to combine site-directed mutagenesis with a variety of spectroscopic techniques to identify the functional roles of specific amino acid residues that appear to be involved in the proton pumping mechanism. We have most recently been examining the effects of mutations in residues that appear to be components of two separate channels (D-channel and K-channel) within the enzyme that have been identified by X-ray analysis. We have clearly demonstrated that these residues are critical for oxidase function and that the two channels must play very different roles. In addition, we have demonstrated that different mutations trap the enzyme in different states of oxygenation, providing a useful experimental tool for characterizing these states of the enzyme.

University of Illinois
Urbana, IL 61801

85. Exploratory Studies on the Bacterial Formation of Methane

R.S. Wolfe, Department of Microbiology

\$70,385

The microbial formation of methane is carried out by a unique group of bacteria known as methanogens. These strict anaerobes are widespread in nature, and are found in diverse habitats, wherever active anaerobic degradation of organic matter occurs, such as sediments, the rumen of ruminants, sanitary landfills, and sewage sludge digesters. We are exploring ways of simplifying the culture of methanogens in liquid and solid media, so that these organisms may be more readily employed as research tools by the scientific community. These studies include the sensitivity of methanogens to a variety of factors such as reducing agents, ions, ionic strength, and oxidizing agents. For example, under certain conditions of stress, cells of methanogens may exhibit a bright red fluorescence instead of the typical blue-green fluorescence. We are studying this phenomenon to isolate and characterize the red fluorescent compound, to determine its structure, its role, and its relationship to known coenzymes which may be modified when cells are under stress. Our goal is to define how sensitive, anaerobic methanogenic-cells survive under stress in terms of biochemistry.

Iowa State University
Ames, IA 50011

86. Characterization of the Membrane-Associated Methane Oxidation System in *Methylococcus capsulatus* Bath

A.A. DiSpinto, Department of Microbiology, Immunology, and Preventive Medicine

\$94,000

In methanotrophs, the oxidation of methane to methanol is catalyzed by the methane monooxygenase (MMO). In some methanotrophs, two different MMOs can be expressed depending 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). This project focuses on the pMMO. The pMMO consists of two components. Component A composed of three polypeptides with molecular masses of 47,000, 27,000, and 25,000 Da, in a 1:1:1 ratio, and contains 2 - 3 non-heme iron and 2 - 3 copper atoms per 99,000 Da. Component B is a small polypeptide with a molecular mass of 1,218 Da, and contains 1 - 2 copper atoms. As isolated purified enzyme shows an approximate component A to B ratio of 1: 5 with 2 - 3 iron and 14 to 15 copper atoms.

Component B also shows a number of siderophore-like properties, and can be isolated from the extracellular fraction in cells expressing the pMMO under copper-limiting conditions.

The overall objective of this project is to characterize the mechanism of methane oxidation in methanotrophs expressing the pMMO. Present work is focused on improvements to the pMMO

purification procedure, enzyme characterization, identification of the physiological reductant to the pMMO, and role of component B in methane oxidation.

Iowa State University
Ames, IA 50011-1020

87. Regulation of Carotenoid Biosynthesis: the *immutans* Variegation Mutant of *Arabidopsis*

S. Rodermel and D. Voytas, Department of Botany and Department of Zoology and Genetics \$105,000

The *immutans* mutant of *Arabidopsis thaliana* contains green and white-sectored leaves due to the action of a nuclear recessive gene. The green sectors are homoplastidic for normal chloroplasts, while the white sectors contain plastids with different morphologies (are heteroplastidic). This indicates that the mutation is plastid autonomous and acts independently on the plastids in a cell. The somatic instability in the mutant is modulated by light, temperature and developmental factors. *immutans* is first expressed during seed coat breakage, and plastid phenotypes, once established, cannot be changed. However, defective plastids are not maternally-inherited, indicating that the plastid defect is cured during reproduction. The white sectors of *immutans* accumulate the carotenoid precursor phytoene, but the mutation affects the activity of phytoene desaturase (PDS) only indirectly: *immutans* does not map to the PDS locus, nor does it affect PDS mRNA or protein levels. The fact that PDS expression is normal in the white tissues of *immutans* shows that the expression of this protein is not coupled to pigment content in *A. thaliana*, as it is in other systems. Transgenic plants that express Lhcb promoter/GUS fusions have revealed that *immutans* uncouples Lhcb expression from its normal dependence on chloroplast development, suggesting that *immutans* is an important component in the signal transduction pathways that regulate chloroplast biogenesis. An approximately 30 kb fragment of the *A. thaliana* genome encompassing *immutans* has been subcloned into overlapping cosmid clones that are being used to complement the mutation and define the gene locus.

University of Iowa
Iowa City, IA 52242

88. Molecular Biology of Anaerobic Aromatic Biodegradation

C.S. Harwood, Department of Microbiology \$63,750

We have been investigating anaerobic benzene ring biodegradation, a process that is critical for the recycling of plant material and the degradation of many environmental pollutants. Benzene rings can be completely degraded in the absence of oxygen only by bacteria. Our emphasis has been on pathways for the degradation of benzoate and 4-hydroxybenzoate, since these aromatic acids are the starting compounds for a central pathway of anaerobic benzene ring reduction and ring fission. We have recently identified and sequenced a cluster of genes from the bacterium *Rhodospseudomonas palustris* that encodes many, and possibly all, of the enzymes for benzoate and 4-hydroxybenzoate degradation. These genes provide a valuable source of material for purifying and studying several novel enzymes that are known to be involved in ring reduction and

cleavage. Assignment of gene products to specific enzymatic steps will also allow us to elucidate the correct sequence of catabolic reactions in the degradation pathways. In addition, the cloned genes will be used to determine how gene expression is regulated in response to aromatic compounds and oxygen. This information will be valuable in designing bacterial systems to mediate biomass conversions or for use in bioremediation.

Johns Hopkins University
Baltimore, MD 21218

89. Bacterial Nickel Metabolism and Storage

R.J. Maier, Department of Biology

\$104,000

The growth of many microorganisms that are beneficial to nutrient cycles in nature requires nickel, as this metal is a component of several microbial enzymes. From studies on bacterial mutants in Ni-containing ureases and hydrogenases, it has become clear that the Ni-requiring organisms contain enzymes to deal with intracellular nickel mobilization and metabolism, and subsequent insertion of the metal cluster into the Ni-enzyme(s). To determine the routes of nickel metabolism and incorporation into a bacterial enzyme (hydrogenase) in the N₂-fixing bacterium *Bradyrhizobium japonicum*, gene-directed mutant strains of the bacterium have been studied, and nickel-binding proteins have been overexpressed and purified. Some mutant strains are specifically deficient within histidine rich areas of the nickel-storage protein we call nickelin, encoded by *hypB*. Analysis of mutants within the metal-binding domain have shown that the his-rich area, capable of binding 18 nickel atoms per molecule of protein plays a nickel storage role for subsequent hydrogenase expression. Other proteins involved with the nickel-dependent regulation of hydrogenase expression either in sensing nickel or in activating hydrogenase transcription have been identified and specific mutants in each of the genes have been generated. Some of the genes encode domains which act as nickel-binding ligands, and a nickel dependent sensing and regulatory cascade for Ni-enzyme (hydrogenase) expression involving several multi-subunit complexes is proposed. The long-term goal is to understand the number, nature and function of the components involved in bacterial nickel metabolism, especially for hydrogenase expression.

Johns Hopkins University
Baltimore, MD 21218

90. Transport of Ions Across the Inner Envelope Membrane of Chloroplasts

R.E. McCarty, Department of Biology

\$103,000

In addition to photosynthesis, chloroplasts carry out a number of essential processes, including nitrite reduction to ammonia and sulfate reduction to the level of sulfide. Nitrite is a source of nitrogen for amino acids, and nucleic acids and sulfate, a source of sulfur for the sulfur-containing amino acids, cysteine and methionine. How nitrite, sulfate and other molecules cross the permeability barrier of the chloroplast, the inner membrane of the chloroplast envelope, has been under investigation in my laboratory for several years. Using a biophysical approach, we have shown that nitrous acid can rapidly cross the inner envelope membrane. The rate of unmediated diffusion of nitrous acid is from five to ten times that of nitrite reduction within chloroplasts. The rate of nitrous acid diffusion across the erythrocyte membrane is, in contrast, slow. This difference may

be due to the high cholesterol content of the erythrocyte membrane. Nitrite will, however, enter erythrocytes and we have shown that nitrite transport is mediated by the erythrocyte anion exchange protein.

Carbon dioxide, a substrate of photosynthesis could enter chloroplasts as carbonic acid, bicarbonate or as carbon dioxide. Together with Dr. James V. Moroney, Dr. Shingles developed a sensitive fluorimetric assay for carbonic anhydrase activity and demonstrated that the rate of transport of carbon dioxide across lipid vesicle membranes is accelerated by the presence of carbonic anhydrase on both sides of the membrane. These results are consistent with free diffusion of carbon dioxide across the membrane.

Ca^{2+} influx into inner envelope vesicles was also investigated. In agreement with studies with intact chloroplasts, the inner envelope vesicles exhibit an uptake of Ca^{2+} that is inhibited by the Ca^{2+} channel blocker, ruthenium red. These results suggest that chloroplasts may be involved in Ca^{2+} homeostasis.

KAIROS Scientific Inc.
Santa Clara, CA 95054

91. Macromolecular Scaffolds for Energy Transfer

D.C. Youvan

\$131,000

The Green Fluorescent Protein (GFP) from the jelly fish *Aequorea victoria* is the first known protein that expresses a genetically encoded, autocatalytically formed fluorophore. In the past year, we have discovered that genetically engineered variants of GFP can be attached to micron-sized beads at surface concentrations sufficiently high to enable fluorescence resonance energy transfer (FRET) to occur efficiently. This is surprising because there is an inverse sixth power dependence on FRET efficiency with respect to the distance between donor and acceptor molecules. In such experiments, donor GFP derivatives ("blue", BFP) are typically excited in the violet and fluoresce in the cyan, while acceptor GFP derivatives ("red-shifted excitation", RSGFP) have spectral overlap in the cyan and fluoresce in the green. These fluorescent proteins are typically attached to Nickel ion chelating beads which complex with an engineered N-terminal histidine-tag on these modified GFP derivatives. Since GFP is known to exist as a dimer at high concentrations, there are interesting possibilities for the macromolecular GFP structures on the bead surface. Various models can be envisioned for the FRET-competent macromolecular structure(s): ranging from closely spaced monomers to donor-acceptor heterodimers (BFP/RSGFP). This new bead-based macromolecular scaffold leads to intriguing possibilities for the use of such FRET beads in energy transfer experiments and in the elucidation of the structure of the FRET pair.

University of Kentucky
Lexington, KY 40536-0084

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

E. DeMoll, Department of Microbiology and Immunology; D.A. Grahame, Uniformed Services University of the Health Sciences \$95,000

We are employing biochemical methods to study the acetyl-CoA decarbonylase/ synthase complex found in methanogenic Archaea. The results of our experiments will allow us to elucidate the complicated biochemical mechanism used by the enzyme complex to catalyze the following reaction:



where H₄Spt and CH₃-H₄Spt are tetrahydrosarcinapterin and *N*⁵-methyl-tetrahydrosarcinapterin, respectively and Fd_{red}(Fe²⁺) and Fd_{ox}(Fe³⁺) are the reduced and oxidized forms of the redox protein ferredoxin. Additionally, we will use the biochemical knowledge gained to better understand how the oxidation-reduction potential of the environment regulates methanogen physiology.

University of Kentucky
Lexington, KY 40546-0091

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

R.L. Houtz, Department of Horticulture and Landscape Architecture \$82,034

The methylation of Lys-14 in the large subunit (LS) of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) by Rubisco LS N-methyltransferase (Rubisco LSMT) occurs by an ordered bi-bireaction mechanism. Kinetic and binding analyses of the initial interaction between Rubisco LSMT and des(methyl) forms of Rubisco, revealed a tight (KD 0.1 nM) and specific interaction which was identified as a consequence of a relatively slow *k*_{ass}(7,368 M⁻¹ s⁻¹) and *k*_{diss} (8x10⁻⁷ s⁻¹). The molecular determinants of this tight and specific interaction were investigated by ELISA analyses of binding between truncated forms of Rubisco LSMT expressed in *E. coli* and immobilized Rubisco. The results demonstrated that among 19 different carboxy- and amino-terminal truncations, representing the removal of 4 to 380 amino acids out of a total of 489, all showed positive signals for binding to Rubisco. While some of the truncated Rubisco LSMT constructs showed noticeably weaker binding to Rubisco than full-length Rubisco LSMT, all maintained a high specificity for Rubisco. Thus, the interaction between Rubisco LSMT and Rubisco appears to be a consequence of multiple binding domains spread over a large portion of the Rubisco LSMT protein. Related studies determined the location of a cross-link between the LS of Rubisco and Rubisco LSMT catalyzed by a homobifunctional sulfhydryl-specific cross-linking reagent (BMH). Cross-linking between Rubisco LSMT and the LS of Rubisco generated a 106 kDa product which was a result of cross-linking between Cys-459 in the LS of Rubisco and Cys-119 or Cys-188 in Rubisco LSMT.

University of Kentucky
Lexington, KY 40546-0091

- 94. Studies of a Novel Pathway for Biosynthesis of Straight and Branched, Odd and Even Length, Medium-Chain Fatty Acids in Plants**
G.J. Wagner, Department of Agronomy \$83,037

Fatty acids (FA) of various chain lengths are key components of plant membranes, waxes, reserve oils, and certain compounds volatilized by plants. These natural products are exploited commercially as food components, lubricants, emulsifiers, etc., and are being researched for their use as renewable, biodegradable polymers, biofuels, stimulants for biodegradation of pollutants, etc.

We have described a new pathway (termed α -keto acid elongation, α -KAE) that utilizes enzymes of branched chain amino acid (BCAA) metabolism to synthesize straight-chain, iso- and anteiso-branched, short and medium chain length FA in trichome glands of certain plants. Results of a completed survey indicate that α -KAE appears to be restricted to trichome glands of petunia and Nicotiana species. Branched and straight medium chain FA of seeds, acid components of epicuticular wax esters and at least one petal-volatilized, anteiso-branched alcohol are elongated by fatty acid synthase and not α -KAE. But, iso and anteiso primers for branched species are provided by BCAA metabolism. A direct assay was established to monitor isopropylmalate synthase (key enzyme of BCAA metabolism) that is 4 times more sensitive than conventional assays. This assay has revealed new properties of this enzyme. Efforts to isolate genes involved in BCAA metabolism and α -KAE are in progress.

Lawrence Berkeley National Laboratory
Berkeley, CA 94720

- 95. Biomolecular Materials**
M.D. Alper, C. Bertozzi, D. Charych, J.F. Kirsch, D.E. Koshland, P.G. Schultz, R. Stevens, C.-H. Wong, Center for Advanced Materials \$160,000*

The goal of this research is the use of natural biological concepts, processes, structures and molecules as the basis for the synthesis of new materials.

One component focuses on the use of natural, engineered and "created" enzymes to catalyze synthetic reactions. The unique stereochemical control exerted by enzymes and their ability to catalyze reactions at low temperature allows the synthesis of materials with structures and therefore properties that cannot be achieved using conventional synthetic routes. Efforts are focused on the design of reaction conditions, engineering of enzyme structure and activity, and generation of catalytic antibodies for materials synthesis. Other polymers with structures inspired by biological polymers are being synthesized chemically.

Work is also progressing on the synthesis of organic thin films which mimic the biological membrane. Membranes self-assemble, present defined and controllable surfaces, and detect the presence or absence of specific materials. These properties are exploited to alter interfacial and

surface properties and to fabricate sensor devices. Thin film sensors have been developed to detect influenza virus, botulism, *E. coli*, cholera toxins, and small molecules, e.g., glucose. Similar films have been used to direct the ordered crystallization of inorganic salts. Research is also focused on the modification of the surface of materials to improve their biocompatibility.

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

Lawrence Berkeley National Laboratory Berkeley, CA 94720

96. Transferring Photosynthesis Genes to New Cellular Environments

J.E. Hearst, Structural Biology Division

\$230,000

We seek a genetic and molecular understanding of photosynthetic processes both in bacteria and in plants. In the past our work has centered on the photosynthetic bacterium *Rhodobacter capsulatus*. This bacterium contains a 46 kilobase region in length, which codes for all of the essential genes for photosynthesis, including the genes for the reaction center (RC) proteins, a long wavelength light harvesting antenna (LHI), and the enzymes for the biosynthesis of bacteriochlorophyll (Bchl) and carotenoid (Crt) pigments. Work on the mapping of this gene cluster is complete. The complete nucleotide sequence of this cluster has been determined, denoting a landmark in the molecular biological studies of photosynthesis. We are now directing our efforts toward the genetic transfer of pigment biosynthesis and photosynthesis to organisms which are incapable of performing these functions. The long term goals of our task are i) to gain an understanding of the evolutionary steps which took place in the transfer of photosynthesis to plants, ii) to gain further understanding of the photosynthetic processes in plants, iii) to use the genetically engineered constructs which are now available to transfer biosynthetic enzyme activity into new cellular environments.

Lawrence Berkeley National Laboratory Berkeley, CA 94720

97. Structural Basis of Plant Signal Transduction

S.-H. Kim, Structural Biology Division, LBNL and W. Gruissem, Department of Plant Microbial Biology, University of California, Berkeley

\$150,000

(1) Ethylene Receptor.

Ethylene, a gaseous phytohormone, controls a variety of plant developmental processes ranging from seed germination to cell elongation, fruit ripening, and fruit and flower senescence. In addition, environmental stresses such as chilling, wounding, flooding, and pathogen invasion induce ethylene production as part of the defense response of the plant. There are at least two functional ethylene receptors, ETR1 and ERS, in *Arabidopsis thaliana*.

ETR1 forms a disulfide bridged dimer and each monomer consists of at least three distinct domains: an N-terminal ethylene-binding transmembrane domain, a histidine kinase domain, and a receiver domain. ERS is similar but lacks the receiver domain. Usually, the two components of bacterial two-component systems reside on two separate proteins. However, ETR1, like several

eukaryotic two-component systems, is a hybrid kinase where both domains are contained in a single polypeptide. We recently subcloned, purified, and crystallized the receiver domain of ETR1 from *Arabidopsis thaliana*. The molecular weight of the purified protein was determined by electrospray mass spectroscopy, and found to correspond to that derived from the DNA sequence of the domain.

Preliminary X-ray diffraction experiments show that the flash-frozen crystals diffracted beyond 2.1 Å resolution. The space group was determined to be P422 with the unit cell parameters of $a=b=48.4$ Å, $c=112.3$ Å. Search for heavy atom derivatives is in progress. Concurrently, a selenomethionine-substituted form has been produced to solve the structure by the MAD phasing technique.

(2) Plant Prenyl Transferase.

The two genes encoding the α and β -subunits of tomato farnesyltransferase (LeFTase) were cloned from a cDNA library using a PCR approach. The two genes can complement the yeast deleted with FTase- β subunit gene *RAM1*. Analysis of the transformed cells revealed that the plant FTase can fully complement the yeast FTase.

A search of the database for protein which terminate with a CaaX-box motif was conducted. Among the proteins which were identified was the Arabidopsis MADS-box transcription factor *Apetalla1* (AP1). In vitro prenylation assays with recombinant AP1 protein showed that this protein can be prenylated, in vitro, by a plant farnesyltransferase with Km value of 3.5 mM.

The gene encoding geranylgeranyl transferase-I β was also cloned by yeast two-hybrid screen using the FTase α subunit as a bait. Expression is carried out in baculovirus infected insect cells. The expressed recombinant proteins are of high quality and are His-tagged at their amino terminus to facilitate purification.

Lawrence Berkeley National Laboratory Berkeley, CA 94720

98. Mechanism of Water Splitting and Oxygen Evolution in Photosynthesis

M.P. Klein and V.K. Yachandra, Structural Biology Division

\$210,000

Photosynthetic oxygen evolution in plants and cyanobacteria is catalyzed by a cluster of four Manganese atoms, the site of water splitting and acts as the locus of charge accumulation driven by the energy of four successive photons through the five S-states. Oxidation of Mn has been established from S_0 - S_1 and S_1 - S_2 but not from S_2 - S_3 . Reduction occurs on S_3 - S_4 - S_0 . The structural motif in S_1 and different forms of S_2 is that of a dimer of di- μ -oxo bridged binuclear Mn units. Sr, an active replacement for the required Ca cofactor, is located at 3.5 Å from Mn. Upon advance to S_3 , significant structural changes occur with the shorter Mn-Mn distances increasing. Further advance to the most reduced or S_0 state, induces significant but different changes in the complex. Use of highly oriented photosystem II membranes has permitted determination of the orientation of several internuclear vectors relative to the membrane normal. These structural results have been obtained with X-ray absorption spectroscopy which has also been adapted to the study of the Cl cofactor. A new EPR signal has been discovered in the S_0 states and a previously discovered EPR signal in the S_1 state has been confirmed and established to arise from the Mn cluster. A mechanism for the formation of the O-O bond has been postulated on the basis of the structural and oxidation state information.

Lawrence Berkeley National Laboratory
Berkeley, CA 94720

99. Photosynthetic Pigment Proteins and Photosynthetic Light Reactions

K. Sauer, Structural Biology Division

\$250,000

Light absorption in photosynthetic antenna pigment-proteins is followed by excitation transfer and trapping in reaction centers of photosynthetic membranes. In previous studies we characterized the rapid and extensive excitation transfer in the pigment-proteins C-phycoyanin and the LH2 bacteriochlorophyll (BChl)-protein complex from purple photosynthetic bacteria. In the latter complex, the excitation transfer dynamics is dominated by excitonic interactions among the BChl molecules in LH2. We have explored the relaxation of the excited electronic states of these pigment-proteins using analysis based on an extension of the Kennard-Stepanov relation. These studies show that the low-energy excited states resulting from exciton coupling among BChl molecules in LH2 are energetically stable during the excited-state lifetime (typically several nanoseconds) despite dephasing and apparent delocalization that occurs on the picosecond time scale.

Trans-membrane electric fields generated by salt gradients across thylakoids from higher plants were shown previously in our lab to result in changes in the fluorescence lifetimes and in the kinetics of charge separation in the reaction centers of Photosystem II. The effects were interpreted to result from an increase in the rate of charge separation and a decrease in the rate of charge recombination when the trans-membrane electric field orientation stabilized of the charge-separated state. Chromatophores from the purple photosynthetic bacterium *Rhodobacter sphaeroides* show an opposite behavior with respect to the direction of the applied electric field. Using mutant organisms deficient in either reaction centers or one or more antenna pigment-protein complexes, we find that most of this oppositely directed effect on the fluorescence arises from the antenna complexes. Once the contribution of the reaction center itself is uncovered, it is found to be the same as that seen in PS II. The generality of this effect provides valuable insights into the consequences of the light-stimulated redistribution of electric charge within the reaction centers and their associated membranes.

Los Alamos National Laboratory
Los Alamos, NM 87545

100. Carbon Metabolism in Methylophilic Bacteria

C.J. Unkefer, Chemical Science and Technology Division

\$130,000

The purpose of this project is to understand the unique aspects of the metabolism and biochemistry of methylophilic bacteria which grow on reduced one carbon compounds such as methane, methanol, or methylamine. Methylophilic possess a unique system for oxidizing alcohols which involves the PQQ-dependent methanol dehydrogenase (MDH). The MDH oxidation of alcohols is coupled directly to the electron transport chain, and is used to drive the net synthesis of ATP. When growing on methane or methanol, methylophilic derive all of their energy from this process. The MDH cofactor PQQ (pyrroloquinoline quinone) is one of the three o-quinone enzyme cofactors that have been identified. PQQ is unique among this group in that it must be biosynthesized as distinct molecule. Using ¹³C labeling and NMR spectroscopy have shown that PQQ is

biosynthesized from the amino acids glutamate and tyrosine. We are continuing to probe the biosynthesis of PQQ. Molecular genetic studies in other laboratories have identified PQQ biosynthesis genes. All three organisms studied contain an open reading frame that encodes for a 23-29 amino acid peptide. We are testing the hypothesis that this peptide is the precursor for the biosynthesis PQQ. We are currently synthesizing the *Methylobacterium extorquens* AM1 peptide labeled with L-[3'-5'-¹³C₂]tyrosine and will examine extracts for their ability to oxidize this peptide. During our PQQ biosynthesis studies we generated ¹³C-labeled samples of PQQ which were used to probe the mechanism of MDH. Using NMR spectroscopy we demonstrated that upon formation of the enzyme-substrate complex the alcohol binds to the C-5 carbonyl of PQQ to form a hemi-ketal structure. In addition, when cyanide, a competitive inhibitor of MDH binds reversibly to PQQ as a cyanohydrin at the C-5 carbonyl. The ability of methylotrophs to grow on C₁ compounds gives them considerable industrial potential because they could be used to produce a variety of useful compounds from inexpensive and renewable precursors such as methanol or methane. Their ability to oxidize a wide variety of chemicals make them potentially important for treatment of waste streams. For the potential of these organisms to be realized, we must understand fundamental aspects of their physiology.

University of Maryland
Baltimore, MD 21202

101. Structure and Regulation of L-glutamate Dehydrogenase in Hyperthermophilic Archaea

F.T. Robb and J. DiRuggiero, Center of Marine Biotechnology

\$98,548

The enzymes from hyperthermophiles often exhibit stability and normal enzyme activity at or near 100° C. We now have direct experimental evidence that ion-pairs can directly elevate enzyme stability at extremely high temperatures. We have compared the sequences and crystal structures of the glutamate dehydrogenases (GDHs) from the hyperthermophiles *Pyrococcus furiosus*, *P. endeavori* and the moderately hyperthermophilic archaeon *Thermococcus litoralis* Topt (88° C). A minimum set of two mutations was found in *T. litoralis* GDH that elevated thermal stability four-fold over the wild type enzyme, by restoring a six-member ion pair network. An initial mutation (T138E) resulted in active GDH with decreased thermostability, and an additional complementary mutation (D167T) was required to obtain increased stability. Placement of ionic residues to create electrostatic networks is not yet straightforward. Enhancement of thermostability was only achieved by maintaining a local net charge identical to *P. furiosus* GDH, providing an equivalent context for the residues in the network between the adjacent subunits.

We have now cloned, sequenced and expressed the gene encoding GDH from a thermophilic *Thermococcus* strain WT1 from hot springs in the Waiotapu area of New Zealand. The strain has Topt of 75° C, and its GDH is very much more labile at 100° C than the other enzymes studied so far. This now completes a matched set of highly homologous GDHs ranging widely in thermostability. Interestingly, WT1 GDH has S138 and G167 at these key positions in the exceptionally thermostable GDHs. Crystallography and site directed mutagenesis of this enzyme are planned.

University of Maryland
Baltimore, MD 21202

102. Regulation of Catabolic Gene Transcription in the Methanogenic Archaea
K.R. Sowers, Center of Marine Biotechnology \$182,000 (FY96 funds/2 years)

The controlled expression of catabolic gene products is integral to microbial interactions that mediate biomass conversion to methane. The goal of this project is to determine the mechanisms of transcriptional gene regulation in the methanogenic Archaea which are the catalysts of methane production and have a role in global carbon cycling. Although the Archaea have structural gene characteristics that are similar to those of both the Bacteria and the Eucarya, preliminary evidence suggests that they employ Eucarya-like transcription factors for site-specific transcription initiation. We have shown that transcription of the gene encoding CO dehydrogenase (cdh) from *Methanosarcina thermophila* is highly regulated in response to substrate. A transformation protocol has been developed for *Methanosarcina spp.* and a simple, inexpensive anaerobic plating vessel has been developed that provides a virtually inexhaustible source of growth vessels for colony selection of genetic transformants. A reporter plasmid has been constructed by fusing the cdh promoter with UidA. Regions of DNA promoter sequence and flanking regions are being evaluated for their role in cdh regulation by studying the effects of point mutations and deletions on gene expression using the reporter fusion. Results of this study will determine whether gene regulation in the Archaea functions by mechanisms that are analogous to the other two lineages or by mechanisms that are unique to this phylogenetic line. Regardless of which mechanism(s) is revealed by this investigation, the results will provide further insight into the global molecular strategies of gene regulation.

University of Maryland
College Park, MD 20742

103. Molecular and Genetic Analyses of TSO1 in Arabidopsis
Z. Liu, Department of Cell Biology and Molecular Genetics \$90,000

Higher plant cell division exhibits several properties that are distinct from animal cells. However, relatively little is known about the molecular and genetic basis of higher plant cell division control. Our long-term objective is to understand the fundamental mechanisms employed by higher plants to regulate mitosis and cytokinesis. Currently, our research is focused on the genetic and molecular characterization of an Arabidopsis gene TSO1. Single *tso1* mutants are abnormal in floral meristem structure, and the mutant cells show an increased DNA content and partially formed cell walls. These defects were only observed in the cells of floral tissues. Thus TSO1 is required for proper mitosis and cytokinesis in the floral tissues. Double mutants will be constructed between *tso1* and other Arabidopsis cytokinesis mutations. The TSO1 gene will be isolated using a map-based cloning approach. The mRNA tissue-expression pattern as well as the subcellular localization of TSO1 will be examined using northern analysis, in situ hybridization, immunolocalization, and the Green Fluorescent Protein (GFP) reporter. Through these studies, we hope to define the specific function of TSO1 during cell division cycle, understand the significance and the regulation of the floral-tissue specific effect of *tso1*, and investigate the relationship between cell division regulation and organ development in higher plants.

University of Maryland
College Park, MD 20742

104. Structure and Regulation of Calcium-pumping ATPases in Plants

H. Sze, Department of Plant Biology

\$93,000

Calcium is not only an important intracellular signal for many stimuli-induced responses, it is also essential for the proper functioning of organelles and the secretory system. However the transport systems required to regulate Ca concentrations in different cellular compartments are poorly defined at the molecular level. The current goal is to identify high affinity Ca pumps in plants and understand the specific functions of the different Ca-ATPases. Two biochemical types of Ca pumps were detected in plants, though plasma membrane (PM)-type pumps are localized to several different membranes. To identify and characterize each Ca pump, genes encoding Ca-pumps from Arabidopsis are being identified by functional complementation of a yeast mutant (pmr1 pmc1 cnb1). One gene, ECA1, shared high identity with animal SERCA pumps and restored growth of mutants on Ca-depleted medium. ECA1 represents the first plant gene encoding a functional Endoplasmic reticulum Ca-ATPase from plants. The 116 kD protein is localized to the ER and forms a Ca-dependent phosphoprotein that is blocked by cyclopiazonic acid, a SERCA pump inhibitor. Another gene ACA2 complemented yeast mutant phenotype only if the N-terminus was truncated. Ca transport of the full-length ACA2p, but not the truncated ACA2p, was stimulated by calmodulin. Thus ACA2p encodes a novel calmodulin-regulated Ca-ATPase with an N-terminal autoinhibitory domain. The transport properties, regulatory domains and subcellular location of these and other Ca pumps are being investigated in order to understand their cellular functions.

University of Massachusetts
Amherst, MA 01003

105. Molecular Analysis of the Interactions Between Pollen Tube Components and a Stylar Transmitting Tissue Pollen Tube Growth-Promoting and -Attracting Protein

A.Y. Cheung and H.-M. Wu, Department of Biochemistry and Molecular Biology

\$94,000

Pollination, a key process in plant sexual reproduction, relies to a large extent on extracellular matrix interactions between pollen grains and specific tissues and cells within the female reproductive organ, the pistil. Upon landing on the pollen-receptive stigmatic surface of the pistil, pollen tubes emerge and elongate in the extracellular matrix of specialized pistil tissues to deliver sperm cells to the embryo sacs for fertilization. Our research is focused on identifying factors on the pollen tube surface and in the extracellular matrix along the pollen tube growth pathways that contribute to the pollination process. This project aims to isolate and characterize tobacco pollen tube components that interact with a pistil glycoprotein (TTS) which attracts pollen tubes and promotes their growth. TTS protein adheres to the pollen tube surface tips, and are incorporated into the walls of pollen tubes. The sugar moieties on TTS protein are important for its adhesion to the pollen tube surface; its unglycosylated C-terminal domain is adequate for binding to the pollen tube tips. We take advantage of this information and another structural feature (a cluster of histidine residues) on TTS protein to isolate the relevant pollen tube proteins. Pollen tube proteins

will be prepared and allowed to interact with various forms of TTS protein *in vitro*. The pollen tube protein-TTS protein complexes will be isolated on an affinity resin that selectively binds histidine residues. The pollen tube proteins thus isolated will be characterized, their genes will be isolated for further analysis and manipulations in order to understand how they interact with TTS protein and to determine their biological roles in pollen tube growth.

University of Massachusetts
Amherst, MA 01003

106. Cellulose Fermentation by Nitrogen-Fixing Anaerobic Bacteria

S. Leschine, Department of Microbiology

\$98,000

In anaerobic environments rich in decaying plant material, the decomposition of cellulose and other plant polysaccharides is brought about by complex communities of interacting microorganisms. We are simulating processes occurring in natural environments by establishing biologically-defined heterogeneous bacterial communities that we use as *in vitro* systems for quantitative studies of cellulose degradation, especially under conditions of combined nitrogen deprivation. In particular, we will establish cellulose-decomposing Fe(III)-reducing microbial communities, and isolate cellulolytic and Fe(III)-reducing members of these communities that we will use in biologically-defined microbial consortia to study metabolic interactions that may occur among members of these communities. Also, we will examine the effects of humic substances on the degradation of cellulose by anaerobic microbial consortia. A second aspect of our research involves investigations of the multicomplex cellulase/xylanase system utilized by the nitrogen-fixing, ethanol-producing bacterium, *Clostridium papyrosolvens* C7, for the hydrolysis of crystalline cellulose and xylan. This enzyme system consists of at least seven distinct extracellular, high-molecular-weight multiprotein complexes, each with different enzymatic and structural properties. Based on initial studies of this multicomplex system, we hypothesized that a 125,000-molecular weight glycoprotein (S4), found in all complexes and apparently lacking enzyme activity, functions as a scaffolding protein (a "scaffoldin") in the assembly of the multicomplex cellulase-xylanase system. We have cloned a DNA fragment from *C. papyrosolvens* C7 which is homologous to *cipA*, the gene that encodes the scaffoldin of the *Clostridium thermocellum* cellulosome. We will determine whether this cloned fragment encodes a protein that has a structural organization typical of a scaffoldin. Other experiments will be directed toward determining whether the cloned *cipA*-homologous fragment encodes the S4 protein of the multicomplex system. Also, we will examine the possibility that the multicomplex system is built on more than one scaffoldin. The objective of these studies is to further our understanding of the organization of the many components of the multicomplex cellulase/xylanase system, and generally, to advance understanding of the enzymology of cellulose hydrolysis by anaerobic bacteria. The results of this research will provide fundamental information on the physiology and ecology of cellulose-fermenting, nitrogen-fixing bacteria. This information is intended to fill gaps in our understanding of the complex processes involved in carbon and nitrogen cycling in anaerobic environments. The information obtained will be valuable in the development of practical applications, such as the bioconversion of plant biomass, including agricultural and municipal waste materials, to fuels such as ethanol.

University of Massachusetts
Amherst, MA 01003

107. Isolation of Genes Involved in a Novel Auxin Biosynthetic Pathway

J. Normanly, Department of Biochemistry and Molecular Biology

\$80,441

Towards our long term goal of identifying the enzymes and intermediates involved in the tryptophan-independent auxin biosynthetic pathway, we are employing a screen for Arabidopsis genes that metabolize indole (a presumed precursor to indole acetic acid). We have pooled a collection of 100,000 individual yeast colonies transformed with Arabidopsis cDNAs from either of two expression libraries. We have screened these pools for their ability to metabolize the indole analogs 5-chloro and 5-methyl indole which normally inhibit growth of yeast at millimolar concentrations. Presumably this is due to incorporation of the indole analog into tryptophan and subsequent deleterious effects upon protein structure. An indole-metabolizing gene that diverts the toxic indole analog away from tryptophan synthesis may in fact be involved in the tryptophan-independent IAA biosynthetic pathway. Yeast transformants that are able to grow in the presence of toxic levels of indole analogs are being tested for indole-metabolizing function. We have also been examining the role of nitrilases in IAA biosynthesis. Feeding studies with ¹³C-labeled IAN indicate that one of 4 nitrilases in Arabidopsis (NIT2) is able to convert IAN to IAA in vivo. Lastly, we are examining the role of indole pyruvic acid (IPA) in IAA biosynthesis. We have quantified IPA in Arabidopsis and will conduct feeding studies to determine whether this compound could be a precursor in an IAA biosynthetic pathway.

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

108. Molecular Basis of Symbiotic Plant-Microbe Interactions

F.J. de Bruijn

\$200,800

The induction of nitrogen-fixing root and stem nodules on leguminous plants by soil bacteria belonging to the *Rhizobiaceae* is a highly evolved, complex process, requiring a fine-tuned interaction between the bacteria and their host. The persistence and competitive ability of the microbes in the soil and the rhizosphere of plants are important factors in early stages of rhizobial infection. In order to increase our understanding of these early stages, we wish to understand the molecular basis of the microbial response to common environmental stresses and plant factors secreted into the rhizosphere, to develop user friendly methods for the detection and classification of soil microbes and to explore the use of specific nutritional mediators to create "biased rhizospheres". Once the infection process has been initiated, distinct sets of plant genes are induced, which are involved in nodule ontogeny and in symbiotic nitrogen fixation. In order to understand the regulatory circuits responsible for symbiotic control of the expression of these loci, we wish to elucidate the molecular basis of the signal transduction pathways responsible for nodule-(cell)-specific expression of plant genes encoding symbiosis-specific proteins (nodulins). In addition, we are using the model legume plant *Lotus japonicus* to generate novel symbiotic mutants via chemical mutagenesis and to isolate novel symbiosis-specific genes via differential display of messenger RNA's.

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

109. Molecular Mechanisms That Regulate the Expression of Genes in Plants

P. Green

\$196,800

The steady state level of an mRNA depends both on its rate of synthesis and on its rate of degradation. Rapidly accumulating data indicate that degradation rates of mRNAs in plants and other eukaryotes vary over a wide range and can be differentially regulated. However, relative to transcription, the mechanisms that control mRNA stability are poorly understood. A major goal of our research is to elucidate the mechanisms that target highly unstable mRNAs for degradation in plants because these mechanisms provide plants with a means to make rapid changes in gene expression in response to a variety of stimuli. Our studies have resulted in the identification of specific sequences, (e.g. DST elements or AUUUA repeats), that markedly accelerate the decay of reporter transcripts in stably transformed tobacco cells and decrease mRNA accumulation in transgenic plants. Mutagenesis experiments have begun to provide insight about the regions within the DST element that are required for instability function and similar experiments on other instability sequences are underway. These sequences are also being used as tools in order to develop molecular, genetic, and biochemical approaches aimed at identifying components of the degradation machinery and steps in the corresponding mRNA decay pathways. In addition, we have begun to characterize the RNases of *Arabidopsis* as a first step toward differentiating between the RNases that play a role in mRNA degradation and those with other roles in RNA metabolism.

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

110. Molecular Biology of Plant-Bacterial Interactions

S.Y. He

\$180,800

This project investigates the molecular basis of interactions between plants and bacteria. Previously, we showed that *Pseudomonas syringae* pathovar tomato strain DC3000 (a pathogenic bacterium of tomato and *Arabidopsis thaliana*) produces Hrp pilus, which is associated with interkingdom transfer of bacterial virulence and avirulence proteins into the plant cell. The Hrp pilus was found to be essential for bacterial pathogenesis and bacterial elicitation of plant disease resistance. We have now raised several antibodies against the HrpA protein or Hrp pili. The HrpA antibody was found to bind to Hrp pili protein or Hrp pili. The HrpA antibody was found to bind to Hrp pili and therefore can be used in future study of temporal and spatial expression of Hrp pili in infected plant tissues. Using Hrp pilus antibody, we have found three additional bacterial proteins associated with the Hrp pilus structure. Experiments are being performed to clone the genes that encode these proteins. The HrpA protein has been expressed in *A. thaliana*. Transgenic plants expressing HrpA were found to allow infection of a P.s. pv. Tomato hrpA mutant strain, which otherwise is incapable of infecting any plants. This result suggests that heterologously expressed HrpA may form a functional pilus. Previously, we showed that a bacterial avirulence protein, AvrB of *P. syringae* pv. *glycinea*, triggers the hypersensitive cell death response when expressed inside the plant cell, but not outside the plant cell or in the bacterial cytoplasm. This result led to the proposal that AvrB must be secreted by bacteria directly into the plant cell. We have now identified

several putative *A. thaliana* AvrB-interacting proteins that may play a role in AvrB-mediated pathogenesis or disease resistance response.

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

111. Regulation of Plant Anti-Herbivore Defense

G. Howe

\$140,800

The long-term goal of this research program is to understand the molecular signaling pathways that control the expression of plant defense genes. A useful model system for studying these pathways is the systemic induction of plant anti-herbivore defense genes in response to wounds inflicted by phytophagous insects. In tomato plants, the signal transduction pathway that couples wounding to the systemic activation of target genes is regulated by an 18-amino-acid peptide called systemin. Recent results indicate that systemin exerts its effects on gene activity by up-regulating the synthesis of the fatty acid-derived hormone jasmonic acid (JA), a potent regulator of stress-induced genes in virtually all plant species examined. The focus of our current research is to exploit genetic strategies to develop this model and to identify genes involved in systemin-mediated signal transduction.

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

112. Chloroplast Biogenesis

K. Keegstra

\$210,800

We are continuing studies on the biogenesis of plastids. Our current efforts are aimed at understanding the transport of cytoplasmically synthesized precursors into chloroplasts. In particular, we are focusing on identifying and characterizing the components of the transport apparatus responsible for mediating this translocation process. Previous work has identified three proteins in the Toc (Translocon at the outer envelope membranes of chloroplasts) complex, Toc86, Toc75 and Toc34 as well as one protein of the Tic (Translocon at the innner envelope membrane of chloroplasts) complex, Tic110. In addition, we have identified ClpC, a stromal protein in the heat shock 100 family of molecular chaperones, as a component of the transport apparatus. cDNA clones and antibodies for all five proteins are available. Current studies are focused on determining the function of each polypeptide and investigating how these polypeptides interact with each other and with precursor proteins to accomplish protein transport into chloroplasts. We are also employing the antibodies and nucleic acid probes for these putative transport components to investigate their expression in various plant tissues that have different types of plastids. Finally, we have recently identified a cyanobacterial homolog of Toc75 and are currently investigating its role in cyanobacteria. Our goal in these studies is to gain some understanding into the evolutionary origins of the import machinery.

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

113. Action and Synthesis of Plant Hormones

H. Kende

\$210,800

The objective of this project is to gain knowledge about the biosynthesis and action of the plant hormone ethylene and about the action of gibberellin (GA) in mediating rapid, submergence-induced elongation of deepwater rice. Our research combines physiological, biochemical and molecular approaches. We are elucidating the regulation of ethylene biosynthesis by determining the expression of genes encoding the two enzymes of this pathway, 1-aminocyclopropane-1-carboxylate (ACC) oxidase and ACC synthase. The evolution of the ethylene biosynthetic pathway is being studied in ferns, which have evolved the capacity to synthesize ACC but produce ethylene via an ACC-independent route. We are also localizing the expression of ACC oxidase mRNA and transcripts of the ethylene signal transduction pathway with respect to ethylene-mediated asymmetric growth, e.g., formation of the apical hook. Signal transduction of ethylene action is being followed in *Arabidopsis*, where we have identified a number of ethylene-regulated genes. In deepwater rice, ethylene reduces the level of endogenous abscisic acid (ABA), thereby increasing the sensitivity of the plant to GA. We propose to study the effect of ethylene on ABA metabolism, in particular with respect to the activity of ABA oxidase. Since GA is the immediate growth-stimulating hormone in deepwater rice, we are studying GA-regulated cell wall extension, mainly regarding the role of the wall-loosening proteins, the expansins, in this process, and GA-promoted cell division in the intercalary meristem (IM), using cDNA probes for genes whose products control the progression of meristematic cells through the cell cycle. Using differential display of mRNA, we are also identifying genes in the IM whose expression is regulated by GA the most interesting of which appears to be a novel transcription factor.

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

114. Photoperiodic Induction and the Floral Stimulus

H. Kende, L. McIntosh, and J.A.D. Zeevaart

\$155,800

A large body of physiological evidence indicates that flower formation is hormonally controlled. In plants exposed to the appropriate photoperiod for flowering, a floral stimulus or florigen is formed in the leaves from where it is translocated in the phloem to the apical meristem. Results of grafting experiments show that the floral stimulus is exchangeable between different response types and is, therefore, very similar, if not identical, in different species. The chemical nature of this floral stimulus is one of the major unsolved problems in plant biology. The research is being conducted with the short-day plant *Pharbitis nil*, strain 'Violet' (Japanese morning glory) which in the cotyledon stage can be induced to flower with a single dark period. mRNA from induced (14 h dark period), or non-induced (8 h dark period, and 14 h period with 5 min red light interruption) cotyledons is used for differential display of mRNA to isolate genes whose expression is changed during induction in comparison with the non-inductive treatments. With 200 primer combinations, 190 PCR products showed changes in expression during induction, of which about half were upregulated. Changes in gene expression will be confirmed in northern blots to eliminate false positives. Genes

that are either up- or downregulated during induction will be sequenced and their possible functions will be determined by comparison with sequences in the data bases.

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

115. Interaction of Nuclear and Organelle Genomes

L. McIntosh

\$205,800

Our studies with plant mitochondria involve how the mitochondria communicate with the nucleus to regulate nuclear-encoded mitochondrial proteins. The alternative oxidase, encoded by the nuclear *Aox1* gene family, is employed to study the nuclear/mitochondrial interaction. Alternative pathway respiration is induced by a number of environmental signals including lowered temperature, salt stress, and wounding. A thread that most of these inducing stresses have in common is oxidative stress generated within mitochondria. We have isolated a large number of cDNA's encoding a variety of genes which respond quickly to mitochondrial oxidative stress. Recently, evidence for the mitochondrial signal for this stress has moved toward reactive oxygen species, specifically hydrogen peroxide. Another aspect of this work has focused on downstream -from respiratory electron transport- regulation of carbon balance through reductive potential generated by the plant TCA (tricarboxylic acid) cycle. Our work indicates a key mitochondrial matrix enzyme, NADP-isocitrate dehydrogenase, may be involved.

Research in photosynthesis is centered on the genetic control of carbon flow through Photosystem I. We have created a number of primary mutants to study electron flow in the "core" of Photosystem I. Recently, we have begun to isolate families of pseudorevertants to these primary mutants. The pseudorevertants appear to "correct" many of the primary mutations. These mutants fall into three classes: Photosystem II mutations, Photosystem I subunit mutations, and mutations in proteins involved in assembly of Photosystem I.

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116. Sensory Transduction in Plants

K.L. Poff

\$160,800

The objective of this project has been to understand the mechanisms for the acquisition of environmental information via light reception. In particular, we have studied phototropism in flowering plants. This response is under the control of the blue light photoreceptor pigment system(s) which control(s) numerous directional light responses, and is strongly modulated by phytochrome which typically controls responses based on daylength. We have developed a genetic system with which initial steps in the transduction sequences have been dissected. Based on mutants of *Arabidopsis* with altered phototropism, a major component of phototropism is phototropic adaptation. Under conditions of long term irradiation, plants adapt to the irradiation. Thus, their responses at the beginning and end of the irradiation are not equal. An understanding of this process of adaptation is necessary to understand the plant's response to the long term irradiations

that are important for the plant under field conditions. One particularly interesting result of this work is the finding that both phytochrome A and phytochrome B are required for the normal expression of phototropic curvature. Our approach to the study of the pathways for phototropism and phototropic adaptation has included genetic, physiological and biophysical characterization of mutants and ecotypes that differ in their phototropic response. This genetic/biophysical/molecular approach should permit access into the transduction sequence, and eventual understanding at the molecular level of the events from reception to the bending responses.

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

117. Molecular Mechanisms of Trafficking in the Plant Cell

N.V. Raikhel

\$200,800

Maintenance of separate subcellular compartments in eukaryotic cells depends on the correct sorting and targeting of newly synthesized proteins. Thus, mechanisms must exist in the cell to assure that these proteins are targeted to, and subsequently translocated across, the correct intracellular membranes. We are working with proteins destined for different compartments: vacuoles, cell wall and nucleus. We are interested in understanding the molecular determinants of differential protein compartmentalization and identifying the components of the molecular machinery which carry out the sorting process. We have analyzed and characterized sequences responsible for protein sorting to the vacuole and to the nucleus, and have isolated several putative receptors of the endomembrane system and the nuclear import machinery. We are now elucidating the functional complexity of vesicular trafficking and transport to the nucleus.

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

118. Cell Wall Metabolism

N.V. Raikhel, K. Keegstra, H. Kende, and J.D. Walton

\$155,800

Our goal in this project is to address two questions of cell-wall metabolism. The first is to understand how xyloglucan, the major hemicellulosic polymer in dicots, is synthesized. The second is to understand the role of expansins, proteins that loosen cell walls, during the growth of rice internodes. The first part of the first objective is to isolate and characterize the fucosyltransferase involved in xyloglucan biosynthesis. Purification of the enzyme has been completed, and protein sequence information should be available soon. The next step will be the isolation of cDNA clones encoding the enzyme and the preparation of antibodies against the protein encoded by the cDNA clone. The second part of the first objective is to investigate the function of a Golgi-localized 40-kD protein that is postulated to have a role in xyloglucan biosynthesis. cDNA clones for this protein have been isolated and antibodies against it prepared. A detailed study of the expression patterns of this gene has been completed. Antisense plants containing suppressed levels of this protein have been produced and will be used to investigate its role, if any, in xyloglucan biosynthesis. Finally, we are investigating the involvement of expansins in the growth of rice internodes. Expansins are recently discovered cell-wall loosening proteins. We have studied the expression

of expansin genes and expansin protein by RNA blot analysis and tissue printing. The cellular localization of expansin gene expression is being studied by *in situ* hybridization. Transformation experiments are being planned to assess the role of expansins in the growth of intact rice plants.

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119. Biochemical and Molecular Aspects of Plant Pathogenesis

J.D. Walton

\$196,800

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

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

120. Developmental Biology of Nitrogen-fixing Cyanobacteria

C.P. Wolk

\$190,800

Anabaena and related filamentous cyanobacteria utilize reductant generated by photosynthesis in vegetative cells to fix nitrogen gas within spaced, differentiated cells called heterocysts. We are elucidating the dependency relationships, and the roles, of genes whose collective function allows *Anabaena* to fix nitrogen (an oxygen-sensitive process) in the presence of oxygen. The heterocyst envelope plays a key role by limiting the rate of entry of oxygen into heterocysts. We are studying *hep* genes, required for synthesis or stabilization of the polysaccharide layer of that envelope, and *het* genes, required for morphological differentiation. Overlapping, ca. 100-bp deletions ("windows") were introduced into the region 5' from a *hepA*-reporter fusion, and the constructions transferred

to *Anabaena*. Deletions from -534 bp to -446 bp and from -343 bp to -169 bp relative to the *hepA* transcriptional start site greatly reduced expression of the reporter after nitrogen stepdown. Gel-retardation experiments showed the presence of a protein that binds specifically to the latter region, and may control expression of *hepA*. A mutation in a gene that shows great similarity to N-acetylmuramoyl-L-alanine amidases, but not a mutation in the gene 3' from it that shows great similarity to glutamate racemases, blocks formation of the heterocyst envelope polysaccharide. Perhaps export of that polysaccharide requires prior catabolism of peptidoglycan in the differentiating vegetative cell. Gene *hetR* represses expression of *hetC*, which is active early in differentiation after pattern formation, ca. 100-fold. This work will facilitate understanding of cellular differentiation, pattern formation, and biological conversion of solar energy.

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

121. Frankia Genetics

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

\$155,800

Frankia spp., bacteria in the Gram positive actinomycete group, form nitrogen-fixing nodules with a wide variety of non-leguminous woody plants. *Frankia*-nodulated plants have great, world-wide potential as sources of fuel, fiber, shade, and forage, as well as for reforestation. Their potential, and opportunities for understanding the interesting biology of *Frankia*, are strongly curtailed by the lack of methodology for genetic manipulation of these bacteria. Our short-term objective is to develop techniques for stable, reproducible genetic transformation of *Frankia*. Transfer of DNA to *Frankia* requires a suitable replicon, a means of transferring the DNA, appropriate selective markers, avoidance of restriction, and a suitable choice of strain. To tailor a replicon, we sequenced an 8.5-kb plasmid from *Frankia* strain Cpl1, and have constructed derivatives of it that bear antibiotic-resistance markers whose codon usage is consistent with that of known *Frankia* genes. Transfer of plasmid DNA to relatively fast-growing strains of *Frankia* has been attempted by electroporation, particle-bombardment, and conjugation. To circumvent possible restriction barriers, we have searched for type-II restriction endonucleases (in collaboration with New England Biolabs, Inc.; none yet found), identified putative clones of DNA methyl transferase genes, and are modifying DNA by a coliphage T4 anti-restriction system (in collaboration with J. Elhai). Longer term objectives include the development of techniques for transposon mutagenesis of, and gene replacement and use of reporter genes in, *Frankia* followed by analysis of the generation of N₂-fixing symbioses of *Frankia* with higher plants.

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

122. Environmental Control of Plant Development and its Relation to Plant Hormones

J.A.D. Zeevaart

\$186,800

Plant growth and development are affected by environmental factors such as daylength, temperature, and moisture. Hormones often function as chemical messengers between the perception of an environmental factor and the morphological expression. In long-day rosette plants, stem growth in long days (LD) is caused by an increase in gibberellin (GA) levels, particularly in the shoot tips. The obvious question is: which step(s) in the GA biosynthetic pathway is (are) stimulated by LDs? *GA5* which encodes GA 20-oxidase in *Arabidopsis* was predominantly expressed in the upper parts of stems. With induction of stem growth by LD, *GA5* mRNA gradually increased in the shoot tips. As the plants elongated, *GA5* mRNA in the upper part of the stems increased to a maximum, and then decreased as the growth rate declined. By contrast, expression of *GA4*, encoding 3 β -hydroxylase, showed low expression in stems, and its expression was not correlated with the rate of stem elongation.

Abscisic acid (ABA) is a plant hormone whose synthesis is greatly stimulated when plants experience a water deficit. The objective of our studies is to find out how the biosynthesis of ABA is regulated. In collaboration with D.R. McCarty's laboratory at the University of Florida, Gainesville, the ABA-deficient mutant *vp14* of maize has been characterized. The VP14 GST-fusion protein gene was assayed for cleavage activity, using 9-*cis*-violaxanthin and 9'-*cis*-neoxanthin as substrates. The expected cleavage products, xanthoxin and the C₂₅ apo-aldehydes, were identified. All-*trans*-carotenoids were not cleaved. Because the cleavage reaction is thought to be the key regulatory step in ABA biosynthesis, it may be possible to alter ABA levels in transgenic plants that would affect such physiological processes as drought tolerance, cold hardiness, and seed maturation.

Michigan State University
East Lansing, MI 48824

123. The Role of Membrane and Surface Carbohydrates of Rhizobium in Symbiosis

R.I. Hollingsworth, Departments of Biochemistry and Chemistry

\$86,000

The primary goal of our research is to determine the role that complex saccharide and membrane lipid chemistry in *Rhizobium* plays in the infection and occupancy of legume plants by these bacteria. Our work so far has turned up a startling degree of overlap between lipid, glycolipid and general carbohydrate biosynthesis in *Rhizobium* and in plants. Hence phosphatidyl choline, diglucosyl diacylglycerol, digalactosyl diacylglycerol, surfoquinovosyl diacylglycerol, phosphatidyl inositol and sulfocholine, all typical lipids in plants and other photosynthetic systems and rare or unheard of in bacteria, have been found in species of *Rhizobium*. This suggests the possibility of some pre-arranged common metabolism between these symbiotic partners. We have also found that the surface chemistry of *Rhizobium* is very variable and very sensitive to environmental factors and can be influenced by host factors and by the environment in the rhizosphere and inside of the

plant. A further goal of our work has been to untangle the complex, interdigitized web of carbohydrate metabolism and saccharide biosynthesis in an effort to understand the many pleiomorphic effects of mutations that affect biosynthesis and cloud or complicate the picture.

Michigan State University
East Lansing, MI 48824-1312

124. Biosynthesis of Triacylglycerol in Developing Oilseeds

J. Ohlrogge, Department of Botany and Plant Pathology

\$97,792

Although plant triacylglycerol (TAG) represents a major form of carbon storage in the biosphere, an understanding of the biosynthetic pathway(s) for triacylglycerol assembly, their subcellular location and regulation is not completely established. Some of the major unanswered questions in oilseed biochemistry are: a) How are unusual fatty acids targeted for storage in TAG and excluded from membrane phospholipids. b) What limits the quantity of oil produced by an oilseed? c) Are there spatially distinct pathways for membrane and TAG biosynthesis? Thus, although the biosynthesis of triacylglycerols in oilseeds has received considerable study, and the basic enzymatic reactions are mostly known, our understanding of how plants control the amount of TAG produced and how fatty acids move from the plastid and are incorporated into TAG is still unclear. The experiments are designed to evaluate the *in vivo* metabolism, regulation, and subcellular organization which lead to TAG assembly in oilseeds. In particular, we will address the following specific objectives: I) What is the role of phosphatidylcholine in TAG biosynthesis in Brassica? II) Is a deacylation/reacylation cycle required for unusual fatty acid incorporation into oilseed triacylglycerol? III) Are there spatially distinct pools of PC and DAG involved in membrane and TAG biosynthesis? IV) Can TAG production in oilseeds be influenced by fatty acid or nitrogen supply?

Michigan State University
East Lansing, MI 48824

125. Structure-Function Relationships of ADP-glucose Pyrophosphorylase: Manipulation of the Plant Gene for Increased Production of Starch in Plants

J. Preiss, Department of Biochemistry

\$170,000 (FY96 funds/2 years)

Structure-function relationships of the ADPglucose pyrophosphorylase from higher plants (potato tuber) and bacteria (*Escherichia coli* and *Anabaena*) will be studied. The *Anabaena* structural gene and cDNA clones of the large and small subunits of the potato ADPglucose pyrophosphorylase have been expressed in *E. coli*. The expression of these genes and prior chemical modification studies on the purified spinach leaf ADPglucose pyrophosphorylase enable us to do site-directed mutagenesis at various regions of the plant enzyme to gain more insight on the nature of the catalytic and effector sites and function of the two different subunits in the higher plant ADPglucose pyrophosphorylase. Attempts will also be made to understand why the higher plant enzyme (a heterotetramer, a₂b₂) requires two different subunits for high activity. Partial proteolysis experiments and molecular modeling programs are being used to predict and determine the secondary structure of the three enzymes. Crystallization and X-ray diffraction studies are major

efforts to obtain insight into the three-dimensional structure of the ADP-glucose pyrophosphorylase. "Mutant," active bacterial enzymes that are less sensitive to allosteric inhibition by phosphate have been constructed and it is planned to do the same for the plant enzymes. Also active bacterial or plant enzymes that may not require the allosteric activator for activity; i.e., fully active without activator will be constructed. These could in the future be used to produce transgenic plants having increased amounts of starch.

Michigan State University
East Lansing, MI 48824-1101

126. Physiology and Molecular Biology of Lignin-Modifying Enzymes

C.A. Reddy, Department of Microbiology

\$88,315

Laccases, lignin peroxidases (LIPs) and manganese-dependent peroxidases (MNPs) are the major lignin-modifying enzymes (LMEs) in fungi. While much information is available on lignin degradation by the white-rot fungus, *Phanerochaete chrysosporium*, relatively little is known on LMEs in other white-rot basidiomycetes. This study focuses on the LMEs in *Ganoderma lucidum*, a widely distributed white-rot fungus in North America, and a soil basidiomycete, AX1. *G. lucidum* produces high amounts of laccase, both in defined media and in wood cultures. However LIP production was not seen in any defined media or wood cultures although LIP gene homology was observed when genomic DNA was probed with LIP gene probes of *P. chrysosporium*. Five major laccase activity bands were seen on SDS-PAGE gel. MNP activity was detected when grown on poplar but not on pine wood or other defined media. We are presently characterizing the laccase and determining its role in degrading synthetic lignin (¹⁴C-DHP). Using the Polymerase Chain Reaction technique we have obtained a laccase gene fragment which will be used in isolating the whole laccase gene. AX1 produces high laccase levels, but no LIPs or MNPs. Laccase production varied in different wood cultures suggesting differential laccase gene expression. The laccase enzyme has been partially characterized for substrate specificity, pH optimum, molecular weight and the number of isoforms. Currently, the role of copper in regulating laccase production is also being studied. Furthermore, we plan to clone the laccase gene(s) after purifying and characterizing the laccase enzyme.

Michigan State University
East Lansing, MI 48824-1319

127. Molecular Physiology of Succinic Acid-Based Fermentation in Anaerobes: Control of Chemical Yield by CO₂ Fixation and Electron Donors

J.G. Zeikus, Department of Biochemistry

\$110,000

Fermentation derived succinic acid has many industrial uses as a specialty chemical; and, it is a potential replacement for petro-derived maleic anhydride used for manufacture of engineered plastics and polyesters. This project will develop fundamental and applied understanding on the mechanisms regulating growth and carbon and electron flow in catabolic succinate producing bacteria that utilize CO₂ as an electron acceptor. The physiology, biochemistry and key genes of *Actinobacillus* and *Anaerobiospirillum succiniciproducens* strains will be compared. Both organisms

regulate PEP carboxykinase in relation to increasing CO₂ levels required for succinate production. The PEP carboxykinase genes have been cloned and site directed mutants will be utilized to examine the key enzyme structural elements responsible for determination of substrate affinity, and CO₂ fixing activity. Metabolic studies on *Actinobacillus* are aimed at developing a high yield fermentation based on coupling of hydrogen and CO₂ metabolism to "homo" succinate production. Because *Actinobacillus* contains hydrogenase, the influence of electro chemical control of redox potential and electron donor availability on metabolism and growth will be determined. Preliminary studies on the succinate fermentation under conditions of cathodic reduction linked to redox dyes have resulted in major changes in growth and end product levels.

University of Michigan
Ann Arbor, MI 48109-1048

128. CLV Signaling in Meristem Development

S. Clark, Department of Biology

\$90,000

The shoot meristem of higher plants is responsible for initiating all above-ground organs. Thus the genes regulating shoot meristem function are required for cell proliferation and differentiation, and for the proper number, spacing and size of primordia such as leaves and flowers. To function as a site of continuous organ formation, the shoot meristem must be able to carry out two fundamental processes. The first function is the maintenance of a population of undifferentiated cells at the center of the meristem. As this population divides, it provides new cells for incorporation into organ primordia. The second process is to direct progeny of the undifferentiated cells towards organ formation and eventual differentiation. This transition from an undifferentiated to differentiated state occurs at the flanks of the shoot meristem, and is regulated in strict balance with the proliferation of the undifferentiated cells.

We have found that the CLV loci (CLV1, CLV2 and CLV3) in *Arabidopsis* regulate the balance between proliferation and differentiation. *clv* mutants accumulate over 1000-fold more undifferentiated cells than wild-type plants. CLV1 encodes a receptor-like kinase, and may relay positional information through a signal transduction cascade. Our work focuses on identifying other members of the CLV1 signaling cascade through both genetic and biochemical approaches.

University of Minnesota
Minneapolis, MN 55455

129. Genetics of Bacteria that Utilize One-Carbon Compounds

R.S. Hanson, Department of Microbiology

\$90,017

Methylotrophic bacteria are able to grow on one-carbon compounds including methane, methanol, methylamines and halomethanes. Some facultative methylotrophs also grow on multicarbon compounds including sugars, fatty acids and amino acids and induce the synthesis of enzymes involved in one-carbon metabolism only in the presence of C1 compounds. One enzyme, methanol dehydrogenase, is encoded by *mxoF* and can comprise 20% of the cellular protein. The expression of *mxoF* and other genes in this regulon requires at least three response regulator proteins (*mxoB*,

mxbM and *mxcE*) and two histidine kinases (*mxuD* and *mxuQ*) that are components of two component regulatory systems. The sequences of all these genes has been completed and the MxcE and MxbM proteins have been expressed in *E. coli* and purified. Both bind to regulatory sequences upstream of the *mxuF* gene. The role of the histidine kinases in phosphorylation of each response regulator protein is under investigation.

A soluble methane monooxygenase, required for oxidation of methane to methanol is synthesized only under copper limited conditions. The mechanisms by which copper regulates the synthesis of this enzyme are being examined.

University of Minnesota
St. Paul, MN 55108-1095

130. Isolation and Characterization of Ammonia Monooxygenase of Nitrosomonas
A.B. Hooper, Department of Genetics and Cell Biology \$101,000

Ammonia monooxygenase (AMO) of the autotrophic bacterium *Nitrosomonas* catalyzes $\text{NH}_3 + 2\text{e}^- + 2\text{H}^+ + \text{O}_2 \rightarrow \text{NH}_2\text{OH} + \text{H}_2\text{O}$. Electrons pass to AMO from hydroxylamine oxidoreductase (HAO): $\text{NH}_2\text{OH} + \text{H}_2\text{O} \rightarrow \text{HNO}_2 + 4\text{e}^- + 4\text{H}^+$. Together with a terminal oxidase, oxidation of NH_3 by AMO and HAO provide all the energy for growth in this bacterium. This oxidative step in the N-cycle in nature has a very important role in N-availability to agricultural plants, the production of greenhouse-active and ozone-reactive N-oxide gases and the transformation of ammonia in waste water. AMO oxidizes halogenated aliphatic and aromatic compounds (sometimes producing pure enantiomers) and is thus potentially important to pollutant bioremediation and the production of specialty chemicals.

The prosthetic groups or structure of AMO has not been determined because active enzyme cannot yet be purified. Acetylenic substrates derivatize a protein subunit of AMO allowing isolation and sequence of the genes for three subunits and generating a model containing possible metal binding sites and 12 transmembrane segments, a common property of membrane pumps. This observation and the inhibition of the AMO system in cells by uncouplers and DCCD, suggests that AMO might be driven by an ion gradient, an hypothesis that we are testing. The ultimate goal is to isolate and characterize all protein subunits, the structure of the active site and electron-transfer redox centers by optical, EPR, Mossbauer and other types of spectroscopy and by chemical analysis.

University of Missouri
Columbia, MO 65211-7400

131. Cellulose Synthesis and Morphogenesis
T.I. Baskin, Division of Biological Sciences \$90,339

This research aims to understand plant morphogenesis. The project studies the relationship between morphogenesis and cellulose microfibrils. Experiments have compared growth of well-watered roots of *Zea mays* to thinner roots grown at low water potential. We measured the

spatial distribution of expansion rates, in length and radius, for stele and cortex, and found that expansion in longitudinal and radial directions were regulated independently. To determine whether alignment of microfibrils regulates radial expansion rate, we quantified microfibrillar alignment with electron microscopy of metal replicas of the inner-most wall layer. Microfibrils were transverse to the long axis of the root throughout the growth zone; however, locations with different rates of radial expansion had similar microfibrillar alignment. Quantitative polarized-light microscopy showed that the amount of retardation of the cell walls did not change as a function of position, confirming that different rates of radial expansion occurred without differences in microfibril organization or abundance. We strengthened the conclusion that rates of radial expansion are independent of the organization or abundance of microfibrils by identifying a mutant in *Arabidopsis thaliana*, *rsw7*, in which rates of radial expansion were stimulated compared to wildtype but microfibrils were neither depleted nor disorganized. The work points to components other than microfibrils that help to shape plant organs. This research will uncover mechanisms of plant morphogenesis and can thus be expected to enhance applied efforts to optimize plant growth for human benefit.

University of Missouri
Columbia, MO 65211

132. Dosage Analysis of Gene Expression in Maize

J. Birchler, Division of Biological Sciences

\$104,000

The goal of this project is to understand further the basis of dosage sensitive regulatory effects on gene expression in maize. These effects act directly or inversely on the quantitative level of gene products when the dosage of specific chromosomal segments is varied. It has long been known that addition or subtraction of chromosomal segments relative to the normal diploid has detrimental effects on the vigor of plants. The experiments proposed seek to understand the molecular basis of these aneuploid syndromes as well as to understand the mechanisms of gene expression that are involved. One set of experiments is to test for a correlation between small regions of chromosome arm 10L that produce inverse and direct dosage effects on selected monitored RNAs and that produce aneuploid effects on kernel size. This chromosome arm will be divided into several segments--some with known kernel size effects. These smaller regions will be tested for a correlation with the molecular dosage effects that are found in the larger segment. Also, conditions that make the aneuploid effects more extreme will be tested as to whether the dosage effects are also of greater magnitude. To determine whether the effects can be reduced to the action of single genes, two projects are proposed. First, dosage series including three known regulatory genes (*Vp1*, *C1* and *R1*) effective on the anthocyanin pathway are being examined for effects on the RNAs for the biosynthetic enzymes. One of these, the *C1* locus, is known to give a dosage effect and might well be a single gene example of a direct regulator. Any effects found with the whole arm dosage series will be tested whether they are due to the respective regulatory gene by recombining a loss-of-function allele in each case onto the B-A translocation used to generate the series. If the regulatory gene is responsible for the whole arm effect, then the recombined translocation will no longer be effective. Secondly, modifiers will be induced that increase or decrease the amount of pigment present in kernels that carry a single functional copy of *C2* in the endosperm using the Mutator transposon system. Modulations of this gene encoding chalcone synthase can be recognized phenotypically. Heritable modifiers will be tested for a dosage effect on *C2* RNA. Lastly, run-on transcription assays will be conducted on some of the most extreme inverse and direct effects on *Alcohol dehydrogenase-1* to test whether the rate of transcription is

being modulated by these changes in chromosomal dosage. These experiments will provide information on the mechanisms of gene expression involved with these dosage effects and the relationship to the altered vigor of plants that have changes in chromosome number.

University of Missouri
Columbia, MO 65211

133. Position Effect as a Determinant of Variegated Pigmentation in Maize

K.C. Cone, Division of Biological Sciences

\$125,000

The aim of this project is to understand epigenetic mechanisms of gene regulation. We are studying PI-Blotched, a gene that controls the synthesis of purple anthocyanin pigments in the maize plant. Plants carrying PI-Blotched exhibit an unusual pattern of pigmentation characterized by variegation, rather than the usual uniform pattern of pigmentation. Because the nucleotide sequence of PI-Blotched is nearly identical to the normal PI-Rhoades sequence but the two alleles are differentially methylated, our hypothesis is that the variegated pattern of PI-Blotched expression is due to an epigenetic effect. As one way to address this idea, we are looking at the role of development in regulating PI-Blotched expression. The methylation of PI-Blotched DNA is progressive through development of the plant from seedling to adult. In seedling leaves, PI-Blotched DNA is less methylated than PI-Rhoades DNA whereas in adult leaves, PI-Blotched DNA is more methylated than PI-Rhoades DNA. In PI-Blotched plants carrying the developmental mutation *Teopod1*, which lengthens the juvenile phase of growth, the change in DNA methylation precedes the change in phase-specific leaf features, such that the latest juvenile leaves display the level of methylation seen in fully adult leaves. If we consider that a change in methylation might occur more rapidly than morphological changes in leaf characteristics, this observation suggests that methylation of PI-Blotched may be responsive to the same molecular cue that signals phase change. Such developmental influences on gene expression are a hallmark of epigenetic regulation.

University of Missouri
Columbia, MO 65211

134. Genetics of the Sulfate-Reducing Bacteria

J.D. Wall and B.J. Rapp-Giles, Biochemistry Department

\$95,000

To explore the energy generating processes of the strictly anaerobic bacteria, new genetic tools for the sulfate-reducing bacterium, *Desulfovibrio desulfuricans* G20, have been developed that include a promoter probe vector with *lacZ* as the reporter. Conditions have been established to detect the activity of beta-galactosidase in colonies of the sulfate-reducing bacterium. Procedures for reasonably reliable electroporation of this anaerobe were established. A mutant was constructed in *cycA*, encoding cytochrome *c3*, by plasmid insertion. After confirmation by Southern analysis, preliminary phenotypic characterization of the mutant shows no aberrant features. Although mRNA was dramatically reduced, protein patterns of heme-containing proteins were not significantly different from the wild type. More detailed analysis of the mutation is in progress. Similarly a mutation has been created in a cloned and partially sequenced *hynAB* operon. This

operon appears to be poorly expressed, if at all, in G20. The 16S RNA gene sequence has been determined and a phylogenetic comparison made with other such sequences from sulfate-reducing bacteria available in the database.

Mount Sinai School of Medicine New York, NY 10029

135. The Respiratory Chain of Alkaliphilic Bacteria

T.A. Krulwich, Department of Biochemistry

\$112,000

The long term goals of this project are to understand those features of the alkaliphile respiratory chain that are required for this ability of the alkaliphile to grow optimally at pH values that place severe energy demands upon these bacteria, i.e., to elucidate the basis for apparently remarkable energy conservation by alkaliphilic bacteria. The extreme, facultative alkaliphile *Bacillus firmus* OF4 grows on malate at a slightly faster rate and to at least as high a molar growth yield at pH 10.5 as it does at pH 7.5. The robust non-fermentative growth of the alkaliphile at pH 10.5 depends upon its full, pH 10.5-induced complement of a *caa*₃-type terminal oxidase. Moreover, a second, *bd*-type, terminal oxidase that is up-regulated upon disruption of the *caa*₃ oxidase-encoding *cta* operon cannot support growth on malate even at pH 7.5. Current studies are focused upon cloning the operon encoding the *bd*-type oxidase so that the effects of its overexpression in the *cta*-disrupted mutant can be tested along-side expression of a cloned *cta* operon. The studies should clarify whether overexpression of the genes encoding the *bd*-type oxidase will render it competent to support non-fermentative growth at pH 7.5, 10.5, both, or neither. If, as hypothesized, the *caa*₃-type oxidase will turn out to be necessary for oxidative phosphorylation and hence non-fermentative growth at pH 10.5, the properties of this oxidase that are essential for alkaliphily will be probed.

The National Renewable Energy Laboratory Golden, CO 80401

136. The Water-Splitting Apparatus of Photosynthesis

M. Seibert, Center for Basic Sciences

\$134,000

The goal of this research is to understand structural and functional relationships in photosystem II (PSII) with emphasis on the reaction center (RC) and water-oxidation processes that generate photosynthetic reductant. Femtosecond flash-probe studies of the isolated PSII RC using a novel analysis procedure has resolved a long standing controversy regarding the rate of primary charge separation. Biphasic kinetics (8 and 50 ps⁻¹) were detected and represent the effective rate of charge separation within the RC equilibrated core and charge transfer, limited by energy transfer from accessory chlorophyll in the RC, respectively. A new way of investigating the water-oxidation process by limiting the availability of substrate water was also developed. Water content was decreased by adding ethylene glycol to the solvent, and both reversible and irreversible changes in various functional properties of PSII were explained in terms of structural and electronic effects of the increased organic solvent environment. Studies of high affinity Mn ligands (associated with functional Mn) in PSII using the DPC-inhibition assay have identified four ligands (two carboxyl and two histidyl residues). Only one of these ligands, a carboxyl, is specific for Mn, and this Mn is

photooxidizable by a single-turnover flash of light. This ligand is probably Asp170 on the D1 RC protein, and its chemical modification uncovers a low affinity, photooxidizable Mn ligand that is neither a carboxyl nor a histidyl residue. The other three ligands (including His337 on D1) bind Mn that is not photooxidizable by a single flash, but they may also bind other metals.

University of Nebraska
Lincoln, NE 68588-0118

137. Tomato Bushy Stunt Virus and DI RNAs as a Model for Studying Mechanisms of RNA Virus Replication, Pathogenicity, and Recombination

T.J. Morris, School of Biological Sciences and A.O. Jackson, University of California, Berkeley \$129,992 (FY96 funds/20 months)

Our research on Tomato bushy stunt virus (TBSV) has focused on determinants affecting replication, pathogenicity and recombination. TBSV is a small isometric virus that contains a single stranded RNA genome with five major genes. We analyzed the importance of an additional small gene designated pX, at the 3' end of the genome. Bio-assays showed that mutations of the translational initiation codon designed to disrupt translation of the pX encoded protein were unable to accumulate to detectable levels in cucumber protoplasts. Although these results suggested a role for the putative pX protein, introduction of a premature stop-codon to truncate expression of the pX protein were unable to replicate. In contrast, a comparable pX mutation that affected the same nucleotides without changing the predicted amino acid sequence greatly reduced RNA accumulation. Therefore, the pX RNA sequences rather than the predicted pX protein influence genome replication. The requirement for pX also appears to be host dependent because comparisons revealed that subtle pX gene mutations that interfere with accumulation of TBSV RNA in cucumber or *Nicotiana benthamiana*, did not affect replication in *Chenopodium quinoa* protoplasts or plants. Irrespective of the host, the cis-acting pX gene sequences were not required for replication of defective interfering RNAs that require helper TBSV for replication in trans. These experiments thus suggest that the pX cis-acting element interacts with one or more host components whose composition differ slightly between different plants.

University of Nebraska
Lincoln, NE 68583

138. Enzymology of Aceticlastic Methanogenesis

S.W. Ragsdale, Department of Biochemistry \$104,000

An understanding of the mechanism of methane formation is critical since methane is an important fuel and a significant greenhouse gas whose concentration is rising at a rate of 1% per year. We are studying the mechanism of methanogenesis from acetate, which is the major substrate for methanogens in nature. We are focusing on three important steps in aceticlastic methanogenesis: (i) the initial step of acetyl-CoA disassembly by the molecular aggregate that contains CO dehydrogenase/acetyl-CoA synthase (CODH/ACS) and a corrinoid/iron-sulfur protein (C/Fe-SP); (ii) the methyl-CoM reductase (MCR) catalyzed reaction that generates methane and a heterodisulfide (CoB-S-S-CoM) from methyl-Coenzyme M (methyl-CoM) and Coenzyme B (CoB);

and (iii) the heterodisulfide reductase (HDR) reaction that reduces CoB-S-S-CoM to the free thiols, CoB-SH and CoM-SH for the next round of methanogenesis. We are using a combination of kinetics, electrochemistry, and spectroscopy to establish the reaction mechanisms of these enzymes. In the last year, a protocol to generate highly active MCR has been developed and the HDR has been characterized as a heme/iron-sulfur protein. 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.

University of Nebraska
Lincoln, NE 68588-0664

139. Role of the Rubisco Small Subunit

R.J. Spreitzer, Department of Biochemistry

\$92,000

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyzes the rate-limiting step of photosynthetic CO₂ fixation. Because of its low carboxylation efficiency and competitive inhibition by O₂, Rubisco has been viewed as a potential target for engineering enhanced crop-plant productivity. The Rubisco holoenzyme is comprised of two subunits, each present in eight copies. Much is known about the structure-function relationships of the chloroplast-encoded large subunit. It contains the active site. Much less is known about the role of the nuclear-encoded small subunit. There is a family of small-subunit genes in crop plants, making it difficult to substitute engineered small subunits into the holoenzyme. A photosynthesis-deficient mutant of the green alga *Chlamydomonas reinhardtii* has recently been recovered that lacks both members of the small-subunit gene family. Because this mutant can be rescued via transformation with a single small-subunit gene, it is now possible to answer questions about small-subunit function. Alanine-scanning mutagenesis is being used to elucidate the significance of small-subunit structural regions that are unique to the Rubisco of eukaryotes. Most substitutions do not eliminate enzyme function, indicating that these regions are not essential for holoenzyme assembly or catalysis. In contrast, random mutagenesis is being used to define those regions of the small subunit that are essential. Because Rubisco enzymes from different species display differences in CO₂/O₂ specificity, heterologous small-subunits will also be introduced into *Chlamydomonas* to assess the contribution of small subunits to catalytic efficiency.

New York University
New York, NY 10003

140. Asparagine Synthetase Gene Regulation and Plant Nitrogen Metabolism

G. Coruzzi, Department of Biology

\$107,000

We are studying the genes controlling asparagine biosynthesis using a molecular-genetic approach in *Arabidopsis thaliana*. Since asparagine serves to transport and store assimilated nitrogen, these may have significance for improving nitrogen use efficiency. We have uncovered two distinct classes of ASN genes. ASN1, the major expressed gene, is transcriptionally repressed by light and this mirrors the light repression of asparagine accumulation in plants. These results suggest the ASN1 gene controls the synthesis of transported asparagine. The ASN2 gene, cloned by complementation of a yeast asparagine auxotroph, is activated by light and may encode an enzyme

with enhanced ammonia-dependent activity possibly involved in ammonia detoxification. The ASN1 and ASN2 genes are also reciprocally controlled by metabolites. ASN2 is induced by light or sucrose and repressed by organic nitrogen. By contrast, ASN1 expression is repressed by light or sucrose and induced by organic nitrogen. We propose that this metabolic regulation of the ASN1 gene serves to channel assimilated nitrogen into asparagine under conditions of carbon limitation (in the dark) or under conditions of organic nitrogen excess. We have begun to study the signal transduction components involved in the light and metabolic regulation of ASN1 expression. We identified cis-elements and nuclear factors involved in the light or sucrose repression of AS1. We are using a transgenic Arabidopsis line (AS1-Hph) in a positive selection to isolate mutants impaired in light or sucrose repression. The phenotypic analysis of such mutants may define how the regulation of asparagine synthesis by light or metabolites affects plant growth and nitrogen use.

North Carolina State University
Raleigh, NC 27695-7905

141. Bioenergetic and Physiological Studies of Hyperthermophilic Archaea
R.M. Kelly, Department of Chemical Engineering \$95,645

The objectives of this project are to examine the biochemical characteristics of several hydrolytic enzymes produced by heterotrophic, hyperthermophilic archaea and relate the function of these enzymes to particular physiological and bioenergetic roles. The study focuses on two members of the Thermococcales, *Thermococcus litoralis* (T_{opt} 88°C) and *Pyrococcus furiosus* (T_{opt} 98°C). Both are obligately anaerobic heterotrophs which grow in the presence or absence of reducible sulfur compounds. A novel, intracellular protease (PfpI) has been isolated from *P. furiosus* which was shown to exist *in vitro* in several functional homomultimeric forms of an 18.8 kDa subunit, including a trimer, a hexamer and a dodecamer, with the largest assembly having the highest specific activity. The gene corresponding to PfpI is homologous to putative proteins in organisms ranging from *Escherichia coli* to *Homo sapiens*. An affinity-based chromatography method has been developed to facilitate the purification of PfpI homologs from a variety of cells and organisms. Its function is being examined in relation to archaeal proteasome in *P. furiosus* which was also isolated and characterized biochemically. Bioenergetic and physiological studies focusing on *T. litoralis*, both in pure and co-culture with *Methanococcus jannaschii*, are also underway. *T. litoralis* was shown to produce an extracellular polysaccharide in continuous culture which was composed mainly of mannan and which was involved in biofilm formation. Energetics issues related to biofilm formation, sulfur utilization and co-culture dynamics are under investigation.

North Carolina State University
Raleigh NC 27695-8008

142. Transcription Factors in Xylem Development
R. Sederoff, M. Campbell, R. Whetten and D. O'Malley, Department of Forestry
\$109,953

Trees form large amounts of wood, or secondary xylem, which is important in both ecological and economic terms. Wood includes a large fraction of the global biomass, and is therefore a key factor

in the global carbon cycle as well as the basis of a major industry. Trees are not convenient experimental organisms, and much of the progress made in plant molecular biology using model species has yet to be transferred to woody plants. We are working to unravel the genetic mechanisms that control formation of wood, using as a guide the understanding of gene regulation that has emerged over the past several years from study of model plant and animal species. Proteins called transcription factors are known to control gene expression during both plant and animal development, as well as modulating responses of organisms to environmental stimuli. We are working to characterize transcription factors found in pine xylem, in order to begin defining the regulatory networks that control wood formation. We began working with the Myb class of transcription factors, and identified two different Myb proteins expressed in xylem. Interactions of these proteins with different DNA sequences is being analyzed to test the hypothesis that they control different aspects of wood formation. We are also working with proteins of the Myc family, another major class of transcription factors known to control growth and development in both plants and animals. Members of other families of transcription factors have also been identified, and will be investigated further in the coming year.

University of North Carolina
Chapel Hill, NC 27599-3280

- 143. Novel Control of Signal Delivery from the *Pseudomonas syringae* avrRPM1 Gene to *Arabidopsis thaliana***
J.L. Dangl, Department of Biology \$104,000

We are interested in determining molecularly how plants sense and respond to pathogens. Recently, we and others have cloned several of the plant genes controlling recognition of pathogens. Products of these "disease resistance" genes, "recognize" the product of a particular pathogen's "avirulence" gene, and this recognition leads to disease resistance. If the plant lacks the correct resistance gene function, or if the pathogen lacks the corresponding avirulence gene, disease results. We use *Arabidopsis* as a model plant and a bacterial pathogen, *Pseudomonas syringae*, which can cause leaf spot disease on a variety of crop plants. Our DOE funded research concerns specifically how the avirulence gene signal triggers disease resistance. In the last year we have demonstrated that expression of the bacterial pathogen avirulence gene product inside the plant cell can trigger disease resistance when the plant expresses the correct resistance gene. We have also shown that in plants which do not express the resistance gene that this bacterial protein can trigger a delayed, symptom like reaction. We additionally know that some plant genotypes express this reaction and others do not. This result implies that the bacterial protein can act as a plant-genotype dependent toxin, as expected in it has a role in causing disease on susceptible plants. This is consistent with our genetic analysis of this particular bacterial avirulence gene. We also have shown that this protein is localized to the plant cell membrane fraction. We are now using *Arabidopsis* genetics to identify these novel plant targets.

University of North Carolina
Chapel Hill, NC 27599-3280

144. Map Based Cloning of the Enhancer of Gene Silencing 1 Locus Which Enhances the Silencing of a Foreign Gene in Arabidopsis

S.R. Grant, Department of Biology

\$94,000

The ability to introduce foreign genes into plants has changed the way that agricultural technologists can approach crop improvement. However in many cases, genes introduced into plants do not behave as we would expect due to gene silencing. Gene silencing inhibits expression of some foreign genes and simultaneously inhibits expression of plant genes with strong structural similarity to an introduced gene. It is unpredictable occurring spontaneously in late stages of development or appearing after two genetically manipulated plants are mated. The mechanism that leads to gene silencing is completely unknown. It does not cause permanent mutation of the affected genes since they can become reactivated. Silencing may be caused by a system of gene regulation that scientists could only begin to see working when they started to introduce genes into organisms. We have identified mutations in two Arabidopsis genes that affect the mechanism of gene silencing. These mutations enhance the silencing of a highly expressed foreign gene so that it occurs in embryonic tissues in all plants. The goal of this project is to clone one of these genes called *enhancer of gene silencing1* in order to better understand the mechanism of gene silencing. Taking advantage of the powerful Arabidopsis genome database and the materials collected by the Arabidopsis Stock Center in Ohio, we have mapped the gene to a small interval (4 centimorgans) on the first chromosome. The Stock Center provided us with large Arabidopsis DNA clones that overlap to cover the interval. We are now using sequences from the overlapping clones to reduce the interval in which the gene must be to a region small enough to introduce into plants. The *egs1* gene will finally be identified by its ability to restore the expression of the silenced gene when introduced in the mutant.

Ohio State University
Columbus, OH 43210-1292

145. Control of Sugar Transport and Metabolism in *Zymomonas mobilis*

T. Conway, Department of Microbiology

\$100,000

Glucose transport in *Zymomonas mobilis* is via facilitated diffusion and the corresponding gene, *glf*, is co-transcribed with the genes encoding the first three intracellular steps of glucose metabolism. Regulation of the *glf-zwf-edd-glk* operon is intriguingly complex. Current work is designed to elucidate facilitator expression, function, and communication with the glycolytic enzymes. Fusions of the *glf* promoter to *lacZ* are being used to discern the architecture of this efficient promoter and have implicated a region that appears to be involved in increasing transcription in the presence of fructose. Kinetic studies of the facilitator prove that the *glf* gene product is indeed a facilitator and that it can function properly in recombinant *E. coli*. Experiments indicate that Glf does not interact with glucokinase to regulate sugar transport. The facilitator exhibits low affinity, high velocity transport properties, with an affinity for glucose that is orders of magnitude greater than for fructose. Catabolite choice at the level of solute exclusion is indicated.

Ohio State University
Columbus, OH 43210

146. The Role of Multiple Transcription Factors in Archaeal Gene Expression

C.J. Daniels, Department of Microbiology

\$99,000

Examination of the archaeal transcription system has indicated that these organisms, while procaryotic in nature, possess an eucaryal transcription apparatus. *In vivo* analysis of the *Haloferax volcanii* transcription system indicated that promoter and termination signals in this organism are similar to those used by the eucaryal RNA polymerase II system, and this organism was found to contain genes encoding eucaryal-like transcription factors, TBP and TFIIB. Unexpectedly, we observed that *H. volcanii* has three TBP and six TFIIB genes, an unprecedented redundancy in these proteins that has not been observed in other Archaea or Eucarya. Gene expression studies also indicated that one of these transcription factors, TFIIB2, is specifically induced during heat shock. This has led to the proposal that this organism uses alternative transcription factor pairing in regulating gene expression. Current studies are focused on determining the role of TFIIB2 in regulating the heat shock response. *In vivo* approaches are being employed to examine the sequences involved in the heat shock regulation of the TFIIB2 gene and to identify regions of this protein that are needed to specifically direct heat shock transcription. *In vitro* approaches will also be used to examine the interaction of this protein with the TBP-DNA complexes and other transcription factors and these studies will aid in the development of an *in vitro* transcription system for this organism. Long range goals include the analysis of the remaining transcription factors and their possible roles in differential gene expression.

Ohio State University
Columbus, OH 43210

147. Biosynthesis of Hydrocarbons and Other Wax Components

P.E. Kolattukudy, Departments of Biochemistry and Medical Biochemistry

\$98,000

Pea leaf particulate preparation obtained by a sucrose discontinuous gradient centrifugation was solubilized with 0.2% octyl glucoside and subjected to protein fractionation. The purified decarboxylase obtained by this procedure requires phosphatidyl choline for enzymatic activity. SDS-PAGE showed a subunit molecular weight of 67 kDa. Antibodies prepared against this protein showed a single immunologically cross-reacting protein at 67 kDa in solubilized particulate preparation. Anti-Rubisco antibodies did not cross-react with this protein showing that the 67 kDa protein is not derived from cross linking of the large and small subunits of Rubisco. Furthermore, the antibodies prepared against the purified decarboxylase did not cross-react with Rubisco but it inhibited the decarboxylase activity of the purified enzyme. Further characterization of the enzyme and cloning are in progress.

Ohio State University
Columbus, OH 43210

148. Transmethylation Reactions During Methylo trophic Methanogenesis in *Methanosarcina barkeri*

J.A. Krzycki, Department of Microbiology

\$96,000

We have been characterizing enzymes and genes involved in methanogenesis from methylo trophic substrates. These pathways are comprised of highly regulated enzymes and are significant routes of methane formation in natural environments. Methylated amines and thiols are converted to methane with methyl-coenzyme M (CoM) as an intermediate. We have reconstituted CoM methylation from growth substrates like trimethylamine (TMA), monomethylamine (MMA), dimethylsulfide (DMS), and methylmercaptopropionate (MMPA) with highly purified proteins. CoM methylation from methylo trophic substrates involves members of at least two different families of proteins. Each family member display specificity for one or more of the methylo trophic substrates. Generally, each substrate is used by a specific methyltransferase to methylate a substrate specific corrinoid binding protein, which is then demethylated by a substrate specific CoM methylase.

For example, MMA is converted to methyl-CoM via a 29 kDa corrinoid protein (MMCP) which acts only in this pathway. This is one of four known homologous corrinoid proteins involved in the metabolism of methylo trophic substrates which bind their cofactor with the corrinoid binding motif also found in methionine synthase. MMCP is methylated with MMA by a 52 kDa MMA specific methyltransferase (MMAMT). CoM methylation with methyl-MMCP is then catalyzed by a homologue of methylcobamide:CoM methyltransferase, termed MT2-A, which is specific for methylamine utilization. Similarly, TMA:CoM methyl transfer can be achieved with another corrinoid protein (TCP) which appears to be methylated by a TMA specific methyltransferase (TMAMT). Methyl-TCP can be demethylated by either of two MT2 homologues, MT2-A or MT2-M. Currently, we are isolating the enzymes mediating CoM methylation with dimethylamine (other than MT2-A), isolating the enzymes responsible for activation of MMCP, completing the sequencing of the gene clusters encoding the MMA and TMA methyltransferases, and studying the interaction of MMCP with its two methyltransferases when in the methylated and unmethylated states.

Methanogenesis from DMS or MMPA occurs via a single methylthiol:CoM methyltransferase. *M. barkeri* induces this enzyme during growth on acetate. This 480 kDa protein is composed of one corrinoid binding subunit (homologous to TCP, MMCP, and methionine synthase) tightly bound to larger subunit (homologous to MT2-A and MT2-M methylcobamide:CoM methyltransferases). Unlike the MMA and TMA dependent CoM methylation enzymes, methylthiol:CoM methyltransferase mediates its reaction solely with the corrinoid protein and MT2 homologue, and we are investigating how the corrinoid is methylated by substrate. In addition, we are characterizing the enzyme and gene from other species of methylated thiols utilizing *Methanosarcina* to ascertain if a single two subunit enzyme with similar substrate specificity is employed for growth on both substrates.

Ohio State University
Columbus, OH 43210

149. Structure and Regulation of Methane Genes

J.N. Reeve, Department of Microbiology

\$120,006

Methanobacterium thermoautotrophicum (Mt) generates energy by using hydrogen to reduce carbon dioxide to methane, and assimilates these same gaseous substrates plus nitrogen or ammonium ions and inorganic salts into biomass. The goal of this project is to understand how these catabolic and anabolic pathways are coordinated and regulated. Many steps in methanogenesis are catalyzed by two or more functionally-equivalent enzymes or isoenzymes, and hydrogen availability, the source of energy for Mt, regulates which of these alternative enzymes are synthesized. The molecular mechanisms by which Mt cells sense the concentration of dissolved hydrogen, and communicate this information into gene regulation, are currently under investigation. The rate of hydrogen dissolution in a fermentor sparged with hydrogen plus carbon dioxide depends on the mixing rate. Changing the mixing rate therefore results in methane gene activation and inactivation, and provides a very simple procedure that is being used to manipulate methane gene regulation *in vivo*. As the sequence of the entire Mt genome is now known, hybridization probes are immediately available and are being used to identify and quantitate all methane gene transcripts, with two dimensional gel electrophoresis used to identify and quantitate their encoded polypeptides. In parallel studies, purified Mt RNA polymerase, with recombinant Mt TATA-binding protein and TFIIIB are being used to reconstitute and investigate *in vitro* the molecular basis of hydrogen-dependent methane gene transcription.

Ohio State University
Columbus, OH 43210

150. Photosynthetic Electron Transport in Genetically Altered Chloroplasts

R.T. Sayre, Departments of Biochemistry and Plant Biology

\$106,999

We have developed structural models of the photosystem II reaction center D1 and D2 polypeptides to guide us in characterizing protein/chromophore interactions which regulate energy transfer processes in photosystem II. Our experimental strategy is to use these models to guide us in selecting D1 and D2 polypeptide amino residues for site directed mutagenesis followed by characterization of the mutant phenotypes. We propose to identify and characterize residues which: 1) are involved in binding and orientation of chromophores, and 2) participate in or regulate charge transfer processes in a chloroplastic photosystem II complex. Recently, we have characterized D1 and D2 polypeptide residues which are presumably involved in the coordination of accessory chlorophylls and the chlorophyll special pair. Additional mutations are proposed to characterize the binding sites for pheophytin and to further characterize the effects of the protein environment on the primary photochemical events. We will also extend our diagnostic capabilities through the use of resonance Raman studies of specific chromophores in isolated *Chlamydomonas* photosystem II reaction center particles. In addition, we will probe the fine structure of the D1 and D2 proteins through chemical modification of cysteine residues with fluorescent and spin-labeled probes which will be used to determine intra- and inter-molecular distance relationships.

Ohio State University
Columbus, OH 43210-1292

151. Regulation of Alternative CO₂ Fixation Pathways and Control of Redox Status of Photosynthetic Microorganisms

F.R. Tabita, Department of Microbiology

\$104,000

In addition to serving as a primary source of carbon, CO₂ is used by photosynthetic microorganisms as an important electron sink for the dissipation of excess reducing power. Recent studies in our laboratory have shown that molecular controls for the expression of several processes for redox balancing are tightly coupled in nonsulfur purple bacteria. This control is particularly manifest when the major CO₂ assimilatory route (Calvin-Benson-Bassham or CBB pathway) is abolished after inactivating structural genes that encode essential enzymes of the CBB path. Thus, when grown in the presence of organic carbon, these CBB negative organisms must employ other electron sinks to balance their redox poise. We have found that one way these organisms cope is to derepress nitrogenase and the *nifHDK* genes under these growth conditions. This allows the cells to reduce protons to molecular hydrogen, despite the presence of ammonia in the growth media. Derepression of *nif* in *Rhodobacter sphaeroides* and growth on molecular nitrogen is controlled by a global two-component signal transduction system which also regulates expression of the *cbb* system in this organism. Current studies are directed at elucidating molecular links that exist between the *cbb* and *nif* control circuits in *R. sphaeroides* and related organisms. Our studies also encompass molecular-based studies on an alternative CO₂ fixation route, the reductive tricarboxylic acid (or RTCA) cycle. For these studies, a genetically tractable and fast-growing moderate thermophile, the green sulfur photosynthetic bacterium *Chlorobium tepidum*, has been employed. The RTCA pathway is a route also employed by many extremophiles that use CO₂ as their sole source of carbon. *C. tepidum* thus appears to be an excellent model system for studies of this major CO₂ assimilatory route. All of the key enzymes have now been purified to homogeneity, including ATP-citrate lyase, pyruvate synthase, α -ketoglutarate synthase, phosphoenolpyruvate carboxylase, as well as several redox carriers. The two synthase enzymes are iron-sulfur proteins that require a specific reduced ferredoxin isolated from *C. tepidum* and other necessary cofactors for activity. The purified proteins are also being employed to isolate the structural genes so that we may determine how the RTCA pathway is regulated at the molecular level. Isolated structural genes will also be employed to prepare recombinant proteins to better relate their structure to specific catalytic events.

Ohio State University
Columbus, OH 43210

152. Mechanisms of Microbial Adaptation

F.R. Tabita, Department of Microbiology

\$48,420 (two years)

Graduate programs specializing in microbial biology have declined in recent years, greatly reducing the opportunities for advanced training in this important research area. As one mechanism to rebuild interest in microbial physiology and to train new scientists in the area, we have developed a four week summer course focusing on the theme "Mechanisms of Microbial Adaptation". In 1997 the course contained both lecture and laboratory components targeted to senior level graduate

students and industrial scientists who desire an in-depth introduction to research in the area of microbial physiology. Current topics on the mechanisms of microbial adaptation were presented in a 20 lecture seminar series given by the course faculty and 12 distinguished invited guest lecturers. Topics included: adaptive response of microorganisms in fermentations, global response mechanisms, regulation of microbial autotrophism, carbon metabolism in methanogens, and biodegradation and bioconversion by microorganisms. The accompanying laboratory exercises illustrated current practical problems in microbial physiology and introduced the students to modern experimental techniques and conceptual approaches. These experiments provided the students with experience in fermentation technology, characterization and quantitation of proteins and metabolic intermediates, enzyme activity measurements and basic kinetic analyses, genetic approaches for the identification of regulated genes, and the anaerobic techniques needed to purify oxygen-sensitive proteins. A second inherent goal of the course was to establish a significant interactive community of research scientists in the field. Informal presentations by the guest lecturers were used to stimulate discussions between students and faculty about both research problems and career development. A detailed description of the course is available at our web site: <http://www.biosci.ohio-state.edu/~microbio/summer/course.html>

Oklahoma State University
Stillwater, OK 74078-3035

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

A.J. Mort, Department of Biochemistry and Molecular Biology

\$100,695

In work over the last eleven years on pectins from cotton suspension culture cell walls we have concluded that pectins are based on only two backbone structures. One is a (1-4) linked galacturonan and the other is a repeated disaccharide of galacturonic acid and rhamnose (rhamnogalacturonan).

The homogalacturonan based pectin regions fall into three common types. 1) True homogalacturonans with varying degrees of methyl esterification, 2) Rhamnogalacturonan II, a homogalacturonan section with complex sidechains clustered such that it can be isolated as an ~ 5,000 Dalton fragment after endopolygalacturonase digestion of cell walls, and 3) xylogalacturonan, a homogalacturonan backbone with frequent single xylose substituents linked to the galacturonic acid residues.

The goal of the project is to learn more about the structure, and if possible, function of the various pectic regions and to determine which regions are covalently attached to each other. We also want to determine how they are linked to each other by isolating and characterizing junction zones between them.

To help achieve these goals we are developing highly sensitive methods for carbohydrate analysis and sequencing using capillary electrophoresis, enzyme and chemical digestions, and spectroscopic methods.

In addition, we are comparing the pectins of cotton cotyledons to those of the tissue cultures in case culturing induces major modifications in the structures.

University of Oklahoma
Norman, OK 73019-0245

154. Energetics and Kinetics of Syntrophic Aromatic Degradation

M.J. McInerney, Department of Botany and Microbiology

\$92,000

The anaerobic bacterium "*Syntrophus acidotrophicus*" strain SB in coculture with the methanogen, *Methanospirillum hungatei*, degraded benzoate to a minimum threshold concentration at which no further benzoate degradation occurred, even with extended incubation. The addition of sodium acetate but not sodium chloride affected the threshold value; increased acetate concentrations resulted in increased benzoate threshold concentrations. Cocultures that initially contained 2.5 mM benzoate with either 0 or 10 mM acetate degraded benzoate to very low threshold concentrations, less than 500 nM. In contrast, cocultures with 30 or 60 mM acetate added, degraded benzoate to threshold concentrations of 4.4 μ M and 1.5 mM, respectively. The final partial pressure of hydrogen in the methanogenic cocultures ranged from 1.5 to 3.0 Pa. At threshold, the Gibb's free energy change under physiological conditions was still favorable, ranging from -14 to -21 kJ per mole. These data show that substrate degradation thresholds do occur under methanogenic conditions and that the threshold values can be substantial under conditions where hydrogen is low if acetate is allowed to accumulate to high concentrations. Similar free energy values obtained under sulfate-reducing and methanogenic conditions when the threshold is reached support the hypothesis that substrate thresholds are thermodynamically controlled and that substrate degradation may be precluded even though the free energy change is still favorable.

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

155. Biochemical Genetics of Lignin Degradation by *Phanerochaete chrysosporium*

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

\$134,000

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 and Mn peroxidases (LiP and MnP) via spectroscopic, kinetic, and bioorganic methods and in collaboration by crystallography. Using our homologous expression system that produces recombinant MnP in *P. chrysosporium*, we have isolated and characterized by kinetic, spectroscopic, and crystallographic methods several mutants of the Mn binding site of MnP. We also have characterized mutants of Phe 190, an amino acid residue in the heme cavity of MnP, which stabilizes the protein. A similar expression system is being developed for LiP. Finally, we are studying the enzymes and genes involved in lignin degradation by the white rot fungus *Dichomitus squalens*.

We are also characterizing an intracellular quinone reductase which is involved in the further metabolism of monomeric quinones produced as lignin degradation products. We have isolated and sequenced cDNA and genomic clones encoding this enzyme from *P. chrysosporium*, and a

variety of studies, including homologous expression, are being carried out to determine the enzyme's mechanism and its role in lignin degradation.

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

- 156. Cellobiose Dehydrogenase and β -glucosidase from *Phanerochaete chrysosporium*: Effect on Cellulose Hydrolysis, Cloning, and Characterization**
V. Renganathan, Department of Biochemistry and Molecular Biology \$90,000

Cellobiose dehydrogenase (CDH) is an extracellular hemoflavoenzyme produced by cellulose-degrading cultures of fungi such as *Phanerochaete chrysosporium*, *Sporotrichum thermophile*, *Trametes versicolor*, and *Coniophora puteana*. In this study, we plan to purify and characterize the thermostable CDH from *Sporotrichum thermophile*. We will also study the interaction of CDH and β -glucosidase from *P. chrysosporium* with cellulose using electron microscopy.

Oregon State University Corvallis, OR 97331-2902

- 157. Characterization of the Genes Involved in Nitrification**
D.J. Arp and L.A. Sayavedra-Soto, Botany and Plant Pathology \$88,000

Nitrification is a bacterial process in which ammonia is oxidized to nitrate. In croplands fertilized with ammonia or urea-based compounds, oxidation of ammonia by these bacteria leads to a loss of available N. Our research is directed at characterizing the genes involved in nitrification with the intention of providing a basis for supporting development of biotechnology aimed at controlling nitrification. The genes in *Nitrosomonas europaea* which code for ammonia monooxygenase (AMO, which catalyzes the oxidation of ammonia to hydroxylamine) and hydroxylamine oxidoreductase (HAO, which catalyzes the oxidation of hydroxylamine to nitrite) were cloned and sequenced. The genes for AMO (*amoA* and *amoB*) are each present in two copies. The gene for HAO (*hao*) is present in three copies. We recently developed a protocol for the transformation of *N. europaea* based on electroporation and homologous recombination of introduced DNA. These observations and developments provide the basis for our proposed experiments. The following specific objectives are proposed: 1) Complete the physical mapping and characterization of the multiple copies of the genes coding for ammonia monooxygenase (*amoA* and *amoB*) and hydroxylamine oxidoreductase (*hao*). 2) Determine which individual copies of *amoA*, *amoB*, and *hao* are dispensable, which are functional and which are expressed. 3) Determine if the individual copies of *amo* and *hao* are expressed differentially under various physiological conditions. 4) Identify genes near *amo* and *hao* and determine the function of these genes. These studies are expected to provide a better understanding of the genetic basis for ammonia oxidation to nitrite.

University of Oregon
Eugene, OR 97403

158. Genetic Analysis of Chloroplast Translation

A. Barkan, Institute of Molecular Biology

\$97,000

The accumulation of components of the photosynthetic apparatus in the appropriate ratios and within the correct cell types requires the regulated expression of chloroplast genes. This project concerns the control of chloroplast protein synthesis by nuclear-encoded factors. The project is focused on *crp1*, a nuclear gene in maize, mutations in which disrupt the translation of the *petA* mRNA and the metabolism of *petB* and *petD* mRNAs. Thus, the *crp1* gene activates the expression of a small set of chloroplast genes. We recently cloned the *crp1* gene by taking advantage of the *Mu* transposon tag. The sequence of a full length cDNA clone revealed no significant homologies with any proteins of known function. The cDNA was used to generate recombinant protein and Crp1p antisera. Immunolocalization studies revealed that Crp1p is localized in the chloroplast stroma and is not associated with envelope or thylakoid membranes. The role of *crp1* in activating translation will be investigated by defining the step at which translation is blocked in mutant chloroplasts, by determining whether Crp1p is a component of an RNA binding activity, and by assessing the activities of purified Crp1p in binding RNA, processing RNA, and in activating translation *in vitro*. Towards this last end, we have established methods for the preparation of pea and maize chloroplast extracts that are active in translation. Understanding the mechanism by which *crp1* activates translation will further our understanding of chloroplast translation in general. A related project concerns the role of endonucleolytic processing of polycistronic RNA precursors in chloroplasts. In *crp1* mutants, the absence of monocistronic *petD* mRNA correlates with a loss of *petD* translation, suggesting that *petD* is inefficiently translated in a polycistronic context. Preliminary results of enzymatic RNA structure analyses suggest that the *petD* start codon is sequestered in secondary structures when it is in the polycistronic context but not when it is in the monocistronic context. Experiments in progress will further explore the role of endonucleolytic RNA processing in controlling the translation of chloroplast mRNAs. Finally, a screen is in progress for new *Mu*-induced mutants with defects in the translation of chloroplast mRNAs.

Pennsylvania State University
University Park, PA 16802

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

J.E. Brenchley, Department of Biochemistry and Molecular Biology

\$103,384

Our objective is to characterize psychrophilic microorganisms and to discover, purify, and study novel cold-active glycosidases. Cold-active glycosidases with high activities at low temperatures can be used for low temperature conversions of saccharides in plant biomass, whey, etc. into fuels or fermentation media. Comparisons of cold-active enzymes with their higher temperature counterparts will yield insight into structural features involved in thermal stability. Our goals are to complete the characterization of three β -galactosidase isozymes cloned from one *Arthrobacter* strain, to isolate new psychrophiles producing cold-active glycosidases with even lower temperature optima, to clone and sequence genes for selected enzymes, and to purify cold-active glycosidases

for biochemical studies and comparisons. We have completed the analysis of the three *Arthrobacter* genes. One encodes a lacZ family subunit, except the enzyme has an optimum about 20°C below that of the *Escherichia coli* enzyme. The second gene helped define a new class, which we designated lacG. The third gene encodes a small 52 kDa subunit with homology to the lysosomal acid β -galactosidases found in human and mouse. In addition, we have sequenced a new lacZ-like gene from an Antarctic isolate that encodes a β -galactosidase with an optimum between 10 and 20°C. The significant similarity of this gene (67%) to the one we examined earlier will permit a careful examination of the amino acid changes that might contribute to the lower temperature optimum of this new enzyme.

Pennsylvania State University
University Park, PA 16802-4500

160. Light-Energy Transduction in Green Sulfur Bacteria

D.A. Bryant, Department of Biochemistry and Molecular Biology

\$90,000

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. Secondary goals are to analyze their transcriptional machinery, to develop methods for genetic analyses, and to understand the relationship of green sulfur bacteria to other eubacteria. Genes encoding eight (of ten) *Chlorobium tepidum* chlorosome proteins have been cloned, sequenced, and characterized. These eight proteins have been overproduced, purified, and used to raise polyclonal rabbit antisera. CsmA, CsmB, CsmC, CsmD, and CsmE have been shown to be surface-exposed components of the chlorosome envelope. Experiments to demonstrate the location(s) of CsmH, CsmI, and CsmJ are in progress. The latter two proteins are similar in their N-terminal domains, and each protein contains a cysteine sequence motif suggesting each will contain a [2Fe-2S] cluster. EPR measurements on CsmI after *in vitro* reconstitution has confirmed the presence of the anticipated Fe-S cluster. EPR studies of chlorosomes isolated under anaerobic conditions are in progress. Finally, we have recently characterized the genes encoding sigma factors of RNA polymerase in *Chlorobium tepidum* and *Chloroflexus aurantiacus*. Like cyanobacteria, *C. aurantiacus* produces multiple Group 1 and 2 sigma factors. The SigC protein is expressed at similar levels in cells grown photoheterotrophically under anaerobic conditions or heterotrophically under aerobic conditions. Interestingly, the SigA protein is expressed at a significantly higher level in cells grown under aerobic conditions than under anaerobic conditions.

Pennsylvania State University
University Park, PA 16802

161. Biophysical and Molecular Mechanisms Controlling Plant Cell Growth

D.J. Cosgrove, Department of Biology

\$105,000

Plant cell enlargement is controlled by wall stress relaxation. This process depends on cell wall structure and the combined activity of wall "loosening" and "rigidifying" proteins. We are using biophysical and molecular approaches to elucidate the nature of wall relaxation and its control of cell enlargement. (A) We have developed a biophysical model of cell wall expansion based on the

thermodynamics of hydrogen-bonded polymer networks. Many of the actual properties of expanding walls are described by this model, which predicts that the ratio of cellulose to hemicellulose is a key structural feature that determines wall yield threshold. This prediction will be tested by in-vitro assays of wall extension. (B) The nature of wall rigidification is being examined in several ways. Specific bonds in the wall will be broken or formed by use of chemical and enzymatic agents, and wall extensibility then assayed. The hypothesis that pectin methyl esterase is a significant wall rigidifying enzyme is being tested by a combination of biochemical and molecular approaches. We are also attempting to identify endogenous wall rigidifying activities in protein preparation made from cell walls. (C) The dynamics of cell enlargement is being explored by use of a novel pressure chamber to modulate turgor and record the resulting growth responses. (D) Wall extension properties are being analyzed in brassinolide-deficient mutants (*Arabidopsis*, pea), with the intention of uncovering the mechanism by which this steroid-like hormone modulates plant form. These studies will deepen our understanding of plant cell wall expansion and its control of plant growth.

Pennsylvania State University
University Park, PA 16802

162. Elongation Factor 1-a and the Plant Cytoskeleton
R. Cyr, Department of Biology

\$94,000

The cytoskeleton, and in particular its microtubule (Mt) component, participates in several processes that directly affect growth and development in higher plants. Normal cytoskeletal function requires the precise and orderly arrangement of Mts into several cell cycle and developmentally specific arrays. One of these, the cortical array, is notable for its role in somehow directing the deposition of cellulose, the most prominent polymer in the biosphere. 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-a behaves as a Mt associated protein serving to both stabilize, and bundle, microtubules in vitro. Both of these effects are modulated in vitro by Ca⁺⁺/calmodulin which unbundles and destabilize Mts. Our working hypothesis is that similar events occur in vivo and we are currently undertaking a structure/function approach to identify the regions on this MAP that are involved in these activities.

Pennsylvania State University
University Park, PA 16802-4500

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

J.G. Ferry, Department of Biochemistry and Molecular Biology \$105,000

Several enzymes 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 are under investigation utilizing site-directed mutagenesis to identify active site residues and mass spectrometry to identify covalent intermediates. Efforts are underway to determine the crystal structures of both enzymes. Crystals of acetate kinase have been obtained in collaboration with Miriam Hasson which diffract to beyond 1.7 angstrom resolution. Procedures are being developed for the overproduction of subunits of the CO dehydrogenase/acetyl-CoA synthase (CODH/ACS) complex in *Escherichia coli* and *M. thermophila*. Methods are also being developed for overproduction of a protein suspected to be involved in maturation of subunits of the CODH/ACS complex. Electron paramagnetic resonance and Mossbauer spectroscopies, in collaboration with Drs. Steve Ragsdale and Kristene Surerus, have been used to characterize the FMN and iron-sulfur centers of a novel iron-sulfur flavoprotein (Isf) discovered in *M. thermophila*. The results support a role for Isf in donating electrons to cytochrome b in the membrane-bound electron transport chain.

Pennsylvania State University
University Park, PA 16802

164. Molecular-Genetic Analysis Of Maize Starch Branching Enzyme Isoforms

M. Guiltinan, Department of Horticulture \$91,999

Starch is an important energy storage molecule used by all plants. Its synthesis involves the creation of branch points by a class of enzymes known as starch branching enzymes (SBE, EC 2.4.1.18). Biochemical studies have shown that three isoforms of SBE exist in maize endosperm, SBEI, IIa and IIb.

To fully understand the role of the SBE isoforms in starch biosynthesis, a comprehensive molecular-genetic dissection of the SBE gene family is underway. We are in the process of isolating cDNA and genomic DNA clones for all of the maize SBE family members and we will use these sequences to study the expression patterns and regulatory elements of each *sbe* gene. We will also identify maize lines with mutations in each *sbe* gene. To reach these Objectives, we are using a combination of conventional molecular techniques to isolate and study the genes, together with a reverse genetics transposon based approach to identify insertional mutants. Together, these lines of research will advance our knowledge of the importance of the SBEs in starch biosynthesis and in the life cycle of the plant. Knowledge of the regulatory elements which control *sbe* gene expression may be useful in engineering maize endosperm for the production of novel carbohydrate storage products for industrial feedstocks. The knowledge gained and the tangible products of this

research (genes, cDNAs and novel germplasm) may also be useful in the production of starches with altered structure for use in industry.

Pennsylvania State University
University Park, PA 16802

- 165. Characterization of Lignin and Mn Peroxidases from *Phanerochaete chrysosporium***
M. Tien, Department of Biochemistry and Molecular Biology \$112,000

The initial steps of lignin biodegradation by white-rot fungi are catalyzed by the lignin and Mn peroxidases. These enzymes were first discovered in the fungus *Phanerochaete chrysosporium* and have now been isolated from a large number of lignin-degrading fungi. Our research has been aimed at elucidating the role of these enzymes in lignin degradation. The enzymology of this process is complicated. The lignin and Mn peroxidases are isozyme families, expressed differentially in response to environmental conditions. These peroxidases are unique in their ability to oxidize substrates which other peroxidases cannot oxidize. The mechanism by which these enzymes act on lignin is not well understood; redox mediators have been proposed to be involved in oxidation of lignin by these enzymes. We have performed site-directed mutagenesis of these enzymes to study the nature of their substrate binding site and the basis of their reactivity. We have also developed a transformation system which is presently being used to generate antisense mutants. These studies are attempting to determine the role of these enzymes in lignin degradation.

University of Pennsylvania
Philadelphia, PA 19104-6018

- 166. Structural Basis of Signal and Energy Transduction in Plants**
A.R. Cashmore, Plant Science Institute, Department of Biology \$260,000

The aim of this program is to train students in both molecular genetic and structural studies in plant science. Students are trained in graduate programs in Biochemistry, Chemistry, and Biology. These students pursue research in areas ranging from structural studies of plant enzymes involved in terpene biosynthesis to molecular genetic studies of signalling by the plant hormone ethylene. Each year a retreat is held involving both local researchers and one or more internationally renowned scientists who address the students on molecular genetic or structural studies in plant science. By this mechanism students are being trained with an appreciation of both molecular genetic as well as chemical, biochemical, and biophysical approaches to research in plant science.

University of Pennsylvania
Philadelphia, PA 19104-6018

167. Light Responses and Photoperiodism in *Arabidopsis thaliana*

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

\$129,000

We have characterized several classes of mutants of *Arabidopsis thaliana* that show alterations in their responses to light. The *pef* mutants (*phytochrome/early flowering*) flower early under white light and exhibit elongated hypocotyls when grown under red or far-red light. Because of their selective insensitivity to red/far-red light, these mutants are believed to correspond to mutations in the phytochrome signalling system. A second class of mutants are the *rsp* mutants (*reduced sensitivity to photoperiod*): these mutants exhibit an alteration in their timing mechanism such that they flower earlier than wild-type plants under certain short-day photoperiods. Furthermore, the *rsp* mutants show an alteration in the periodicity of the free-running rhythm for *CAB* gene expression. This alteration in the timing of two distinct physiological parameters suggest that the *rsp* mutants may correspond to lesions in a central component of the circadian clock. Another class of mutants we have characterized are the *elr* mutants (*enhanced light response*), which exhibit unusually short hypocotyls when grown under either red or blue light. As the *elr* mutants show no discernible phenotype when grown in the dark, these mutants (like the *pef* mutants) appear to correspond to lesions in a light-signalling pathway.

University of Pennsylvania
Philadelphia, PA 19104-6018

168. Membrane-attached Electron Carriers in Photosynthesis and Respiration

F. Daldal, Department of Biology

\$114,000

Our overall aim is the molecular characterization of the structure, function and biogenesis of membrane-associated cytochromes (cyt) that act as electron carriers in photosynthesis (Ps) and respiration (Res). We use the facultative photosynthetic bacteria of *Rhodobacter* species as a model system for these studies. The presence of a dual electron transfer (ET) pathway between the cyt *bc₁* complex and the reaction center (RC) in Ps, and the cyt *c* oxidase in Res, was first revealed by our Ps- and Res-proficient mutants of *R. capsulatus* devoid of the soluble cyt *c₂*. We have then demonstrated that the novel membrane-associated cyt *c_y* is the molecular basis of this entirely membrane-confined ET pathway that functions both in Ps and Res growth conditions. Using an epitope-tagged and functional derivative of cyt *c_y* we purified it for the first time, determined its physicochemical properties and its mode of attachment to the membrane. These studies established that cyt *c_y* is composed of an "anchor," a "linker" and a "cyt *c*" domains, and demonstrated that its "anchor-linker" domains can also attach cyt *c₂* to the membrane in a functional form. Our very recent measurements indicate that kinetic characteristics of the cyts *c₂* and *c_y* are very similar, except that the latter is not diffusion-limited during multiple turnovers. This finding strongly supports the presence of supercomplexes formed of the RC, the *bc₁* complex and cyt *c_y*. Unexpectedly, an homologue of cyt *c_y* was also found in *R. sphaeroides*, its nucleotide sequence was completed, a mutant lacking it was obtained, and its role in electron transport is under study. Current work is now focused on defining the molecular differences between the cyt *c_y* molecules of these two closely related *Rhodobacter* species which behave very differently in

respect to their ability to use membrane-attached electron carrier cytochromes in photosynthesis and respiration.

University of Pennsylvania
Philadelphia, PA 19104-6018

169. Molecular and Genetic Analysis of Hormone-Regulated Differential Cell Elongation in Arabidopsis

J.R. Ecker, Department of Biology

\$213,000 (FY96 funds/2 years)

The aim of this project is to understand how plant hormones act coordinately to regulate the elongation of cells. Ethylene and auxin are known to modulate cell elongation in the hypocotyl and apical hook in etiolated seedlings. The hook is formed by asymmetric elongation of cells on opposite sides of the hypocotyl. For genetic studies, the apical hook provides an excellent model system to identify genes required for hormone-regulated growth since this structure is dispensable for plant viability. Mutants in Arabidopsis that exhibit altered patterns of differential growth in the hypocotyl have now been identified and include those lacking an apical hook or showing constitutive apical hook curvature. One of these genes, HOOKLESS1 (HLS1) a putative N-acetyltransferase, is required for apical hook formation and for normal expression of auxin response genes in the hypocotyl and apical hook. Interestingly, HLS1 transcription is regulated by ethylene, thus providing a link between the ethylene and auxin response pathways. This novel protein may regulate cellular responses to auxin by one of several mechanisms, such as by alteration of gene transcription, or a change in hormone sensitivity or transport. In order to further explore this connection, we are now identifying transcription factors that interact with the promoter of HLS1. Through the identification of additional mutants defective in differential growth and by characterization of their corresponding gene products, we hope to gain new insight into this little understood process. It is expected that the general mechanisms found to be important for controlling cell growth in this model system will also be utilized in other plants.

University of Pennsylvania
Philadelphia, PA 19104-6018

170. Biochemical Basis of YCF1-Dependent Vacuolar Glutathione-S-Conjugate Transport

P.A. Rea, Department of Biology

\$98,000

The *Saccharomyces cerevisiae* YCF1 (yeast cadmium factor) gene was originally isolated according to its ability to confer cadmium resistance. YCF1 encodes a 1,515 amino acid, cystic fibrosis transport regulator- (CFTR-) like ATP binding cassette (ABC) transporter which is closely related to the human multidrug resistance-associated protein (MRP1 or GS-X pump) implicated in the resistance of a number of cancer cell lines to chemotherapeutic drugs. Recent experiments by our group demonstrate that YCF1 is both a functional and structural homolog of the mammalian GS-X pump. YCF1 participates in the MgATP-energized vacuolar uptake of bis(glutathionato)cadmium (Cd.GS₂) and a wide range of organic toxins after their S-conjugation with glutathione (GSH).

This program of research is concerned with several fundamental aspects of the mechanism of YCF1: (i) Its mode of interaction with GS-conjugates. We have shown that YCF1 is necessary for MgATP-energized vacuolar GS-conjugate transport but we have not yet determined if it directly interacts with these compounds. (ii) Its sufficiency for transport. Although the inferred structure of YCF1 is consistent with its direct participation in MgATP-energized GS-conjugate transport, it is not known if YCF1, alone, is sufficient. (iii) Its facility for transporting metal ions other than Cd²⁺ after their complexation with GSH. While the wild type *YCF1* gene is required for Cd²⁺ resistance, nothing is known of its involvement in the vacuolar sequestration of other GSH-complexable metal ions. (iv) Its regulation by phosphorylation. YCF1 is unusual in possessing a CFTR-like regulatory domain containing dibasic PKA phosphorylation motifs whose modification may modulate activity. (v) The necessity of the N-terminal extension unique to it and other members of this subclass of ABC transporters for substrate recognition and/or transport.

In view of the involvement of YCF1 in both heavy metal and organic toxin transport and its equivalence to MRP1 and the vacuolar GS-X pumps of plants, these studies are likely to provide fresh insights into the membrane transport phenomena associated with mammalian tumor biology and plant herbicide resistance, in particular, and heavy metal tolerance and bioremediation, in general.

Purdue University
West Lafayette, IN 47907

171. Molecular Studies of β -D-Glucan Synthesis in Cereals

N.C. Carpita, Department of Botany and Plant Pathology

\$97,000

We continue our search in rice for genes that encode the Golgi-localized synthases of the (1A_E3),(1A_E4)- β -D-glucan (β -D-glucan), a cell-wall polysaccharide unique to the cereal grasses. β -D-Glucan is synthesized transiently during the expansion phase of cell growth and is hydrolyzed during cell differentiation once cells have ceased growing. The glucan is synthesized again in the endosperm walls of the grain. The β -D-glucan constitutes a majority of the cell wall mass of the endosperm, whereas cellulose content is very low. Based on several enzymic properties which are shared by the synthases of β -D-glucan *in vitro* in isolated Golgi apparatus and callose at the plasma membrane (Gibeaut, D.M., and N.C. Carpita, 1993, Proc.Natl. Acad. Sci USA 90, 3850-3854), we proposed that the genes that encode the cereal β -D-glucan synthases are derived from ancestral cellulose synthase genes. We have sequenced five rice cDNAs with high homology to cotton and Arabidopsis *CelA* genes, each of which likely encode the cellulose synthase catalytic subunit. These rice cDNAs fall into three groups, and we are using a PCR-based approach to identify members of these three and other *CelA*-like genes from a genomic library of rice. Each of the cDNAs contains a hypervariable region between regions of high conservation that are predicted to encode highly conserved UDP-Glc binding domains of the deduced polypeptides. These regions are suitable for construction of gene specific probes, and experiments are designed to differentiate between cellulose synthase and β -D-glucan synthase by differential expression in seedling and endosperm. Further proof will come from the immunolocalization of unique peptides of the β -D-glucan and cellulose synthases at the Golgi or plasma membrane, respectively.

Purdue University
West Lafayette, IN 47907-1153

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

C.C.S. Chapple, Department of Biochemistry

\$190,000

Lignin is a phenolic polymer that imparts strength and decay resistance to the plant secondary cell wall. In flowering plants, lignin is composed mainly of two aromatic monomers which differ in their methoxyl substitution pattern. These monomers are derived from ferulic acid and sinapic acid and give rise to guaiacyl- and syringyl-substituted lignin monomers, respectively. The balance between guaiacyl and syringyl units in lignin varies among plant species, within a given plant, and even within the wall of a single plant cell.

Two cytochrome P450-dependent monooxygenases are required for the synthesis of lignin monomers, cinnamate-4-hydroxylase (C4H) and ferulate-5-hydroxylase (F5H). Ectopic overexpression of F5H in *Arabidopsis* under the control of the C4H promoter abolishes tissue-specific lignin monomer deposition and generates a novel lignin that is almost entirely comprised of syringyl units. These data demonstrate that the composition of the lignin polymer is dictated by the temporal and tissue-specific expression pattern of F5H. We have further shown that the CaMV 35S promoter fails to promote F5H gene expression in cells undergoing or providing precursors for lignification. The promoter of the C4H gene used in this study is far more efficient in this regard. Finally, we have shown that it is possible to genetically engineer plants to accumulate lignin that is highly enriched in syringyl residues. Thus, it seems possible to increase the syringyl content of crop species and trees, thereby generating lignins that are easier to digest or extract without detrimental consequences on agricultural performance.

Purdue University
West Lafayette, IN 47907

173. A Genetic Analysis of the Lumenal Proteins of the Photosystem II O₂-evolving Complex in Cyanobacteria

L.A. Sherman, Department of Biological Sciences

\$98,000

The primary objective of this project will be to determine the proteins in the cyanobacterium *Synechocystis* sp. PCC6803 that interact with the Mn-stabilizing protein (MSP) and help form the lumenal component of the PSII O₂-evolving complex. We have cloned the *psbU* gene (encoding the 12 kDa protein) and the *psbV* (encoding cyt C550) and have produced insertional mutations. We have also produced the double mutants $\Delta psbO \Delta psbU$, $\Delta psbO \Delta psbV$ and we will analyze the characteristics of all of these mutations. We will be particularly interested in O₂-flash yields and fluorescence properties, as well as the growth characteristics of these mutants. We have also utilized a digital imaging spectrometer (DIS) to isolate a series of randomly-induced mutants of *psbO*. The DIS permits many colonies to be analyzed for fluorescence and we have utilized the high sensitivity of this instrument to obtain some interesting mutations. We will continue this analysis throughout the course of the year to determine which residues provide particularly

interesting lesions in MSP. One goal is to determine the domains within MSP that give rise to specific categories of mutations.

We are also analyzing the characteristics of PSII in the unicellular, diazotrophic cyanobacterium *Cyanothece* sp. ATCC 51142. This strain demonstrates great heterogeneity in PSII organization throughout the N₂-fixing cycle, which we interpret as a means of downregulation of PSII. We will concentrate on factors which affect the oxidizing side of the photosystem, including the status of the luminal proteins and factors which lead to the degradation of PSII.

Rice University
Houston TX 77005

174. Genetic and Biochemical Analysis of Solvent Formation in *Clostridium acetobutylicum*

G.N. Bennett and F.B. Rudolph, Department of Biochemistry and Cell Biology

\$135,000 (FY95 funds/30 months)

The anaerobic organism *Clostridium acetobutylicum* has been used for commercial production of important organic solvents due to its ability to convert a wide variety of crude substrates to acids and alcohols. The objectives are to improve our knowledge of the molecular genetics and enzymology of Clostridia in order to make genetic alterations which more effectively channel cell metabolism toward production of desired products. Two factors that limit butanol production in continuous cultures are: 1) The degeneration of the culture, with an increase in the proportion of cells which are incapable of solvent production. Isolated degenerate strains are being evaluated to analyze the molecular mechanism of degeneration. Recent studies show two general types of degenerates, one which has lost essential solvent pathway genes and a less complete type which has not lost all solvent production capability and retains the DNA bearing solvent pathway genes. 2) The production of hydrogen which uses up reducing equivalents in the cell. If the reducing power could be more fully directed to the reduction reactions involved in butanol production, the process would be more efficient. We have studied oxidation reduction systems related to this process. We have also analyzed some genes related to ATP production and membrane energetics.

Rice University
Houston, TX 77251

175. A Molecular-Genetic Approach to Studying Source-Sink Interactions in *Arabidopsis thaliana*

S.I. Gibson, Department of Biochemistry and Cell Biology

\$99,000

Sources (such as leaves) and sinks (such as roots and seeds) of carbohydrate interact in complex ways that play an important role in plant development and physiology as well as in determining crop yields. The mechanisms by which sources and sinks of carbohydrate interact are poorly understood, but are likely to involve the regulation of key genes by soluble sugar levels. Our research is focused on determining the role of sugar-regulated gene expression in plant development and on elucidating the molecular mechanisms by which this type of gene regulation

occurs in plants. Towards this end, mutants that are defective in sugar-regulated gene expression have been identified in the model plant *Arabidopsis thaliana* and are currently being characterized. This work should lead to a better understanding of source-sink interactions, which is a pre-requisite to developing more rational approaches to improving crop yields.

The final aspect of our work is focused on characterizing fumaric acid accumulation in a wide variety of plant species. Our interest in this area stems from our discovery that fumaric acid accumulates to high levels in a number of plant species, including model plants like *Arabidopsis thaliana* and economically important plants like soybean. Fumaric acid is of interest because it can be used by plants to synthesize sugar and starch. Therefore, understanding fumaric acid accumulation could be critical to understanding sugar and starch metabolism in plants, a topic of great economic importance to agriculture.

University of Rochester
Rochester, NY 14627-0166

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

J.H.D. Wu, Department of Chemical Engineering

\$78,000

The *Clostridium thermocellum* cellulosome is a multicomponent cellulase complex. Its largest subunit, CipA (formerly called CelL), contains nine repeated domains (R's), each functioning as a binding receptor to various cellulosomal catalytic subunits, and a cellulose binding domain (CBD) in between the second and the third repeats. We have previously reported that CelS, an exoglucanase and the most abundant catalytic subunit of the cellulosome, forms a stable complex with CipA. Furthermore, binding of CelS to crystalline cellulose, as its activity toward the same substrate, is enhanced by CipA. Thus the activity of a cellulosomal exoglucanase subunit can be enhanced by the anchorage function of CipA.

In this work, we examined the effect of anchorage on the activity of CelD, an endoglucanase subunit of the cellulosome, using recombinant CelD (rCelD) and the CipA functional domains, R3 (the repeat next to CBD) and CBD/R3, expressed in *Escherichia coli*. rCelD formed a stable complex with CBD/R3 as analyzed by a gel-shift assay on a nondenaturing polyacrylamide gradient gel. Binding of rCelD to crystalline cellulose, as its activity toward both phosphoric acid-swollen and crystalline cellulose, was dependent on CBD/R3. These results indicate that the activity of an endoglucanase subunit of the cellulosome, as that of the exoglucanase subunit, is enhanced by the anchorage function of CipA. Such anchorage function may thus augment the potential endo-exo synergism in the cellulosome. The results shed more light into the structure of the cellulosome and are important for engineering the cellulase complex for biomass energy conversion.

Rockefeller University
New York, NY 10021

- 177. Characterization of the Systemic Acquired Resistance Immediate-Early Response to Salicylic Acid**
N.-H. Chua, Laboratory of Plant Molecular Biology \$97,000

Plant disease resistance is a phenomenon which, in part, requires the biosynthesis and perception of salicylic acid (SA) for the induction of defense gene expression. We have used differential display analysis of tobacco cell cultures to identify SA early response genes. We cloned fragments of 15 genes with this behavior and estimated the total number of SA early response genes at 30-60. The kinetics of induction and response to cycloheximide (CHX) treatment allowed classification of genes into four groups. Classes I-III are characterized by immediate-early responses, showing increased accumulation of mRNA within 30 minutes of SA treatment. Moreover, CHX did not block induction of these genes, indicating that latent cellular factors mediate the SA response. Class IV genes were induced more slowly, but still within 2-3 hr of SA treatment, and required protein synthesis for expression. Although identified in this study as SA-responsive genes, several could also be induced by other compounds. Two genes were characterized in more detail, including isolation of cDNA sequences and additional analysis of gene expression. Sequence analysis revealed that one is the previously identified Ethylene Response Element Binding Protein 1 (EREBP1), an ethylene induced transcription factor for basic Pathogenesis-Related (PR) genes, whereas the other, G8-1, is a novel sequence. *EREBP1* was found to be activated not only by SA, but also by auxin and methyl jasmonate. In contrast, *G8-1* was found to be strongly induced only by SA and its active analogs and was exquisitely sensitive to low SA concentrations. In addition, these and other genes were found to be activated at early times following TMV infection of resistant tobacco genotypes.

Rutgers University
Piscataway, NJ 08855-0759

- 178. Corn Storage Protein: A Molecular Genetic Model**
J. Messing, Waksman Institute \$115,000

Corn is largely used to produce animal protein. Therefore, livestock receives its protein from corn meal. The major proteins in corn meal are a family of proteins, called zeins, whose main function is to store amino acids in the seed. These proteins 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. But recent advances in the manipulation of large DNA fragments has made it possible to make long-range physical maps. This has allowed us to map a tandem array of 22-kDa zein genes within 250 kilobases of genomic DNA. This array is divided into subclusters separated by about 60 kilobases containing mainly highly repetitive retroelements, the latter preventing us from forming a contiguous set of genomic clones. This was solved with DNA probes from a closely related cereal, Sorghum, whose repetitive DNA does not cross-hybridize to the corn genome, while its gene sequences do. Furthermore, the smaller genome size of Sorghum

seems to correlate with shorter distances of gene sequences. Therefore, the synteny between a smaller and a larger cereal genome will facilitate new approaches to genome analysis.

Salk Institute for Biological Studies
La Jolla, CA 92037

179. Signal Transduction Pathways that Regulate CAB Gene Expression

J. Chory, Plant Biology Laboratory

\$112,000

The process of greening, or chloroplast differentiation, involves the coordinate regulation of many nuclear- and chloroplast-encoded genes. The cues for the initiation of this developmental program are both extrinsic (e.g., light) and intrinsic (cell-type and plastid signals), but very little is known of the signaling pathways that regulate nuclear photosynthetic gene expression. The research program focuses on the genetic, biochemical, and molecular characterization of new *Arabidopsis thaliana* mutants in which a photoregulated promoter (CAB) is expressed aberrantly with respect to light, intrinsic developmental signals, and signals from the chloroplast. These genetic screens have allowed us to identify 18 new genes that play a role in the signal transduction pathways controlling photoregulated gene expression and chloroplast development in *Arabidopsis*. Specifically, we have obtained mutants in which CAB is expressed either: (1) in the light at abnormally low levels, defining positive regulators (9 genes); (2) in the dark at abnormally high levels, defining negative regulators (3 genes); or (3) in the presence of inhibitors that eliminate chloroplast function, defining an intracellular signaling pathway from chloroplasts to the nucleus (6 genes). During the past year, we have cloned three genes, mutations which result in lower CAB mRNA levels in response to light. One of the mutations lies in phytochrome B and two of the genes encode proteins that are localized to the chloroplast envelope. These genes thus represent a step toward elucidating how information is transmitted from photoreceptors and organelles in the cytoplasm to regulatory factors in the nucleus.

The Scripps Research Institute
La Jolla, CA 92037

180. Regulated Expression of the RTBV promoter by RF2a, a Transcriptional Regulator

R.N. Beachy, Department of Cell Biology

\$140,200 (18 months)

Rice tungro bacilliform virus (RTBV) is one of two viruses which together are responsible for causing rice tungro disease. In previous studies we characterized the viral genome, including the major promoter, and several proteins encoded by the virus, including the reverse transcriptase (RT) and viral protease that is responsible for processing the viral polyprotein. The major promoter regulates accumulation of the genome length transcript: the transcript serves as template for production of viral DNA (by the reverse transcriptase) and as messenger RNA for synthesis of viral structural and non-structural proteins.

In transgenic rice plants the RTBV promoter is expressed in elements of the vascular system, including primary xylem and primary phloem; this is in agreement with the observation that RTBV

infection is limited to vascular tissues in plants. After identifying the DNA sequences (*cis* elements) to which rice nuclear proteins bind, we determined the importance of each *cis* element for expression of the promoter in transgenic plants. One of the *cis* elements, referred to as Box II, was used in a yeast one plasmid system to isolate the protein that binds the element. The protein, referred to as RF2a, was cloned and expressed in *E. coli*, and following purification and renaturation was shown to bind Box II as homodimer; we also determined that a heterodimer was likely responsible for regulation *in vivo*. RF2a is active in an *in vitro* transcription assay system derived from rice cells, and in such assays promotes expression from the RTBV promoter but not selected other promoters. Transgenic plants which contain reduced levels of RF2a due to transgenes that produce (-)sense RNA derived from the RF2a gene exhibit abnormal vascular development in seedlings, but not in adult plants. Research in progress is directed to characterizing sub-domains of RF2a that regulate expression of the promoter, and to develop mutants of RF2a that restrict infection by RTBV.

The Scripps Research Institute

La Jolla, CA 92037

181. Membrane Targeting of P-type ATPases in Plant Cells

J. Harper, Department of Cell Biology

\$96,000

Our long term goal is to understand how integral membrane proteins are targeted to different subcellular locations. This knowledge is needed to engineer plants with new solute transport systems. Such systems include pumps for heavy metal uptake and salt efflux, which may be useful for bioremediation and salt tolerance, respectively. The general focus of our research is on a family of ion pumps called P-type ATPases. We are carefully examining the subcellular localization of two proton pumps (AHA2 and AHA10), two calcium pumps (ACA2 and ACA3) and a putative copper pump (AXA2) in a model plant system, Arabidopsis. Our approach is to assess localization using immunocytology, cell fractionation, and imaging of pumps fused to a green fluorescent protein. Our primary focus is on an unusual pump, ACA2, which is most similar to a plasma membrane-type calcium pump in animals, but is distinct since it is localized to an internal membrane. We are testing three hypotheses using mutant pumps expressed in transgenic plants. 1) Does the N-terminal domain contain targeting information? 2) Does the tonoplast become a "default" destination when normal targeting information is deleted? 3) Can ACA2 be retargeted to the plasma membrane by making chimeras with different regions of a plasma membrane proton pump?

The Scripps Research Institute

La Jolla, CA 92037

182. Nuclear Genes Regulating Translation of Organelle mRNAs

S. Mayfield, Department of Cell Biology

\$93,301

We have identified a set of proteins that bind with high specificity and affinity to the 5' untranslated region (UTR) of the chloroplast *psbA* mRNA. Binding of these proteins to the 5' UTR is required for ribosome association of the mRNA and hence for translation of the downstream coding region. We have also identified the RNA elements contained within the 5' UTR of the *psbA* mRNA that are recognized by this protein complex and that are required for high levels of translation. Cloning and

characterizing of the RNA binding proteins has revealed that one of these proteins is a member of the poly(A) binding protein family. Binding activity of this protein is regulated in response to light, resulting in light regulated translation within the chloroplast. Regulated binding is achieved by altering the redox status of the RNA binding protein by an associated protein disulfide isomerase. Protein disulfide isomerase are involved in the formation and reduction of disulfide bonds normally associated with protein folding. We have constructed chimeric genes containing the psbA 5' RNA elements and the coding region of either a bacterial luciferase gene or a single chain antibody gene. Both of these heterologous marker genes are translated at high levels within the chloroplast. These marker genes should allow us to precisely define the factors that are involved in translational regulation of psbA mRNA, and provide important insight into the molecular mechanism by which protein/RNA interactions trigger translational activation of specific chloroplast mRNAs.

Medical University of South Carolina
Charleston, SC 29525-2501

183. Molecular Mechanisms Controlling Proton Pumping by Bacteriorhodopsin
R.K. Crouch, Department of Ophthalmology and Biochemistry \$129,000

Bacteriorhodopsin (bR) is the simplest biological system for the transduction of light energy. This single, small membrane protein directly converts light energy to transmembrane proton gradient. The extraordinary stability of bR makes it an outstanding subject for bioenergetic studies.

The focus of our studies is the determination of the key mechanisms of light-induced proton transfer during light energy transduction in bR. Site directed mutagenesis, spectroscopic methods and chemical modification techniques are being developed and applied. The most important accomplishments are: 1) discovery of the complex titration of the primary proton acceptor, Asp85; 2) development of the concept of coupling of the primary proton acceptor Asp85 with the proton release group; 3) discovery of the role of transient protonation of Asp85 in catalysis of thermal isomerization of the chromophore; 4) development of methodology to routinely map the technically challenging bR by mass spectroscopy.

These findings comprise a new approach for the investigation and interpretation of the properties of the pigment in the initial (ground state) and the photolyzed intermediate states. These properties are particularly important for understanding of early and late light-induced proton release in bR and its mutants. The insights gained from these results are fundamental to the understanding of proton transport through membrane proteins and hence, the conversion of light into energy.

Southern Illinois University
Carbondale, IL 62901

184. Regulation of Alcohol Fermentation by Escherichia coli
D.P. Clark, Department of Microbiology \$98,000

We are studying the regulation of alcohol and lactate fermentation in the facultative anaerobe *Escherichia coli*. The protein encoded by the adhE gene expresses both alcohol and acetaldehyde dehydrogenase activities and is responsible for ethanol synthesis. Its N-terminal half is

homologous to other aldehyde dehydrogenases and its C-terminus to Fe-activated alcohol dehydrogenases. The mechanism of anaerobic induction of *adhE* has been characterized by means of gene fusions. The buildup of reduced NADH during anaerobic conditions plays the major role in regulating the *adhE* gene. The regulatory gene, *adhR*, has been cloned and preliminary work indicates that the AdhR protein binds to DNA in the *adhE* upstream region. We are presently isolating and characterizing regulatory protein mutants which result in high expression of *adhE* even when the level of NADH is low. Other mutations resulting in constitutive expression of the *adhE* gene, even in air, are known. The upstream regions of several of these have now been sequenced, confirming that they are in the *adhE* promoter region. The *ldhA* gene, encoding the fermentative lactate dehydrogenase has also been cloned and sequenced. Although it is induced by low pH, the *ldhA* gene does not respond to the same regulators as other known acid-inducible genes. The mechanism of *ldhA* induction in response to acidic conditions and growth phase is being investigated using *ldhA-lacZ* gene fusions. The *ldhA* upstream region is presently being dissected by PCR to locate the promoter and regulatory sites.

Stanford University
Stanford, CA 94305-5020

185. Nodulation Genes and Factors in the Rhizobium Legume Symbiosis

S.R. Long, Department of Biological Sciences

\$256,174

We study the bacterium *Rhizobium meliloti* and its legume host, alfalfa, which interact to form symbiotic root nodules. Within nodules, *Rhizobium* carries out nitrogen fixation; the plant thus can grow and produce protein without requiring nitrogen fertilizer. Because fertilizer manufacture requires intensive fossil fuel use, improvement of biological nitrogen fixation is an important strategy for maintaining productive agriculture as fossil fuels become scarce. We have defined and cloned the nodulation (*nod*) genes in *Rhizobium* that cause specific host plants to develop root nodules, providing a site for bacterial colonization and nitrogen fixation. The *nod* genes direct the synthesis of a chemical signal emitted by the bacteria that influences plant development, and we have recently defined early stages in the plant response to the bacterial signal. These early responses include calcium oscillation, a mechanism for signal transduction known in animal cell biology. What other bacterial and plant genes are involved in the symbiosis? We have begun several new projects to define these. We have purified the DNA for the symbiosis plasmid of *R. meliloti* for the purpose of creating two libraries of random DNA fragments, to be used to determine its sequence. We have also used a set of four independent strategies to discover the genes involved in invasion of the plant by the bacteria. In future work, we hope to define the plant genes involved in symbiosis, through generation of a library of expressed plant genes, and through classical and molecular genetics of the model legume *Medicago truncatula*.

University of Tennessee
Knoxville, TN 37996-0845

186. Plant Recognition of *Bradyrhizobium japonicum* Nod Factors

G. Stacey, Department of Microbiology

\$86,000

We are studying the agronomically important, nitrogen-fixing symbiosis between *Bradyrhizobium japonicum* and soybean. *B. japonicum* infects soybean roots and induces the formation of a nodule, a new organ, in which the bacteria reside. Organogenesis of the nodule is induced by substituted lipo-chitin molecules synthesized by the products of the bacterial nodulation genes. The potency of these molecules, as well as their high specificity, suggests the presence of plant receptors. However, past work has shown that nod signal perception is complex requiring at least two, structurally distinct lipo-chitin signals. These data argue for the presence of two plant receptors with differing structural specificity. In collaboration with other research groups, we have isolated cDNA clones of a nod signal binding protein from soybean. The encoded protein shows significant similarity to a family of lectin-like proteins that possess NTP phosphatase activity. A major objective of our work is to characterize this soybean lectin and ascertain its role in nodulation. In addition, we are pursuing a biochemical approach to identify nod signal binding activity in soybean tissue. The purpose of this work is the biochemical isolation of a nod signal receptor. Our eventual goal is to elucidate the complete signal pathway involved in the soybean nodulation response. Detailed knowledge of legume symbioses is important for the possible extension of biological nitrogen fixation for energy conservation.

University of Tennessee
Knoxville, TN 37996-1100

187. Molecular Mechanism of Light-Regulated Protein Transport Across the Nuclear Envelope

A.G. von Arnim, Department of Botany

\$96,000

The compartmentalization into nuclear and non-nuclear compartments affords a straightforward means by which eukaryotic cells limit the access of transcriptional regulators to their nuclear target genes. However, little is known about the cellular mechanisms that regulate nuclear uptake and depletion of proteins. The Arabidopsis COP1 protein, a light inactivated repressor of transcription essential for plant development, can confer a light regulated nuclear localization pattern on the fused reporter protein beta-glucuronidase (GUS). We have determined by genetic complementation that COP1, when fused to GUS or green fluorescent protein (GFP), can function normally in Arabidopsis. We are now using protein tagging with the reporter proteins GFP and GUS to delineate domains in the COP1 protein that are essential and sufficient for light regulated nuclear accumulation. In addition, we are determining why and how COP1 localizes to specific subnuclear domains (speckles), reminiscent of diverse animal proteins involved in chromatin-mediated processes. First, specific mutations are introduced into the COP1 protein. The mutant proteins are then examined for their ability to undergo light regulated nuclear localization. The localization data are correlated with data on the proteins' ability to function properly during plant development. We have identified a constitutive nuclear localization signal in COP1 and are now defining a domain required for cytoplasmic retention of the protein under light conditions as well as a domain that targets COP1 to subnuclear speckles.

Texas A&M University
College Station, TX 77843

188. Novel Biomaterials: Genetically Engineered Pores

H. Bayley, Health Science Center

\$181,104*

An extensive collection of nanometer-scale pores is being constructed by remodeling α -hemolysin (α HL), a protein secreted by the bacterium *Staphylococcus aureus*. The single polypeptide chain of 293 amino acids self assembles to form heptameric pores in membranes ~ 14 Å in internal diameter. By examining the properties of mutant and chemically modified α HLs, and the three-dimensional structure of the pore a working model for assembly has been devised. Monomeric α HL first binds to lipid bilayers and then aggregates to form a heptameric prepore complex. Finally, the open pore is formed when subunits in the complex undergo a cooperative conformational change, involving both the central glycine-rich loop and the N-terminus of the polypeptide. The central loop lines a section of the transmembrane channel in the fully assembled pore. Continuing studies of assembly and function are allowing point mutagenesis, combinatorial mutagenesis and targeted chemical modification to be used to create pores with new properties. For example, triggers and switches have been built into α HL to gain control over the opening and closing of the pores. Inputs that actuate the reengineered molecules can be biochemical in nature (activation by specific proteases), chemical (modulation of activity by covalent and non-covalent interactions with small molecules) and physical (activation by light). We have also begun to build pores based on the α HL β barrel, by *de novo* design. The new pores will be used to confer novel permeability properties upon materials such as thin films, which might then be used as components of energy conversion and storage devices, selective electrodes, electronic devices, and ultrafilters.

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

Texas A&M University
College Station, TX 77843-3122

189. Role of Glycolytic Intermediates in Global Regulation and Signal Transduction

J.C. Liao, Department of Chemical Engineering

\$101,000

The goal of our research is to probe the existence of signal metabolites in global regulation, such as Ntr regulon. We perturbed the metabolic pools by overexpression of related enzymes, and characterized the effects on specific regulons. One of the problems in this approach is the disturbance of physiological state caused by protein overexpression itself. To control for this adverse effect, we constructed mutants that are enzymatically inactive but are stable and soluble. These mutants showed that high-level protein overexpression per se decreases ribosome synthesis. Although the phenomenon resemble the stringent response, it is not mediated by guanidine tetraphosphate, (p)ppGpp. Fortunately, the decrease in ribosome synthesis was not significant for the level of protein overexpression used in detecting signal metabolites. Overexpression of phosphoenolpyruvate carboxykinase (Pck) was used to introduce perturbation to glycolytic metabolite pools. The overexpression of Pck in a wild-type background has no effect on *glnA* expression. However, overexpression of Pck in a *pta* strain caused a non-inducible phenotype for *glnA* operon. In addition, the adenylation of glutamate synthetase remained high

during Pck overexpression even during nitrogen starvation. This result suggests that a signal metabolite is perturbed to mask the effect of nitrogen limitation. Fructose 1,6-diphosphate and pyruvate levels correlate with such an effect, although the causal relationship remains to be established.

Texas Tech University
Lubbock, TX 79409

190. Ferredoxin-Linked Chloroplast Enzymes

D.B. Knaff, Department of Chemistry and Biochemistry

\$93,000

The ferredoxin-binding site of spinach nitrite reductase has been partially mapped using differential chemical modification of the enzyme and of the enzyme/ferredoxin complex with arginine- and lysine-modifying reagents. Two arginine residues, Arg 375 and Arg 556, and one lysine residue, Lys 436, are protected by ferredoxin against labeling by phenylglyoxal and pyridoxal-5'-phosphate, respectively. Positive charges are present at these three positions in all ferredoxin-dependent nitrite reductases for which sequences are available, suggesting that these amino acids are directly involved in electrostatic binding of ferredoxin to the enzyme. Characterization of the ferredoxin-dependent spinach glutamate synthase after treatment of the enzyme with the tryptophan-modifying reagent N-bromosuccinimide, suggests that at least one (and perhaps two) tryptophans are directly involved in electron transfer from reduced ferredoxin to glutamate synthase. Experiments with site-specific ferredoxin mutants, using a cyanobacterial ferredoxin as a homolog for spinach ferredoxin, have established the importance of a negative charge at the position equivalent to Glutamate 92 in spinach ferredoxin for efficient electron transfer from ferredoxin to spinach glutamate synthase. The midpoint potential of the regulatory disulfide/dithiol couple of spinach chloroplast phosphoribulokinase (PRK), an important regulatory enzyme of the carbon fixation pathway, was shown to be -295 mV at pH 7.0. Oxidation-reduction titrations of PRK mutants confirmed that Cysteine 16 and Cysteine 55 form the regulatory disulfide.

University of Texas
Austin, TX 78713

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

R.M. Brown, Jr. and K. Sathasivan, Department of Botany

\$92,000

We have been continuing the use of the multidisciplinary approach in the area of structure, biochemistry, genetics, and molecular analysis for our studies of cellulose biosynthesis in *Arabidopsis thaliana*. *Vigna radiata* has been used as a pilot research organism for development of suitable biochemical protocols. For the first time, a complete separation of *in vitro* (1,3)- β - and (1,4)- β -glucan synthase activities has been achieved in mung bean using non-denaturing electrophoretic conditions. Several polypeptides are common to cellulose and callose activities, and some polypeptides very specific for each separated fraction. Particles with the "rosette" organization are exclusively associated with cellulose assembly, while in callose synthesis, smaller particles never aggregated into the "rosette" structures. Similar solubilization conditions and native

gel electrophoresis were tested with *A. thaliana* membrane fractions. The enzymes and product bands were successfully separated. Identification of proteins involved in cellulose synthesis is in progress. Recent application of isoelectric focusing in combination with native gel electrophoresis allowed for the first time exclusive synthesis of cellulose *in vitro* from mung bean enzyme fractions. We plan to apply the same conditions to *A. thaliana* to produce fractions active in cellulose assembly for kinetic studies, antibody production, sequence analysis and TEM observations. Using a sequence motif for processive β -glycosyl transferases we have identified an EST from *A. thaliana* which could be a cellulose synthase. We anticipate a combined methodology will enable purification of the proteins and eventual isolation of the genes involved in cellulose biosynthesis and its regulation.

University of Texas
Austin, TX 78712

192. Phosphorylation of Plant Protein Synthesis Initiation Factors

K.S. Browning, Department of Chemistry and Biochemistry

\$90,000

Our goal is to identify plant protein synthesis factors that may be phosphorylated and determine their potential as sites of regulation for plant protein synthesis. The phosphorylation of eIF-2a in mammals and yeast and the phosphorylation of cap-binding protein in mammals both play a critical role in the control of protein synthesis, gene expression and signal transduction in mammalian cells. The complexity of the regulation translation by phosphorylation of mammalian cap-binding protein and eIF-2a, underscores the need for greater understanding of translational control mechanisms in plants. The unique presence of two forms of the cap-binding protein in plants (p26 of eIF-4F and p28 of eIF-(iso)4F) offers an excellent system to study the role of phosphorylation of cap-binding proteins in translational control of plants.

We propose to determine the phosphorylation state of initiation factors from wheat by *in vivo* labeling wheat sprouts with [³²P]orthophosphate and establish which initiation factors, particularly eIF-2a, eIF-4A, eIF-4B and the subunits of eIF-4F and eIF-(iso)4F, are (or are not) phosphorylated. The effects of growth and heatshock on the phosphorylation state of the initiation factors will also be determined.

The completion of the goals will open an area to be explored by both *in vitro* and *in vivo* methods. Of particular interest will be what kinases/phosphatases will be found to be involved in regulation and their roles in gene expression.

The Institute for Genomic Research
Rockville, MD 20850

193. Tandem-pore Outward-rectifying K⁺ Channels: Molecular Partners of the Proton ATPase in Membrane Potential Regulation

K.A. Ketchum

\$94,000

Ion and nutrient transport across the plasmalemma of living cells is governed by membrane potential. The goal of this project is to provide a comprehensive understanding of the bioenergetic

principles that influence membrane potential in plants and fungi. Several lines of evidence indicate that two distinct transport proteins, the outward-rectifying K⁺ channel and the H⁺-pumping ATPase, set the limits of membrane voltage and maintain negative cell polarity in these organisms. Our recent cloning of the yeast outward-rectifying K⁺ channel, TOK1, lets us test this hypothesis directly by investigating the molecular, biophysical, and biochemical properties of the channel protein and by characterizing its interaction with the yeast H⁺-ATPase. To determine the factors that regulate TOK1 activity, we are conducting two-electrode voltage-clamp recording and patch-clamp recording of the channel expressed in *Xenopus laevis* oocytes. We are investigating the voltage-dependence of channel activation and deactivation along with the regulatory effect of extracellular monovalent ions, cellular pH, and cytosolic second messengers. To evaluate TOK1 function *in vivo*, we are disrupting the coding sequence of the TOK1 gene in wild type yeast and in the pma1-105 hygromycin B-resistant mutant yeast strain that has altered membrane potential and K⁺ channel activity. By these means, the role of this channel in cell physiology can be analyzed and its interaction with other yeast transport proteins may be determined. Finally, to assess the broader merits of these experiments in plant and fungal physiology the electrophysiological and pharmacological properties of the outward-rectifying K⁺ channel(s) from a closely-related fungus, *Neurospora crassa*, and the flowering plant *Arabidopsis thaliana* are being compared with those of TOK1. This research will elucidate the role of K⁺ channel currents in maintaining electrical homeostasis and pave the way for a better understanding of membrane transport phenomena.

The Institute for Genomic Research Rockville, MD 20850-3319

194. Arabidopsis Genome Sequencing Using Random Shotgun Sequencing of BAC Clones

J.C. Venter

\$230,800

The objective of this program is to produce high quality, annotated genomic sequence within the framework of the international *Arabidopsis* Genome Initiative (AGI). We have focused our initial efforts on BAC clones from chromosome II, submitting fully annotated sequence to GenBank when each clone is complete (over 1.3 million base pairs to date), as well as releasing preliminary sequence for those BAC clones in progress (over 1 million base pairs to date). In addition, we have developed a high throughput protocol for generating BAC end sequences which provide a powerful resource for both mapping and selection of minimally overlapping BAC clones. Initially we focused on chromosome II-associated clones, but recently switched to a genome wide strategy in collaboration with the French Genome Center. We have produced over 6,000 end sequences from two different BAC libraries, and it is expected that the sequence for each end of 22,000 BAC clones will be completed in the coming year. The combination of the BAC end sequence database and the production of high quality finished sequence will facilitate the completion of the genome sequence and will make a critical contribution to the worldwide plant biology community.

Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

195. Enzymology of Acetone-butanol-Isopropanol Formation

J.-S. Chen, Department of Biochemistry

\$101,000

Several species of *Clostridium* produce acetone, butanol and isopropanol (solvents), which are important industrial chemicals and fuel additives. Commercial production of acetone and butanol by fermentation began with the starch-based Weizmann process using *C. acetobutylicum*. After the 1930s, *C. acetobutylicum* was replaced by newly isolated clostridia that produced high levels of solvents from sugars. Using DNA reassociation and other tests, *C. beijerinckii* and two other *Clostridium* species were identified as the organisms used in industrial solvent production from sugars. *C. beijerinckii* does not require an acidic pH for solvent production, and some strains produce isopropanol in addition to acetone. We have been using *C. beijerinckii* in our study with an aim of establishing mechanisms and strategies for regulating the expression of solvent-production genes and the flow of metabolites. The long-term goals are to prevent the degeneration of strains, to regulate the product ratio, and to prolong the solvent-producing phase of growth. Our present study has an emphasis on characterizing the aldehyde and alcohol dehydrogenases that convert butyryl-CoA to butanol under different conditions, on determining the structure and organization of solvent-production genes in *C. beijerinckii*, and on studying the control of expression of the solvent-production genes. The organization of genes (*ctfA*, *ctfB* and *adc*) for acetone formation and the promoter regions differ between *C. beijerinckii* and *C. acetobutylicum*, suggesting mechanistic differences in the regulation of expression of solvent-production genes in the two species.

University of Virginia
Charlottesville, VA 22901

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

M.P. Timko, Department of Biology

\$96,000

Chlorophylls are major components of the energy absorption and transduction apparatus in all photosynthetic organisms. In vascular plants, chlorophyll biosynthesis is regulated in part at the level of protochlorophyllide (Pchl_{id}) to chlorophyllide (Chl_{id}) catalyzed by NADPH: protochlorophyllide oxidoreductase (POR). POR is completely dependent upon light for its activity. In dark-grown seedlings, POR accumulates along with its substrates, Pchl_{id} and NADPH, in stable ternary complexes in the prolamellar bodies of the etioplasts. Illumination of POR ternary complexes with stoichiometric amounts of light quanta result in the trans-reduction of the C17-C18 double bond in the D ring of Pchl_{id} and the formation of the Chl_{id} product. The action spectra for the enzyme closely matches the absorption spectra for Pchl_{id} indicating the Pchl_{id} is the primary light-absorbing species involved in the reaction. We are interested in understanding the mechanism of light-dependent Pchl_{id} reduction and the structural determinants in POR proteins required for substrate (NADPH and Pchl_{id}) binding, catalytic activity, and proper intraorganellar assembly within the plastid. We have generated and characterized a series of site-directed and clustered charged-to-alanine scanning mutants of the POR from PEA and have identified potential residues and domains required in the enzyme for Pchl_{id} photoreduction. In addition we have

located some of the structural factors required for proper association with the plastid membrane. Work is currently underway to define in considerably more detail the effects of various mutations on the kinetic properties (i.e., K_m for substrate or cofactor, turnover rate, etc.) of the enzyme and to refine our analysis of the structural factors required for enzyme function. We are particularly interested in clarifying the role of the various conserved cysteine residues as well as the role of the C-terminal region of the POR protein in forming the active site pocket or in binding/stabilizing the substrate. We are also working towards solving the three-dimensional structure of the protein by using X-ray crystallography. These studies should provide significant information on one of the most crucial biosynthetic steps in the development of photosynthetic organisms.

Washington State University
Pullman, WA 99164-6340

197. Membrane Function in Lipid Mutants of Arabidopsis

J. Browse, Institute of Biological Chemistry

\$109,000

Our investigations of the biochemistry of wild-type and mutant plants have provided us with new information about the enzymology and regulation of lipid metabolism in higher plants. More importantly, our isolation and characterization of *Arabidopsis* mutants laid the groundwork for important discoveries about the role of membrane lipids in the cell biology and physiology of plants as well as the means to clone genes that encode fatty acid desaturase enzymes. Recently, the scope of our work has been expanded by the isolation and cloning of desaturase genes from the model nematode *Caenorhabditis elegans*. The many genetic tools available in *C. elegans* means that it may be possible to develop this worm as an additional model for studying the relationship between lipid composition and membrane function.

Washington State University
Pullman, WA 99164-6340

198. Regulation of Terpene Metabolism

R. Croteau, Institute of Biological Chemistry

\$106,000

Terpenoid oils, resins and waxes from plants are important renewable resources with a range of pharmaceutical, agricultural and industrial uses. The objective of this project is to control terpenoid production by targeting regulatory steps for genetic engineering to increase the yields and expand the types of terpenoid natural products that can be made available for commercial exploitation. Model systems used are (+)-camphor metabolism in sage and (-)-menthone metabolism in mint. Developmental studies indicate that the yield of these terpenoids is controlled by the balance between biosynthetic and catabolic capacity. Experiments utilizing antibodies directed against, and cDNAs coding for, key metabolic enzymes indicate that regulation of terpene biosynthesis resides primarily at the level of gene expression, that several compartments within the secretory gland cells (plastids, endoplasmic reticulum and cytosol) participate in terpene biosynthesis, and that the bulk of terpene production occurs at the early stages of gland development. Two genes have been selected for overexpression in transgenic plants to increase terpene yield, and the experiments to test this possibility are in progress. Experiments are also underway to examine flux control within, and communication between, the cytosolic mevalonate (acetyl CoA-dependent) pathway and the

plastidial non-mevalonate (pyruvate/glyceraldehyde-3-phosphate-dependent) pathway to the central precursor isopentenyl diphosphate. The cDNA encoding the first dedicated enzyme of the non-mevalonate pathway (catalyzed by 1-deoxy-D-xylulose-5-phosphate synthase) has recently been isolated from mint.

Washington State University
Pullman, WA 99164-6340

199. Carbon Metabolism in Symbiotic Nitrogen Fixation

M.L. Kahn, Institute of Biological Chemistry

\$95,000

Nitrogen fertilizer is an important input for increasing crop yield because nitrogen deficits often limit plant growth. However, producing nitrogen fertilizer is expensive and energy intensive and excess fertilizer can increase nitrate in groundwater and soil acidity. Some plants are able to obtain nitrogen through symbiotic associations with nitrogen-fixing bacteria in which the plant exchanges carbon compounds for ammonia produced by the bacteria. Enzymes of the plant and bacterial tricarboxylic acid (TCA) cycles are at the center of this exchange, generating energy, reductant and biosynthetic intermediates from the catabolism of photosynthate. We are investigating the genetics and biochemistry of symbiotic carbon metabolism in both soybean and alfalfa. Our immediate goal is to define the role of the decarboxylating leg of the TCA cycle, a series of reactions needed to synthesize amino acid precursors that may also be required to generate energy and reductant for nitrogen fixation. We are investigating TCA cycle mutants, including those with defects in citrate synthase, isocitrate dehydrogenase and oxoglutarate dehydrogenase, and are developing conditional TCA cycle mutants to probe the relationship between the TCA cycle and nodule development and metabolism. Recent studies suggest that rhizobia use the decarboxylating leg of the cycle much more in the symbiosis with alfalfa than they do when associated with soybean. The difference may be related to the form of nitrogen exported from the nodules or with the way in which energy is generated by the bacteria.

Washington State University
Pullman, WA 99164-6340

200. A Comprehensive Approach to Elucidation of Lignification at the Plasma Membrane/Cell Wall Interface

N.G. Lewis, Institute of Biological Chemistry

\$170,000

Lignification, suberization and lignin-forming systems in plants are exquisitely orchestrated yet poorly understood biological processes involving phenolic coupling, and account for up to 40% of all organic carbon in living systems. The first example of control of phenolic coupling was described this year, in our discovery of dirigent proteins. The dirigent protein is thought to function by trapping and orienting free-radical intermediates in such a way as to engender both regio- and stereoselective coupling. In this first case of a presumed new class of proteins, only (+)-pinosresinol is obtained from E-coniferyl alcohol, rather than diffusion-controlled non-specific coupling. The dirigent protein has no homology with any other protein of known function, nor does it possess oxidative capacity to generate the free-radical species from coniferyl alcohol. Free-radical generation involves one-electron oxidizing enzymes, such as laccase. The gene encoding the

dirigent protein has been cloned, and the recombinant functional protein obtained using a Spodoptera/baculovirus system.

Studies are now directed towards establishing the existence of other members of this class of protein, with particular attention being placed upon lignification. Emphasis is being placed upon determining how preferential formation of the beta-O-aryl linkage within native lignin polymers occurs, and how this is controlled at the protein level.

Related studies have established that during active phenylpropanoid metabolism in lignifying plants, suberizing tissues and fungi, the ammonium ion released during phenylpropanoid metabolism is reassimilated via GS/GOGAT to regenerate glutamate, which is used as amino donor for phenylalanine regeneration and hence completion of the nitrogen cycle.

Washington State University **Pullman, WA 99164-6340**

201. Interdisciplinary Plant Biochemistry Research and Training Center *N.G. Lewis, Institute of Biological Chemistry* **\$315,000**

The Plant Biochemistry Research and Training Center at Washington State University has this year extended the range of programs from 13 to 17 participating faculty. In its third year, research training opportunities have been provided for 7 undergraduate students for both academic year and summer student research appointments. Additionally, a total of 15 graduate students (Ph.D. and M.S.) and 3 post-doctorals are currently enrolled in the Center activities and training. The multi- and interdisciplinary programs have been extended to include: the Plant Biochemistry Seminar Series; the biennial Plant Biochemistry Summer Course (held this year); and the annual review of the Center by eminent plant biochemists. The areas of strategic importance emphasized in the Training Program include: regulation of biochemical pathways; plant cell wall formation; and signal transduction mechanisms. Each trainee overlaps research activities between at least two laboratories in order to gain the necessary interdisciplinary insight and experience.

Washington State University **Pullman, WA 99164-6340**

202. Enhancement of Photoassimilate Utilization by Manipulation of ADP-glucose pyrophosphorylase *T.W. Okita, Institute of Biological Chemistry* **\$190,000 (FY96 funds/2 years)**

The goal of this project is to increase the conversion of photoassimilate into starch via manipulation of ADP-glucose pyrophosphorylase (AGP), an enzyme which controls one of the key steps in starch biosynthesis. Using a powerful mutagenesis-glgC complementation system we have generated and identified several up-regulatory enzyme mutants that display increase sensitivity to activation by 3-phosphoglyceric acid (3-PGA) while simultaneously having increased resistance to Pi inhibition. For example, one mutant, UpReg-1, requires only 2 uM of 3-PGA to achieve 50% of maximum enzyme activity. This level of 3-PGA is 50-fold less than that required by the wildtype enzyme. Moreover, when measured in the presence of 250 uM 3-PGA, UpReg-1 requires more than 4700

uM of Pi for 50% inhibition, or 67-fold greater amounts of Pi than that required by the wildtype enzyme. The study of these AGP mutants will not only increase our knowledge on the structure-function relationships of this enzyme and the role of this enzyme in carbon partitioning in source and sink tissues but will lead to novel strategies of increasing starch production and, in turn, increasing overall productivity for many crop plants.

Washington State University
Pullman, WA 99164-6340

203. Targeting and Processing of the Thiol Protease Aleurain

J.C. Rogers, Institute of Biological Chemistry

\$96,000

We study a plant vacuolar thiol protease, aleurain, that is structurally and functionally equivalent to a mammalian lysosomal protease, cathepsin H. These unique enzymes are poor general endoproteases but have active aminopeptidase activity; they undergo unusual processing such that a short minichain is cut from the enzyme prosequence and disulfide-linked to an extra cysteine in the mature large chain. Aleurain is expressed in most cells in barley but its functional importance to the plant is unknown. We have shown that aleurain is a marker for an acidified, lytic vacuolar compartment distinct from the protein storage vacuole compartment in plant cells that contain storage proteins. As the vacuolar compartment increases in size, the two separate types of vacuoles appear to merge; this may be a mechanism by which storage proteins are exposed to proteases to initiate their degradation. An understanding of mechanisms regulating targeting of aleurain and of storage proteins to their separate vacuoles, and mechanisms by which the two compartments may merge, may provide new approaches towards more effective mobilization of protein reserves. An understanding of mechanisms that control the activation of protease proenzymes may enhance our ability to protect or mobilize those reserves at specific times during plant development. We have identified and cloned a potential receptor protein that binds proaleurain and directs it into the clathrin-coated vesicle pathway to the acidic, lytic vacuole. Future work will expand an understanding of that pathway by identifying and characterizing other soluble vacuolar proteins that are bound by the same receptor. Cytoplasmic proteins that interact with the cytoplasmic tail of the receptor, and thereby participate in the process by which the receptor with its ligand is segregated into vesicles for transport to the vacuole, will be identified and characterized. Another marker for the specific acidic, lytic vacuolar compartment will be obtained by purifying the protease that specifically "clips" proaleurain upon its entry into that compartment. The functional importance of aleurain will be investigated by constructing mutant *Arabidopsis* plants that do not express the *Arabidopsis* equivalent of that enzyme.

University of Washington
Seattle, WA 98195-1750

204. Genetics in Methylophilic Bacteria

M.E. Lidstrom, Department of Chemical Engineering

\$103,000

The purpose of this project is to study the transcriptional regulation of methanol oxidation (Mox) functions in the facultative methylophilic bacterium, *Methylobacterium extorquens* AM1. Mox functions include a 14-gene operon (mxafJGIRSACKLDHEB) containing genes required for

production, assembly, and regulation of methanol dehydrogenase, a gene immediately upstream of the *mx*a cluster, but divergently transcribed (*mx*aW), a 4-gene cluster (*pqq*ABC/DE) and a 2-gene cluster (*pqq*FG) required for production of the methanol dehydrogenase prosthetic group, pyrroloquinoline quinone (PQQ), a two-gene cluster (*mx*dRS) of unknown function, and two additional sets of Mox regulatory genes, *mx*bDM and *mx*cQE. Sequence analysis of these regulatory genes suggests that they comprise two sets of sensor protein kinase/response regulator systems. In addition, *mx*aB appears to encode a third response regulator, but the sensor protein kinase that couples with *mx*aB is unknown. Methanol-inducible promoters have been isolated for *mx*aF, *mx*aW, *pqq*A, and *mx*bD, and reporter gene fusion experiments have demonstrated that *mx*cQE are required for expression of *mx*bDM, and *mx*bDM are required for expression of the *mx*aF, *mx*aW and *pqq*A promoters, but not for the *mx*bD promoter. *mx*aB is required for expression of only the *mx*aF and *pqq*A promoters. Experiments are underway to purify each of the putative response regulators and map their binding to the four methanol-inducible promoters.

**University of Wisconsin
Madison, WI 53706-1381**

**205. Biochemical Characterization of the Ethylene Receptor ETR1 from
Arabidopsis thaliana**
A.B. Bleecker, Department of Botany \$97,000

Ethylene regulates a number of developmental processes in plants including fruit ripening and abscission. The goal of our project is to elucidate the sensory system that utilizes ethylene as a signal. The ETR1 gene codes for an ethylene receptor in Arabidopsis. We are seeking to understand how this receptor senses and transduces the ethylene signal. Using recombinant receptor protein expressed in yeast, we have shown that the receptor binds ethylene with high affinity. A recombinant protein in which the first 128 amino acids of ETR1 are fused to a bacterial glutathione-S-transferase sequence binds ethylene, indicating that the N-terminal domain of the receptor is necessary and sufficient for ethylene binding. Binding activity of the recombinant protein is increased 20-fold when copper ion is supplied in the buffer, supporting the long-standing hypothesis that ethylene interacts with a transition metal at the receptor binding site. We have shown by affinity purification that the copper ion co-purifies with the recombinant protein using wild type ETR1 binding site, but not in the *etr1-1* mutant protein that does not bind ethylene. We are developing a molecular model of the ethylene binding domain which accounts for the coordination of a copper ion at the active site. We are also developing procedures to purify the full length ETR1 protein to determine whether ethylene binding regulates activity of the C-terminal kinase domain of the protein.

**University of Wisconsin
Madison, WI 53706**

206. Molecular Genetics of Ligninase Expression

D. Cullen, Department of Bacteriology

\$107,000

In addition to playing a key role in the carbon cycle, lignin-degrading fungi have demonstrated potential in a number of emerging technologies. These include biomechanical pulping, bleaching and otherwise improving chemical and mechanical pulps, converting lignin to useful chemicals, effluent treatments, and remediation of contaminated soils. The mechanism(s) involved in these processes are poorly understood, and this represents a barrier to further development.

The long term goals of this research seek to elucidate the basic genetics and physiology involved in the degradation of lignin and related aromatic compounds. Fungal genes expressed in wood and in organopollutant-contaminated soil are being identified using specialized cloning techniques. Focus has been on regulated genes which encode extracellular enzymes of *Phanerochaete chrysosporium* and *Ceriporiopsis subvermispota*. The structure, genomic organization and regulation of these genes are under investigation. Key genes are expressed in heterologous systems and recombinant proteins prepared for basic biochemical investigations.

This research furthers our understanding of mechanism(s) involved in the degradation of lignin and related aromatic compounds. These studies also provide insight into lower eukaryote genome organization with particular emphasis on complex gene families and chromosome dynamics. Development of commercial processes will be greatly facilitated by the identification of key genes and the production of recombinant enzymes.

**University of Wisconsin
Madison, WI 53706**

207. Microbial Formaldehyde Oxidation

T.J. Donohue, Department of Bacteriology

\$90,000

Our research dissects microbial pathways for formaldehyde oxidation and sensing. This work capitalizes on our discovery of a glutathione-dependent formaldehyde dehydrogenase (AdhI) in the facultative phototrophic bacterium *Rhodobacter sphaeroides*. In photosynthetic cells, AdhI is required to use methylated compounds like methanol as a sole carbon source. Under respiratory conditions, AdhI removes the formaldehyde produced when methanol is co-metabolized. Our experiments also take advantage of mutations that alter *adhI* expression. Under respiratory conditions, the sensor/histidine kinase-response regulator pair (GfdRS) represses *adhI* transcription. A second class of *trans*-acting mutations appear to alter function of an activator of *adhI* transcription (SpdA).

One set of experiments is asking how cells generate energy and one-carbon skeletons from formaldehyde oxidation. Specifically, we are characterizing mutants that are defective in either respiratory methanol co-metabolism or photosynthetic utilization of methanol as a sole carbon source. By determining where individual lesions block formaldehyde metabolism, we will trace the

flow of carbon and identify enzymes that remove this compound under respiratory conditions or assimilate it in photosynthetic cells.

Simultaneously, we will determine how cells sense formaldehyde and control *adhI* transcription. Specifically, we are testing the prediction that GfdS is a sensor/histidine kinase, asking if formaldehyde controls phosphorylation of this presumed sensor, monitoring GfdR phosphorylation by GfdS, and testing if GfdR phosphorylation increases its binding to a presumed *adhI* operator. To test if SpdA is an activator of *adhI* expression, we are defining presumed gain-of-function mutations that increase transcription of this gene.

**University of Wisconsin
Madison, WI 53706**

208. Identification of the Primary Mechanism for Fungal Lignin Degradation

K.E. Hammel, Department of Bacteriology

\$78,000

Microbial ligninolysis is an essential link in the terrestrial carbon cycle that is thought to be carried out almost entirely by wood- and litter-decomposing basidiomycetous fungi. These organisms produce a variety of extracellular oxidative agents, including lignin peroxidases (LiPs) that have the unusual ability to cleave the recalcitrant nonphenolic structures that predominate in lignin. However, many ligninolytic fungi appear to lack LiP activity, and the mechanisms by which lignin is initially degraded in wood remain unclear. Work on this project has shown that one LiP-negative fungus, *Ceriporiopsis subvermispota*, is able to cleave the nonphenolic arylglycerol-2-aryl ether lignin structure that predominates in lignin. The fungus was given new polyethylene glycol-linked ¹³C-labeled arylglycerol-2-aryl ether lignin model compounds, and the high molecular weight products that resulted from extracellular fungal attack were analyzed by ¹³C nuclear magnetic resonance spectrometry. The data showed that the lignin structure underwent two major degradative reactions: cleavage between C1 and C2 of the propyl side chain and cleavage of the 2-aryl ether linkage. These results suggest that *C. subvermispota* produces a ligninolytic agent other than LiP and that this agent oxidizes lignin structures either by ionizing them to cation radicals or via oxyradical addition to an aromatic ring. Further investigation has shown that these reactions are strongly dependent on the presence of Mn: when *C. subvermispota* cultures were grown in the presence of low (0.5 uM) Mn and a polymeric lignin model compound, they exhibited almost no degradative activity towards the compound, but the addition of 150 uM Mn to these cultures elicited rapid degradation within less than 24 h. Since past work on this project has demonstrated that the Mn-dependent peroxidases of *C. subvermispota* are able to initiate lipid peroxidation reactions that cleave nonphenolic lignin *in vitro*, it may be that these Mn-requiring enzymes are responsible for the ligninolytic activity found *in vivo*. Current work is aimed at determining which Mn-dependent enzymes are involved in this process, and whether they are induced at the level of gene transcription.

University of Wisconsin
Madison, WI 53706

209. Organization of the R Chromosome in Maize

J. Kermicle, Laboratory of Genetics

\$88,000

The maize *r* gene product activates expression of structural genes in the 3-hydroxy anthocyanin pathway. Different *r* genes (individual alleles) confer pigmentation on different plant parts. In some strains multiple *r* genes are clustered on chromosome 10 forming complexes. In certain heterozygotes the level of *r* action is heritably silenced, i.e., paramutated. We want to know how the potential of one *r* complex to silence genes in the homologous chromosome is organized and how the change is brought about. Is silencing facilitated when the inciting and responding genes are in the same rather than in homologous chromosomes? When two responding genes and one inciting gene are present in trisomic plants, are the responding genes affected coordinately or independently? When during plant development does silencing occur? We also want to know what other loci are necessary for *r* silencing to occur. Our general strategy is to dissect silencing genetically, then characterize selected aspects molecularly.

University of Wisconsin
Madison, WI 53706-1569

210. The Biochemistry, Bioenergetics and Physiology of the CO-Dependent Growth of *Rhodospirillum rubrum*

P.W. Ludden, Department of Biochemistry

\$99,304

Rhodospirillum rubrum is a purple, non-sulfur photosynthetic bacterium that is capable of growth with carbon monoxide as the carbon and energy source. CO-dependent growth requires Carbon Monoxide Dehydrogenase (CODH), a NiFeS-containing enzyme that oxidizes CO to CO₂, and a CO-induced, CO-insensitive hydrogenase. The *coo* regulon includes the *cooFSCTJ* operon and the *cooMKLXUH* operon, both under the control of *CooA*, which serves as the transcriptional regulator for the system. Alterations of specific amino acid residues by site-directed mutagenesis of the *cooS* gene have yielded important information on the residues suspected of serving as ligands to the NiFeS prosthetic group (C cluster) and the FeS group (B cluster) involved in the redox reactions of the enzyme. Continued characterization of mutant forms of the enzyme will be a goal of the coming grant period. The *cooCTJ* gene products have been implicated in processing of Ni for the C cluster, and the *CooJ* protein has been purified and characterized. Its role in binding and processing Ni for CODH will continue to be investigated. The *CooC* protein has been overexpressed in *R. rubrum* and antibodies to the protein have been generated. The protein will be purified and characterized in the coming grant period. Anti *CooC* antibodies will be employed in the study of its role in Ni processing. Crystals of CODH have been obtained and the structural analysis of CODH continues.

University of Wisconsin
Madison, WI 53706

211. Feedback Regulation of Photosynthetic Processes

T.D. Sharkey, Department of Botany

\$96,259

In this project the interaction between photosynthesis and sucrose synthesis are studied using naturally occurring mutant plants and transgenic plants. Using nuclear magnetic resonance we have shown that during darkness carbon is exported from the chloroplast primarily as hexose phosphate. The hexose transporter must be highly regulated; it must be off during the day, forcing carbon through cytosolic FBPase, and active at night. Characterization of the hexose transporter has been started. The export of hexose phosphate at night bypasses the cytosolic FBPase allowing FBPase-lacking plants to survive. The effects of increasing the capacity for sucrose synthesis on yield are studied using plants expressing sucrose phosphate synthase (SPS) on either a rubisco small subunit promoter or the cauliflower mosaic virus 35S promoter. The SSU promoter increases SPS in the leaf 6 fold and in the fruit 2 fold. The 35S promoter increases SPS in the leaf 2 fold and in the roots 2 to 10 fold. We are manipulating the SPS level using classical breeding techniques to test whether there is an optimum SPS level for yield. To test whether SPS in roots affects yield, young plants were grafted so that either the root or the shoot only was transgenic. Most, but not all, of the effect of transformation results from the extra gene in shoot material. One tomato line routinely exhibits substantially increased fruit yield, as do potatoes with excess SPS. This work will contribute to our understanding of how photosynthesis works and to increased plant yield through biotechnology.

University of Wisconsin
Madison, WI 53706

212. Molecular Mechanism of Energy Transduction by Plant Membrane Proteins

M.R. Sussman, Department of Horticulture

\$110,000

Proton pumps are membrane-bound proteins that act as biological energy transducers. The plasma membrane of plants and fungi contains an ATP-coupled proton pump (H^+ -ATPase) that converts chemical into electrical energy, and provides the driving force to catalyze the uptake and accumulation of solutes. The plant/fungal plasma membrane H^+ -ATPase contains a single polypeptide of Mr=100,000. Its simple polypeptide structure makes this enzyme an attractive candidate for studying the molecular basis of energy transduction. A major aim of this project is to identify aspects of the enzymes' primary structure that are essential for converting chemical into electrical energy. DNA cloning and sequencing techniques are being used to obtain the complete amino acid sequence for twelve H^+ -ATPase structural genes present in *Arabidopsis thaliana*, a model higher plant with a small genome and a rapid generation time. Mutant *A. thaliana* plants in which each of these ATPase genes is disrupted are being isolated. The phenotypes of these plants will help to determine the biological role of each pump isoform *in situ*. Expression of the genes is being studied using Northern blots and 'epitope-tagging' to produce isoform-specific polypeptide probes. Overall, these studies are essential for testing hypotheses concerning the biological role of ion pumps and the molecular mechanism of protein-mediated energy transduction in plants.

**University of Wisconsin
Madison, WI 53706**

213. Analysis of Structural Domains Required for Phytochrome Function by *in vitro* and *in vivo* Mutagenesis

R.D. Vierstra, Department of Horticulture

\$108,000

Phytochrome is a red/far-red photoreversible photoreceptor that has a central role in light-regulated plant development. To determine how phytochrome functions at the molecular level, we have exploited a biological assay for active chromoproteins that involves the expression of chimeric phytochrome genes in transgenic tobacco. Such ectopic overexpression induces a striking "light exaggerated" phenotype that can be used as an *in vivo* assay of receptor function. The goal of the project is to combine this transgenic system with *in vitro* mutagenesis to identify phytochrome domains important to synthesis, dimerization, chromophore attachment, Pr/Pfr phototransformation, Pfr-enhanced degradation, and biological activity. Preliminary mapping has discovered two distinct domains near the N-terminus of phytochrome A, one required for the structural integrity and biological activity of the chromoprotein, and a second, serine-rich domain that modulates phytochrome activity. Modification of this second domain creates a hyperactive photoreceptor that is 10-50 more sensitive to light. Biochemical and genetic approaches are now in progress to determine the mechanism(s) that underlie this hyperactivity since it may reflect a novel post-translational step that attenuates phytochrome A function. The first 600 amino acids also was found to contain element(s) responsible for the rapid degradation of phytochrome A as Pfr. Appending these domains to the C-terminal region of the more stable phytochrome B confers instability to this chimeric protein as Pfr. Additional sets of phytochrome A/B chimeras are under construction to localize this degradation signal. Completion of this work will represent an important step in understanding the function of this essential photoreceptor.

**Xavier University of Louisiana
New Orleans, LA 70125**

214. Molecular Characterization of Bacterial Respiration on Minerals

R. Blake II, College of Pharmacy

\$94,040

Aerobic respiration on reduced iron is a principal metabolic activity exhibited by certain chemolithotrophic bacteria that inhabit ore-bearing geological formations exposed to the atmosphere. Each phylogenetically distinct group of iron-oxidizing bacteria expresses one or more unique acid-stable, redox-active biomolecules in conspicuous quantities during aerobic respiration on iron. Structural and functional studies continue on two such novel biomolecules that have been purified to electrophoretic homogeneity, rusticyanin from *Thiobacillus ferrooxidans* and cytochrome₅₇₉ from *Leptospirillum ferrooxidans*. The aim of these studies is to determine the role of each protein in the iron respiratory chain of its respective organism. Other redox-active components present in cell-free extracts of iron-oxidizing organisms continue to be sought, isolated, and investigated with regard to their roles in the same respiratory chains. Another aim is to investigate the mechanisms, consequences, and principal features of bacterial adhesion to insoluble minerals. The specific, selective adhesion of *T. ferrooxidans* to pyrite was recently shown to be mediated by aporusticyanin 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 chemolithotrophic bacteria that respire on insoluble minerals. It is anticipated that this project will provide useful information toward manipulating *T. ferrooxidans* and related organisms for commercial use.

Xavier University of Louisiana
New Orleans, LA 70125

215. Biochemistry of Dissimilatory Sulfur Oxidation

R. Blake II, College of Pharmacy

\$93,000

Dissimilatory sulfur-oxidizing bacteria obtain all of their energy for metabolism from the aerobic oxidation of reduced inorganic sulfur compounds. Despite the environmental and economic importance of these organisms, there is still much uncertainty regarding the actual metabolic pathways and the stoichiometries of these bacterial oxidation reactions. This project has two major experimental goals: (i) to quantify the adherence of sulfur-oxidizing bacteria to their insoluble elemental substrate; and (ii) to define the substrate oxidation pathways, the electron transport mechanisms, and the modes of energy conservation employed by various species of the thiobacilli. Studies on the interaction between bacteria and insoluble sulfur exploit field flow fractionation, laser Doppler velocimetry, electrical impedance, static and dynamic light scattering, surface tension, contact angle, and other measurements commonly employed to characterize colloidal particles. Studies on the latter goal currently focus on the enzymology of polythionate degradation in those thiobacilli that accumulate polythionates in the culture medium. Efforts to isolate and characterize trithionate hydrolase, thiosulfate dehydrogenase, and tetrathionate hydrolase from cell-free extracts of both neutrophilic and acidophilic thiobacilli are in progress. It is anticipated that this information will be of value in the eventual manipulation of the thiobacilli and related organisms to benefit both the environment and the mining industry.

Yale University
New Haven, CT 06511-7444

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

T. Nelson, Department of Molecular, Cellular and Developmental Biology

\$116,989

The dicot genus *Flaveria* includes species utilizing C3, C4, and C3/C4 intermediate schemes of carbon fixation. In the C4 scheme, leaf bundle sheath (BS) and mesophyll (M) cells cooperate to make carbon fixation more efficient. CO₂ is assimilated in M cells, then reduced in BS cells, via a C4 pathway that relies on expression of the genes for pathway enzymes in M- or BS-specific patterns. In less efficient C3 species, M cells independently fix CO₂, and the BS is not generally photosynthetic. C3/C4 intermediate species exhibit anatomical and biochemical characteristics between the C4 and C3 extremes. In both C3 and C3/C4 species, C4 pathway genes are present, but used in different spatial patterns. The variety of *Flaveria* species provides an opportunity to compare the structure and regulation of C4 pathway genes in closely related C3 and C3/C4 species, and to determine the feasibility of increasing the efficiency of the non-C4 species through

reregulation of their C4 pathway genes. We have isolated and characterized genes encoding malic enzyme (ME), ribulose biphosphate carboxylase (rbcS), and malate dehydrogenase (MDH) from C3 and C4 species of *Flaveria*. We are characterizing the DNA elements associated with the different activity patterns of these genes in *Flaveria* species, by means of both transient and stable introduction of reporter constructs into C3, C4 and C3/C4 species. The individual C4 pathway genes appear to be spatially regulated via distinct rather than common DNA elements.

Yale University
New Haven, CT 06510

217. Calcium Mapping and Signalling in Yeast

C.L. Slayman, Department of Cellular and Molecular Physiology \$121,000

The long-range object of these experiments is to develop a comprehensive and quantitative picture of calcium transport and signalling in a model cell, *Saccharomyces cerevisiae*, whose ion-physiology closely resembles that of higher plant cells. Recent public release of the complete genome sequence for the organism has yielded ~45 genes which may be directly or indirectly involved with calcium metabolism. This number is small on the scale of most eukaryotic genomes, and invites both systematic investigation and surgical engineering. The basis of quantitative functional models must necessarily be quantitative measurements, of intracellular calcium localization and of both intra- and extra-cellular Ca⁺⁺ fluxes.

To facilitate such measurements, we are constructing giant yeast cells in which light-microscopic localization should be simpler than in haploid yeast, and on which electrophysiological measurements should also be greatly simplified. In addition to cytoengineering, patch-clamp measurements are being carried out on a tonoplast calcium channel (YVC1; *P.N.A.S.* 87:7824, 1990), which is not yet identified with a structural gene. A systematic search for this coding sequence is being conducted by motif-scanning, test deletion, and patch-clamping of isolated yeast vacuoles. Two other potential approaches are the identification of high-affinity inhibitors, to be used as protein-purification tags; and drop-out searches of 2-D protein gels following deletion of genes for YVC1-associated proteins. A systematic patch-clamp search is also being conducted for the electrophysiological counterpart to a major plasma-membrane calcium channel, YGR217w.

An important spinoff of the plasma-membrane Ca⁺⁺-channel search is proving to be the detailed electrophysiological description of other small ion currents in the yeast plasma membrane, including a conspicuous low-affinity inward K⁺ current.

PROJECT CATEGORIZATION

What follows is a grouping of Energy Biosciences projects into a few major topic areas that characterize the overall objectives of the program. Projects that overlap different categories are marked by an asterisk (*), and are listed in both categories. Each project has been assigned a number which identifies the abstract found within this report.

1. PHOTOSYNTHESIS

The conversion of solar energy into chemical energy by photosynthesis is the energetic underpinning for life on earth that ultimately results in renewable resources. Important research topics include dissecting the numerous aspects of photosynthesis such as carbon fixation, oxygen evolution, photorespiration, photophosphorylation, structures of photosynthetic elements and other topics relating to photosynthesis under natural conditions. A great diversity of techniques ranging from ultrafast laser spectroscopy to site directed mutagenesis are being utilized from the molecular to the whole plant level. The intent is to understand the most critical biological energy conversion process upon which most life depends.

- Abs. 4 Consequences of Altering Rubisco Regulation
A.R. Portis, Jr., USDA - University of Illinois
- Abs. 5 Structure, Function and Regulation of Antenna Complexes of Green Photosynthetic Bacteria
R.E. Blankenship, Arizona State University
- Abs. 6 Chlorophyll-Binding and Biogenesis of Photosystem II
W. Vermaas, Arizona State University
- Abs. 17 Molecular Bases of Photobiological Consequences of Light Intensity Adaptation in Photosynthetic Organisms
P.G. Falkowski, Brookhaven National Laboratory
- Abs. 18 Regulation of Energy Conversion in Photosynthesis
G. Hind, Brookhaven National Laboratory
- Abs. 47 Molecular Genetic Analysis of Biophotolytic Hydrogen Production in Green Algae
L. Mets, University of Chicago
- Abs. 62 Molecular, Genetic and Physiological Analysis of Photoinhibition and Photosynthetic Performance in the Green Alga *Chlamydomonas reinhardtii*
J.E. Boynton, N.W. Gillham and C.B. Osmond, Duke University

- Abs. 81 Mechanisms Regulating psbD Transcription in Higher Plants
D.A. Christopher, University of Hawaii
- Abs. 82 Violaxanthin De-epoxidase: Structure, Regulation and Function
H.Y. Yamamoto, University of Hawaii
- Abs. 90 Transport of Ions Across the Inner Envelope Membrane of Chloroplasts
R.E. McCarty, Johns Hopkins University
- Abs. 91 Macromolecular Scaffolds for Energy Transfer
D.C. Youvan, KAIROS Scientific Inc.
- Abs. 93 Mechanism and Significance of Post-Translational Modifications in the Large
Subunit of Ribulose-Bisphosphate Carboxylase/Oxygenase
R.L. Houtz, University of Kentucky
- Abs. 96 Transferring Photosynthesis Genes to New Cellular Environments
J.E. Hearst, Lawrence Berkeley National Laboratory
- Abs. 98 Mechanism of Water Splitting and Oxygen Evolution in Photosynthesis
M.P. Klein and V.K. Yachandra, Lawrence Berkeley National Laboratory
- Abs. 99 Photosynthetic Pigment Proteins and Photosynthetic Light Reactions
K. Sauer, Lawrence Berkeley National Laboratory
- Abs. 136 The Water-Splitting Apparatus of Photosynthesis
M. Seibert, National Renewable Energy Laboratory
- Abs. 139 Role of the Rubisco Small Subunit
R.J. Spreitzer, University of Nebraska
- Abs. 150 Photosynthetic Electron Transport in Genetically Altered Chloroplasts
R.T. Sayre, Ohio State University
- Abs. 151 Regulation of Alternative CO₂ Fixation Pathways and Control of Redox Status of
Photosynthetic Microorganisms
F.R. Tabita, Ohio State University
- Abs. 160 Light-Energy Transduction in Green Sulfur Bacteria
D.A. Bryant, Pennsylvania State University
- Abs. 168 Membrane-Attached Electron Carriers in Photosynthesis and Respiration
F. Daldal, University of Pennsylvania
- Abs. 173 A Genetic Analysis of the Lumenal Proteins of the Photosystem II O₂-evolving
Complex in Cyanobacteria
L.A. Sherman, Purdue University

- Abs. 183 Molecular Mechanisms Controlling Proton Pumping by Bacteriorhodopsin
R.K. Crouch, Medical University of South Carolina
- Abs. 190 Ferredoxin-Linked Chloroplast Enzymes
D.B. Knaff, Texas Tech University
- Abs. 196* Structural Domains in NADPH: Protochlorophyllide Oxidoreductases Involved in
Catalysis and Substrate Binding
M.P. Timko, University of Virginia
- Abs. 211 Feedback Regulation of Photosynthetic Processes
T.D. Sharkey, University of Wisconsin
- Abs. 216 Spatial Regulation of C4 Genes in C3, C4, and C3/C4 Intermediate *Flaveria*
Species
T. Nelson, Yale University

2. MEMBRANES OR ION TRANSPORT

Membranes provide a selective barrier around a cell as well as delineate the organelles within the cell. Many critical metabolic processes are membrane "bound" or occur across membranes including key steps in the energy transducing processes of photosynthesis and respiration. Knowledge about the structure and properties of different membrane systems is essential to an understanding of how plants use the available photosynthetically derived energy and how organisms absorb, transport and utilize mineral ion nutrients in sustaining their growth, development and other synthetic activities.

- Abs. 11 Molecular Characterization of the Role of a Calcium Channel in Plant
Development
K.S. Schumaker, University of Arizona
- Abs. 14* Osmoregulation in Methanogens
M.F. Roberts, Boston College
- Abs. 35 Protein Translocation and Assembly in Chloroplasts
S.M. Theg, University of California - Davis
- Abs. 36* Vacuole Biogenesis in Differentiating Plant Cells
T.A. Wilkins, University of California - Davis
- Abs. 39 Molecular Structure, Function and Physiology of K⁺ Uptake Channels in Plants
J.I. Schroeder, University of California - San Diego
- Abs. 43 Regulation of Vacuolar pH in *Citrus limon*
L. Taiz, University of California - Santa Cruz

- Abs. 57 Effects of Freezing and Cold Acclimation on the Plasma Membrane of Isolated Cereal Protoplasts
P.L. Steponkus, Cornell University
- Abs. 104 Structure and Regulation of Calcium-Pumping ATPases in Plants
H. Sze, University of Maryland
- Abs. 117 Molecular Mechanisms of Trafficking in the Plant Cell
N.V. Raikhel, Michigan State University-DOE Plant Research Laboratory
- Abs. 170 Biochemical Basis of YCF1-Dependent Vacuolar Glutathione-S-Conjugate Transport
P.A. Rea, University of Pennsylvania
- Abs. 183 Molecular Analysis of a Thylakoid K⁺ Channel
C. Chin, Rutgers University
- Abs. 181 Membrane Targeting of P-type ATPases in Plant Cells
J. Harper, The Scripps Research Institute
- Abs. 187* Molecular Mechanism of Light-Regulated Protein Transport Across the Nuclear Envelope
A.G. von Arnim, University of Tennessee
- Abs. 193 Tandem-pore Outward-rectifying K⁺ Channels: Molecular Partners of the Proton ATPase in Membrane Potential Regulation
K.A. Ketchum, The Institute for Genomic Research
- Abs. 212 Molecular Mechanism of Energy Transduction by Plant Membrane Proteins
M.R. Sussman, University of Wisconsin
- Abs. 217 Calcium Mapping and Signalling in Yeast
C.L. Slayman, Yale University

3. PLANT METABOLISM AND BIOSYNTHESSES

One of the greatest resources plants have to offer is the ability to synthesize a massive variety of products that provide food, fibers, structural components, pharmaceuticals and numerous materials and chemicals for other uses. In order to have greater ability to use plants as a resource in the rapidly growing biotechnology industry, it is essential to build the base of understanding of not only the metabolic capabilities of plants, but how the various pathways are regulated. The availability of newer techniques for chemical analyses, in addition to the formidable tools of molecular genetics, have made it possible to probe questions that previously were almost intractable. Aspects of research on the major storage products, carbohydrates and lipids are listed as subheadings of this category.

- Abs. 20 δ -Aminolevulinic Acid Biosynthesis in Oxygenic Prokaryotes
S. Beale, Brown University
- Abs. 32 Plant Physiological Aspects of Silicon
E. Epstein, T.W-M. Fan, W.K. Silk, and R.M. Higashi, University of California -
Davis
- Abs. 49 The Magnesium Chelation Step in Chlorophyll Biosynthesis
J.D. Weinstein, Clemson University
- Abs. 51* Microbial Production of Isoprene
R. Fall, University of Colorado
- Abs. 65 Gene-Enzyme Relationships of Aromatic Amino Acid Biosynthesis in Higher
Plants
R.A. Jensen, University of Florida
- Abs. 77 Nitrogen Control of Chloroplast Development and Differentiation
G.W. Schmidt, University of Georgia
- Abs. 140 Asparagine Synthetase Gene Regulation and Plant Nitrogen Metabolism
G. Coruzzi, New York University
- Abs. 196* Structural Domains in NADPH: Protochlorophyllide Oxidoreductases Involved
in Catalysis and Substrate Binding
M.P. Timko, University of Virginia
- Abs. 198 Regulation of Terpene Metabolism
R. Croteau, Washington State University
- Abs. 201 Interdisciplinary Plant Biochemistry Research and Training Center
N.G. Lewis, Washington State University

3a. CARBOHYDRATE METABOLISM

Carbohydrates comprise a dominant component of the storage products and biomass of plants mostly in the forms of starch and the polymers of the cell wall. In addition to supplying the critical building blocks for plant metabolism and plant structure, carbohydrates are increasingly identified as important components of glycoproteins, glycolipids and polysaccharides, which have important regulatory functions in plant growth and development.

- Abs. 3 Regulation of Sucrose-Phosphate Synthase and Other Cytosolic Proteins by
Reversible Protein Phosphorylation
S.C. Huber, USDA - North Carolina State University

- Abs. 7 Polyol Functions in Stress Protection of Photosynthesis
H.J. Bohnert and R.G. Jensen, University of Arizona
- Abs. 63 Role of Sucrose in Modulating Stomatal Aperture
W.H. Outlaw, Jr., Florida State University
- Abs. 125 Structure-Function Relationships of ADP-glucose Pyrophosphorylase:
Manipulation of the Plant Gene for Increased Production of Starch in Plants
J. Preiss, Michigan State University
- Abs. 145 Control of Sugar Transport and Metabolism in *Zymomonas mobilis*
T. Conway, Ohio State University
- Abs. 164 Molecular-Genetic Analysis of Maize Starch Branching Enzyme Isoforms
M. Gultinan, Pennsylvania State University
- Abs. 175* A Molecular-Genetic Approach to Studying Source-Sink Interactions in
Arabidopsis thaliana
S.I. Gibson, Rice University
- Abs. 199* Carbon Metabolism in Symbiotic Nitrogen Fixation
M.L. Kahn, Washington State University
- Abs. 202* Enhancement of Photoassimilate Utilization by Manipulation of ADP-glucose
pyrophosphorylase
T.W. Okita, Washington State University

3b. LIPID METABOLISM

Within the past decade many of the enzymes involved in the biosynthesis of a variety of fatty acids and oils in plants have been identified, and commercial research is actively underway to genetically engineer plants to produce higher-value plant oils for food and industrial uses. However, many unanswered questions remain on how the biochemical pathways regulate which fatty acids are synthesized and how they are partitioned among the biosynthesis of membrane components, storage oils and other lipid constituents of the plant cell.

- Abs. 19 Modification of Plant Lipids
J. Shanklin, Brookhaven National Laboratory
- Abs. 44* Production of Lipophilic Materials from Plants
C.R. Somerville, Carnegie Institution of Washington
- Abs. 94 Studies of a Novel Pathway for Biosynthesis of Straight and Branched, Odd
and Even Length, Medium-Chain Fatty Acids in Plants
G.J. Wagner, University of Kentucky

- Abs. 124 Control of Triacylglycerol in Developing Oilseeds
J. Ohlrogge, Michigan State University
- Abs. 147* Biosynthesis of Hydrocarbons and Other Wax Components
P.E. Kolattukudy, Ohio State University
- Abs.197 Membrane Function in Lipid Mutants of *Arabidopsis*
J. Browse, Washington State University

4. PLANT GROWTH AND DEVELOPMENT

Clearly, the productivity of plants for maximal biomass requires knowledge about the nature of how cells and tissues expand and grow, as well as differentiate into the different organs of the plant. Over the last decade molecular genetic approaches have completely revitalized the manner in which plant growth and development are studied and are leading to significant advances in our understanding of these processes.

- Abs. 2 Metabolic Regulation of the Plant Hormone Indole-3-acetic Acid
J.D. Cohen, USDA - Beltsville, MD
- Abs. 10 Role of Zein Proteins in Structure and Assembly of Protein Bodies and
Endosperm Texture
B.A. Larkins, University of Arizona
- Abs. 21 Molecular and Genetic Analysis of LEAFY, a Gene Controlling Floral Induction
and Flower Development in *Arabidopsis thaliana*
E. Meyerowitz, California Institute of Technology
- Abs. 22 The Molecular Genetics of Ligule Induction
M. Freeling, University of California - Berkeley
- Abs. 26 Phytochrome from Green Plants: Properties and Biological Function
P.H. Quail, University of California - Berkeley
- Abs. 28 Analysis of Genes Essential for Floral Development in *Arabidopsis*
P. Zambryski, University of California - Berkeley
- Abs. 29 Where Do Nectaries Fit in the Floral Architecture?
J.L. Bowman, University of California, Davis
- Abs. 33 Regulation of Embryonic Development in Higher Plants
J.J. Harada, University of California - Davis
- Abs. 34* Cellular and Molecular Characterization of Vascular Plasmodesmata
W.J. Lucas, University of California - Davis

- Abs. 36* Vacuole Biogenesis in Differentiating Plant Cells
T.A. Wilkins, University of California - Davis
- Abs. 38 Structure, Biosynthesis and Role of Complex Protein-Bound Glycans
M.J. Chrispeels, University of California - San Diego
- Abs. 40 Suspensor Differentiation During Early Plant Embryogenesis
R.B. Goldberg, University of California, Los Angeles
- Abs. 42* Sensory Transduction of the CO₂ Response of Guard Cells
E. Zeiger, University of California - Los Angeles
- Abs. 48 Analysis of Cell-Cell Interactions during Arabidopsis Reproduction
D. Preuss, University of Chicago
- Abs. 54 Cytoplasmic Male Sterility and Mitochondrial Function During
Microsporogenesis
M.R. Hanson, Cornell University
- Abs. 55 Signal Transduction in the Pollen-Stigma Interactions of *Brassica*
J.B. Nasrallah and M.E. Nasrallah, Cornell University
- Abs. 61 Metabolic Mechanisms of Plant Growth at Low Water Potentials
J.S. Boyer, University of Delaware
- Abs. 66 Genetic Analysis of Abscisic Acid Biosynthesis
D.R. McCarty, University of Florida
- Abs. 97 Structural Basis of Plant Signal Transduction
S.-H. Kim and W. Gruissem, Lawrence Berkeley National Laboratory
- Abs. 103 Molecular and Genetic Analyses of TSO1 in Arabidopsis
Z. Liu, University of Maryland
- Abs. 105 Molecular Analysis of the Interactions Between Pollen Tube Components and
a Stylar Transmitting Tissue Pollen Tube Growth-Promoting and -Attracting
Protein
A.Y. Cheung and H.-M. Wu, University of Massachusetts
- Abs. 113 Action and Synthesis of Plant Hormones
H. Kende, Michigan State University-DOE Plant Research Laboratory
- Abs. 114 Photoperiodic Induction and the Floral Stimulus
H. Kende, L. McIntosh, J.A.D. Zeevaart, Michigan State University-DOE Plant
Research Laboratory

- Abs. 116 Sensory Transduction in Plants
K.L. Poff, Michigan State University-DOE Plant Research Laboratory
- Abs. 120* Developmental Biology of Nitrogen-fixing Cyanobacteria
C.P. Wolk, Michigan State University-DOE Plant Research Laboratory
- Abs. 122 Environmental Control of Plant Development and Its Relation to Plant
Hormones
J.A.D. Zeevaart, Michigan State University-DOE Plant Research Laboratory
- Abs. 128 CLV Signaling in Meristem Development
S. Clark, University of Michigan
- Abs. 162 Elongation Factor 1-a and the Plant Cytoskeleton
R. Cyr, Pennsylvania State University
- Abs. 167 Light Responses and Photoperiodism in *Arabidopsis thaliana*
A.R. Cashmore, University of Pennsylvania
- Abs. 175* A Molecular-Genetic Approach to Studying Source-Sink Interactions in
Arabidopsis thaliana
S.I. Gibson, Rice University
- Abs. 187* Molecular Mechanism of Light-Regulated Protein Transport Across the
Nuclear Envelope
A.G. von Arnim, University of Tennessee
- Abs. 203 Targeting and Processing of the Thiol Protease Aleurain
J.C. Rogers, Washington State University
- Abs. 205 Biochemical Characterization of the Ethylene Receptor ETR1 from
Arabidopsis thaliana
A.B. Blecker, University of Wisconsin

5. PLANT GENETIC REGULATION AND GENETIC MECHANISMS

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

- Abs.9 Regulation of DNA Endoreplication in *Zea mays* Endosperm
B.A. Larkins, University of Arizona
- Abs. 13 Role of HSP100 Proteins in Plant Stress Tolerance
E. Vierling, University of Arizona and S. Lindquist, University of Chicago
- Abs. 15 Differential Regulation of Plastid mRNA Stability
D.B. Stern, Boyce Thompson Institute for Plant Research, Inc.
- Abs. 16 Molecular Plant Genetics
B. Burr and F.A. Burr, Brookhaven National Laboratory
- Abs. 23 Cellular Integration of MVA Synthesis and Protein Prenylation
W. Gruissem, University of California - Berkeley
- Abs. 44* Production of Lipophilic Materials from Plants
C.R. Somerville, Carnegie Institution of Washington
- Abs. 50 The *lojap* Gene in Maize
R.A. Martienssen and M. Byrne, Cold Spring Harbor Laboratory
- Abs. 74 Plant Arginine Decarboxylase, Regulation and Function
R.L. Malmberg, University of Georgia
- Abs. 75 The Determinants of RNA Turnover in Higher Plants: The role of poly(A) binding proteins
R.B. Meagher, University of Georgia
- Abs. 78 Structure, Regulation and Evolution of the R Transcriptional Activators from Maize and Rice
S.R. Wessler, University of Georgia
- Abs. 80 Development of Innovative Techniques That May be Used as Models to Improve Plant Performance
W.W. Hanna and G.W. Burton, University of Georgia
- Abs. 87 Regulation of Carotenoid Biosynthesis: The *immutants* Variegation Mutant of *Arabidopsis*
S. Rodermel and D. Voytas, Iowa State University
- Abs. 107 Isolation of Genes Involved in a Novel Auxin Biosynthetic Pathway
J. Normanly, University of Massachusetts
- Abs. 109 Molecular Mechanisms That Regulate the Expression of Genes in Plants
P. Green, Michigan State University-DOE Plant Research Laboratory

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- Abs. 112 Chloroplast Biogenesis
K. Keegstra, Michigan State University-DOE Plant Research Laboratory
- Abs. 115 Interaction of Nuclear and Organelle Genomes
L. McIntosh, Michigan State University-DOE Plant Research Laboratory
- Abs. 132 Dosage Analysis of Gene Expression in Maize
J. Birchler, University of Missouri
- Abs. 133 Position Effect as a Determinant of Variegated Pigmentation in Maize
K.C. Cone, University of Missouri
- Abs. 144 Map Based Cloning of the *Enhancer of Gene Silencing 1* Locus Which Enhances the Silencing of a Foreign Gene in *Arabidopsis*
S.R. Grant, University of North Carolina
- Abs. 158 Genetic Analysis of Chloroplast Translation
A. Barkan, University of Oregon
- Abs. 166 Structural Basis of Signal and Energy Transduction in Plants
A.R. Cashmore, University of Pennsylvania
- Abs. 169 Molecular and Genetic Analysis of Hormone-Regulated Differential Cell Elongation in *Arabidopsis*
J.R. Ecker, University of Pennsylvania
- Abs. 178 Corn Storage Protein: A Molecular Genetic Model
J. Messing, Rutgers University
- Abs. 179 Signal Transduction Pathways that Regulate CAB Gene Expression
J. Chory, Salk Institute for Biological Studies
- Abs. 180 Regulated Expression of the RTBV promoter by RF2a, a Transcriptional Regulator
R.N. Beachy, The Scripps Research Institute
- Abs. 182 Nuclear Genes Regulating Translation of Organelle mRNAs
S. Mayfield, The Scripps Research Institute
- Abs. 192 Phosphorylation of Plant Protein Synthesis Initiation Factors
K.S. Browning, University of Texas
- Abs. 194 *Arabidopsis* Genome Sequencing Using Random Shotgun Sequencing of BAC Clones
J.C. Venter, The Institute for Genomic Research

- Abs. 202* Enhancement of Photoassimilate Utilization by Manipulation of ADP-glucose pyrophosphorylase
T.W. Okita, Washington State University
- Abs. 209 Organization of the *R* Chromosome in Maize
J. Kermicle, University of Wisconsin
- Abs. 213 Analysis of Structural Domains Required for Phytochrome Function by *in vitro* and *in vivo* Mutagenesis
R.D. Vierstra, University of Wisconsin

6. PLANT CELL WALL DEVELOPMENT

The most dominant biomass products are plant cell walls which consist of polysaccharides, lignins, proteins and other compounds. The category includes research on the synthesis, structure, function and other aspects of cell wall components. In approaching the multiple questions encompassed by this topic, a broad diversity of techniques are employed including fast atom bombardment mass spectroscopy, Raman spectroscopy, Nuclear Magnetic Resonance spectroscopy and numerous molecular biological procedures, among others. The objective is to insure our knowledge about this natural resource, which would result in greater productivity and also make it more utilizable.

- Abs. 1 Molecular Organization in the Native State of Wood Cell Walls: Studies of Tertiary Structure and its Development Using the Raman Microprobe, Solid State ¹³C NMR, Fluorescence Spectroscopy and Photoconductivity
R.H. Atalla, USDA - Madison, Wisconsin
- Abs. 8 Role of Pectolytic Enzymes in the Programmed Release of Cells from the Root Cap of Higher Plants
M.C. Hawes, University of Arizona
- Abs. 30 Biosynthesis of Cellulose and Callose in Developing Cotton Fibers
D.P. Delmer, University of California - Davis
- Abs. 53 Genetic Analysis of Sugar Nucleotide Interconversions in *Arabidopsis*
W.-D. Reiter, University of Connecticut
- Abs. 68 CarbBank - A Structural and Bibliographic Database for Complex Carbohydrates
P. Albersheim and S. Doubet, University of Georgia
- Abs. 69 The University of Georgia Complex Carbohydrate Research Center (CCRC)
P. Albersheim and A. Darvill, University of Georgia

- Abs. 70 The Structures and Functions of Oligosaccharins
P. Albersheim, University of Georgia
- Abs. 71 Structural Studies of Complex Carbohydrates of Plant Cell Walls
A. Darvill, University of Georgia
- Abs. 72 Mechanisms of Lignin Biosynthesis During Xylogenesis in *Zinnia elegans*
K-E.L. Eriksson and J.F.D. Dean, University of Georgia
- Abs. 76 Identification of Novel Cell Wall Components
M. Momany, University of Georgia
- Abs. 118 Cell Wall Metabolism
N.V. Raikel, K. Keegstra, H. Kende, and J.D. Walton, Michigan State
University-DOE Plant Research Laboratory
- Abs. 131 Cellulose Synthesis and Morphogenesis
T.I. Baskin, University of Missouri
- Abs. 142 Transcription Factors in Xylem Development
R. Sederoff, M. Campbell, R. Whetten and D. O'Malley, North Carolina State
University
- Abs. 153 The Structure of Pectins from Cotton Suspension Culture Cell Walls
A.J. Mort, Oklahoma State University
- Abs. 161 Biophysical and Molecular Mechanisms Controlling Plant Cell Growth
D.J. Cosgrove, Pennsylvania State University
- Abs. 171 Molecular Studies of β -D-Glucan Synthesis in Cereals
N.C. Carpita, Purdue University
- Abs. 172 Modification of Lignin Composition in Plants by Manipulation of ferulate-5-
hydroxylase Expression
C.C.S. Chapple, Purdue University
- Abs. 191 Molecular, Genetic, and Biochemical Analysis of Cellulose Synthesis in
Arabidopsis thaliana
R.M. Brown, Jr., and K. Sathasivan, University of Texas
- Abs. 200 A Comprehensive Approach to the Elucidation of Lignification at the Plasma
Membrane/Cell Wall Interface
N.G. Lewis, Washington State University

7. LIGNIN-POLYSACCHARIDE BREAKDOWN

The most abundant biomass consists of complex associations of polymers including lignin, cellulose and other polysaccharides that are not easily broken down to convert these polymers into other usable products. Clearly, organisms have been carrying out such conversions for eons, but the exact details of the mechanisms are lacking. Accordingly, this category dwells on basic studies dealing with the organismal and enzymatic conversion of the major constituents of lignocellulosic biomass into usable feedstocks. Such studies include genetic, biochemical and physiological approaches in learning how organisms do this so that it may be possible to carry forth these processes in a biotechnological manner.

- Abs. 31 Cellulose Binding Proteins of *Clostridium cellulovorans* Cellulase
R.H. Doi, University of California - Davis
- Abs. 59 Studies of the Genetic Regulation of the *Thermomonospora fusca* Cellulase
Complex
D.B. Wilson, Cornell University
- Abs. 73 Fermentation of Cellulose and Hemicelluloses by Clostridia and Anaerobic
Fungi
L.G. Ljungdahl, University of Georgia
- Abs. 106 Cellulose Fermentation by Nitrogen-Fixing Anaerobic Bacteria
S. Leschine, University of Massachusetts
- Abs. 126 Physiology and Molecular Biology of Lignin-Modifying Enzymes
C.A. Reddy, Michigan State University
- Abs. 155 Biochemical Genetics of Lignin Degradation by *Phanerochaete chrysosporium*
M.H. Gold, Oregon Graduate Institute of Science & Technology
- Abs. 156 Cellobiose Dehydrogenase and β -glucosidase from *Phanerochaete*
chrysosporium: Effect on Cellulose Hydrolysis, Cloning, and Characterization
V. Renganathan, Oregon Graduate Institute of Science & Technology
- Abs. 165 Characterization of Lignin and Mn Peroxidases from *Phanerochaete*
chrysosporium
M. Tien, Pennsylvania State University
- Abs. 176 Synergism and Interaction Between *Clostridium thermocellum* Major
Cellulosome Components, CelS and Cell
J.H.D. Wu, University of Rochester
- Abs. 206 Molecular Genetics of Ligninase Expression
D. Cullen, University of Wisconsin

- Abs. 208 Identification of the Primary Mechanism for Fungal Lignin Degradation
K.E. Hammel, University of Wisconsin

8. NITROGEN FIXATION AND PLANT-MICROBIAL SYMBIOSIS

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

- Abs. 46 Organization and Regulation of the Genes for Nitrogen Fixation in
Rhodobacter capsulatus
R. Haselkorn, University of Chicago
- Abs. 108 Molecular Basis of Symbiotic Plant-Microbe Interactions
F.J. de Bruijn, Michigan State University-DOE Plant Research Laboratory
- Abs. 120* Developmental Biology of Nitrogen-Fixing Cyanobacteria
C.P. Wolk, Michigan State University-DOE Plant Research Laboratory
- Abs. 121 Frankia Genetics
C.P. Wolk, T. Newman, S.Y. He and F.J. de Bruijn, Michigan State University-
DOE Plant Research Laboratory
- Abs. 123 The Role of Membrane and Surface Carbohydrates of Rhizobium in
Symbiosis
R.I. Hollingsworth, Michigan State University
- Abs. 157* Characterization of the Genes Involved in Nitrification
D.J. Arp and L.A. Sayavedra-Soto, Oregon State University
- Abs. 185 Nodulation Genes and Factors in the Rhizobium-Legume Symbiosis
S.R. Long, Stanford University
- Abs. 186 Plant Recognition of *Bradyrhizobium japonicum* Nod Factors
G. Stacey, University of Tennessee
- Abs. 199* Carbon Metabolism in Symbiotic Nitrogen Fixation
M.L. Kahn, Washington State University

9. MECHANISMS FOR PLANT ADAPTATION

Plant survival frequently depends on the ability to detect and resist attack from other organisms as well as the ability to grow under sub-optimal environmental conditions. How plants withstand pests and pathogens and contend with drought conditions, heat, salinity and other factors that deter growth is the emphasis of this category. Studies are aimed at discerning the mechanisms by which plants detect, adapt to, and survive such conditions. Once again effects using genetic, biochemical and physiological approaches are revealing the basic mechanisms plants employ to mitigate the organisms and environmental conditions that threaten their growth.

- Abs. 12 Phytoalexin Detoxification Genes and Gene Products: Implication for the Evolution of Host Specific Traits for Pathogenicity
H. VanEtten, University of Arizona
- Abs. 24 Determinants of Environmental Stress Tolerance by Bacteria on Leaves
S.E. Lindow, University of California - Berkeley
- Abs. 27 Molecular Analysis of Pathogen Recognition and Signal Transduction Events Specifying Plant Disease Resistance
B.J. Staskawicz, University of California - Berkeley
- Abs. 34* Cellular and Molecular Characterization of Vascular Plasmodesmata
W.J. Lucas, University of California - Davis
- Abs. 42* Sensory Transduction of the CO₂ Response of Guard Cells
E. Zeiger, University of California - Los Angeles
- Abs. 45 Powdery Mildew Disease Resistance
S.C. Somerville, Carnegie Institution of Washington
- Abs. 110 Molecular Biology of Plant-Bacterial Interactions
S.Y. He, Michigan State University-DOE Plant Research Laboratory
- Abs. 111 Regulation of Plant Anti-Herbivore Defense
G. Howe, Michigan State University-DOE Plant Research Laboratory
- Abs. 119 Biochemical and Molecular Aspects of Plant Pathogenesis
J.D. Walton, Michigan State University-DOE Plant Research Laboratory
- Abs. 137 Tomato Bushy Stunt Virus and DI RNAs as a Model for Studying Mechanisms of RNA Virus Replication, Pathogenicity and Recombination
T.J. Morris, University of Nebraska and A.O. Jackson, University of California, Berkeley

- Abs. 143 Novel Control of Signal Delivery from the *Pseudomonas syringae* *avrRPM1* Gene to *Arabidopsis thaliana*
J.L. Dangl, University of North Carolina
- Abs. 177 Characterization of the Systemic Acquired Resistance Immediate-Early Response to Salicylic Acid
N.-H. Chua, Rockefeller University

10. FERMENTATIVE MICROBIAL METABOLISM

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

- Abs. 64 Ethanol-Tolerant Biocatalysts for Fuel Ethanol Production
L.O. Ingram, University of Florida
- Abs. 88 Molecular Biology of Anaerobic Aromatic Biodegradation
C.S. Harwood, University of Iowa
- Abs. 154 Energetics and Kinetics of Syntrophic Aromatic Degradation
M.J. McInerney, University of Oklahoma
- Abs. 157* Characterization of the Genes Involved in Nitrification
D.J. Arp and L.A. Sayavedra-Soto, Oregon State University
- Abs. 174 Genetic and Biochemical Analysis of Solvent Formation in *Clostridium acetobutylicum*
G.N. Bennett and F.B. Rudolph, Rice University
- Abs. 184 Regulation of Alcohol Fermentation by *Escherichia coli*
D.P. Clark, Southern Illinois University
- Abs. 195 Enzymology of Acetone-Butanol-Isopropanol Formation
J.-S. Chen, Virginia Polytechnic Institute and State University

11. ONE AND TWO CARBON MICROBIAL METABOLISM

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

- Abs. 14* Osmoregulation in Methanogens
M.F. Roberts, Boston College
- Abs. 41 Molecular Biology and Genetics of the Acetate-Utilizing Methanogenic Bacteria
R.P. Gunsalus, University of California - Los Angeles
- Abs. 60 Nitrogen Fixation and its Regulation in the Archaeon *Methanosarcina barkeri*
S.H. Zinder, Cornell University
- Abs. 79 Biochemistry and Genetics of Autotrophy in *Methanococcus*
W.B. Whitman, University of Georgia
- Abs. 85 Exploratory Studies on the Bacterial Formation of Methane
R.S. Wolfe, University of Illinois
- Abs. 86 Characterization of the Membrane-Associated Methane Oxidation System in *Methylococcus capsulatus* Bath
A.A. DiSpirito, Iowa State University
- Abs. 92 Acetyl-CoA Cleavage and Synthesis in Methanogens: Mechanistic, Enzymological, and Metabolic Studies
E. DeMoll, University of Kentucky and D.A. Grahame, Uniformed Services University of the Health Sciences
- Abs. 100 Carbon Metabolism in Methylotrophic Bacteria
C.J. Unkefer, Los Alamos National Laboratory
- Abs. 102 Regulation of Catabolic Gene Transcription in the Methanogenic Archaea
K.R. Sowers, University of Maryland
- Abs. 127 Molecular Physiology of Succinic Acid-Based Fermentations in Anaerobes: Control of Chemical Yield by CO₂ Fixation and Electron Donors
J.G. Zeikus, Michigan State University

- Abs. 129 Genetics of Bacteria that Utilize One-Carbon Compounds
R.S. Hanson, University of Minnesota
- Abs. 138 Enzymology of Aceticlastic Methanogenesis
S.W. Ragsdale, University of Nebraska
- Abs. 148 Transmethylation Reactions During Methylo trophic Methanogenesis in
Methanosarcina barkeri
J.A. Krzycki, Ohio State University
- Abs. 149 Structure and Regulation of Methane Genes
J.N. Reeve, Ohio State University
- Abs. 163 Enzymological Studies of One-Carbon Reactions in the Pathway of Acetate
Utilization by Methanogenic Bacteria
J.G. Ferry, Pennsylvania State University
- Abs. 204 Genetics in Methylo trophic Bacteria
M.E. Lidstrom, University of Washington
- Abs. 207 Microbial Formaldehyde Oxidation
T.J. Donohue, University of Wisconsin
- Abs. 210 The Biochemistry, Bioenergetics and Physiology of the CO-dependent Growth
of *Rhodospirillum rubrum*
P.W. Ludden, University of Wisconsin

12. EXTREMOPHILIC MICROBES

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

- Abs. 25 Phylogenetic Analysis of Hyperthermophilic Natural Populations Using
Ribosomal RNA Sequences
N.R. Pace, University of California - Berkeley
- Abs. 37 Membrane Bioenergetics of Salt Tolerant Microorganisms
J.K. Lanyi, University of California - Irvine

- Abs. 52 Sugar Transport and Metabolism in *Thermotoga*
K.M. Noll and A.H. Romano, University of Connecticut
- Abs. 67 The Metabolism of Hydrogen by Extremely Thermophilic Bacteria
M.W.W. Adams, University of Georgia
- Abs. 101 Structure and Regulation of L-glutamate Dehydrogenase in Hyperthermophilic
Archaea
F.T. Robb and J. DiRuggiero, University of Maryland
- Abs. 141 Bioenergetic and Physiological Studies of Hyperthermophilic Archaea
R.M. Kelly, North Carolina State University
- Abs. 146 The Role of Multiple Transcription Factors in Archaeal Gene Expression
C.J. Daniels, Ohio State University
- Abs. 159 The Characterization of Psychrophilic Microorganisms and Their Potentially
Useful Cold-Active Glycosidases
J.E. Brenchley, Pennsylvania State University

13. MICROBIAL RESPIRATION, NUTRITION AND METAL METABOLISM

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

- Abs. 51* Microbial Production of Isoprene
R. Fall, University of Colorado
- Abs. 56 Regulation of Denitrification in *Rhodobacter sphaeroides*
J. Shapleigh, Cornell University
- Abs. 58 Genetic Control of Nitrate Assimilation in *Klebsiella oxytoca* (pneumoniae)
V.J. Stewart, Cornell University
- Abs. 83 Heavy Metal-lux Sensor Fusions and Gene Regulation
S. Silver, University of Illinois - Chicago
- Abs. 84 Studies on the bo_3 -type Ubiquinol Oxidase from *Escherichia coli*
R.B. Gennis, University of Illinois
- Abs. 89 Bacterial Nickel Metabolism and Storage
R.J. Maier, Johns Hopkins University

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- Abs. 130 Isolation and Characterization of Ammonia Monooxygenase of Nitrosomonas
A.B. Hooper, University of Minnesota
- Abs. 134 Genetics of the Sulfate-Reducing Bacteria
J.D. Wall and B.J. Rapp-Giles, University of Missouri
- Abs. 135 The Respiratory Chain of Alkaliphilic Bacteria
T.A. Krulwich, Mount Sinai School of Medicine
- Abs. 152 Mechanisms of Microbial Adaptation
F.R. Tabita, Ohio State University
- Abs. 147* Biosynthesis of Hydrocarbons and Other Wax Components
P.E. Kolattukudy, Ohio State University
- Abs. 189 Role of Glycolytic Intermediations in Global Regulation and Signal
Transduction
J.C. Liao, Texas A&M University
- Abs. 214 Molecular Characterization of Bacterial Respiration on Minerals
R. Blake II, Xavier University of Louisiana
- Abs. 215 Biochemistry of Dissimilatory Sulfur Oxidation
R. Blake II, Xavier University of Louisiana

14. MATERIALS BIOSYNTHESIS

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

- Abs. 95 Biomolecular Materials
M.D. Alper, C. Bertozzi, D. Charych, J.F. Kirsch, D.E. Koshland, P.G. Schultz,
R. Stevens, and C.-H. Wong, Lawrence Berkeley National Laboratory
- Abs. 188 Novel Biomaterials: Genetically Engineered Pores
H. Bayley, Texas A&M University

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