DOE/ER-0676

Division of Energy Biosciences
Annual Report and Summaries of FY 1995 Activities

April 1996

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

Cover Picture: Azaleas in bloom. Water color by Robert Rabson.

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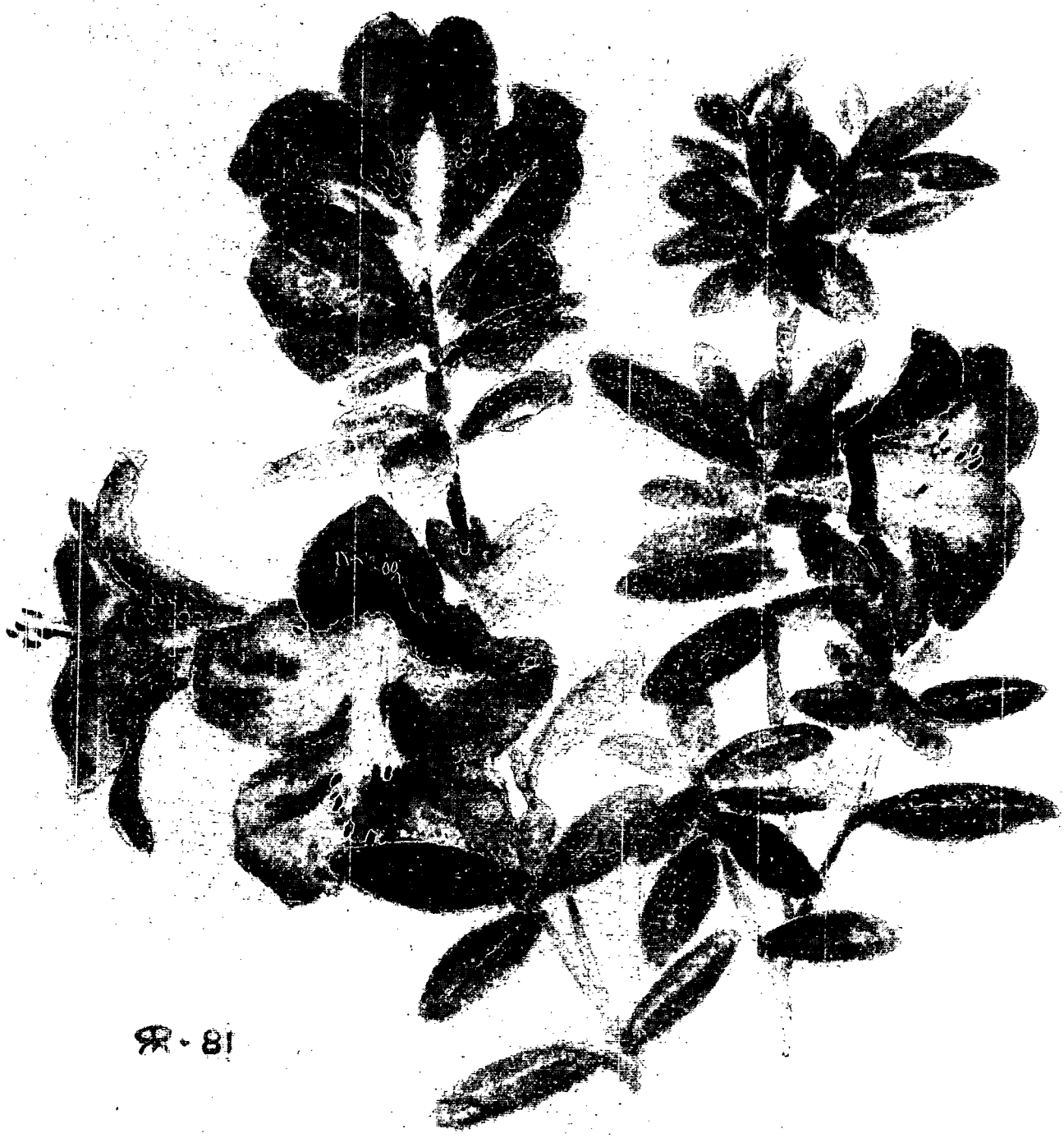

U.S. Department of Energy
Office of Energy Research
Office of Basic Energy Sciences
Division of Energy Biosciences
Germantown, MD 20874-1290

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. The departmental civilian 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 have 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, now supports nine multi-institutional research coordinating group awards and eleven interdisciplinary research training group awards. The three agency programs have done much to stimulate plant science research nationwide on university campuses. The Division is also actively participating in the *Arabidopsis thaliana* Genome Sequencing project, an international effort designed to elucidate the genomic structure of this important model plant system. The National Institutes of Health, the NSF and the USDA are also participating in this coordinated effort.

The Energy Biosciences program has supported many scientific advances in its sixteen years of existence. Current research on the ethylene signal transduction pathway in plants is one example. Ethylene is a relatively chemically simple gas that acts as a plant hormone regulating multiple plant processes ranging from stem elongation to root growth and fruit ripening. The recent isolation of genes in the ethylene signalling pathway reveals that plants sense this gaseous hormone through a combination of proteins that resemble signal transduction pathways previously described in bacteria and yeast. Genetic manipulation of these proteins will provide new tools to modify plant growth and development. Plants monitor the light environment (darkness, sunlight or shade) through the pigment phytochrome The genes that code for a family of phytochromes have given considerable information on specific photosensory functions and how the pigments interact with signal molecules that transduce changes in light quantity and quality into altered plant developmental patterns from leaf expansion to the induction of flowering. There has also been considerable progress made in determining how plants respond to invading pathogens. The recent cloning of plant resistance genes has shown that there appear to be common mechanisms for resisting a wide range of unrelated pathogens. Last summer's commercial production of lauric acid using

a genetically-engineering relative of mustard shows the promise of biotechnology to provide volume chemicals and potential fuels at both low energy and economic costs. The basic research activities that led to the development of this plant involved developing gene transfer technologies, determining how to control the location and timing of gene expression, determining the metabolic pathways leading to plant lipid synthesis, and studies on the genetic regulation of plant lipid synthesis.

While these examples of success are impressive, there are still a number of fundamental research areas in need of attention to continue to progress in the development of energy-related biotechnologies. There is an urgent need for more research related to microbial physiology. The new tools provided by molecular biology are providing new, exciting research opportunities in this area of importance to both science and future biotechnology in industry. Also, extensive characterization of a plant genome is expected to show the scope of the metabolic potential of plants to develop radically new products and processes. Thus, the need for fundamental studies in plant metabolism and biochemistry will become more obvious. Basic studies are also needed to determine the mechanisms of plant ion absorption, transport and accumulation. The mechanistic role microorganisms play in the movement of ions is also understudied. Understanding these complex interactions are essential for future biotechnologies to be effective in environmental restoration and sustainability.

In an effort to facilitate the exchange of scientific data and concepts, as well as bring potential research opportunities to the attention of the scientific community, the Division provided partial support during fiscal year 1995 for the following conferences, workshops or training activities:

1. Bacterial Locomotion and Signal Transduction, Austin, TX, January 12-16, 1995

2. Conference on Signal Transduction in Plants, Hilton Head Island, SC, March 29-April 4, 1995

3. Plant Mitochondria: From Gene to Function, Durham, NC, April 7-12, 1995

4. Symposium on Topics in Rumen Microbiology, The Environmental Applications of Microorganisms and Biodiversity, Urbana, IL, May 19-20, 1995

5. Sixth International Conference on *Arabidopsis* Research, Madison, WI, June 7-11, 1995

6. 15th International Conference on Plant Growth Substances, Minneapolis, MN, July 14-18, 1995

7. Investigations into the Metabolic Diversity of Microorganisms as Part of Microbial Diversity, Woods Hole, MA, Summer 1995

8. Workshop to Establish Databases of Carbohydrate Spectra, Seattle, WA, August 18-20, 1995

9. 8th International Symposium on Microbial Growth on C1 Compounds, San Diego, CA, August 27-September 1, 1995

10. Conference on Biodiversity, Ecology and Evolution of Thermophiles from Yellowstone National Park: Overview and Issues, Yellowstone National Park, September 17-20, 1995

11. Conference on Signalling in Plant Development, Cold Spring Harbor, NY, September 27-October 1, 1995

12. Conference on the Extracellular Matrix of Plants: Molecular, Cellular and Developmental Biology, Tamarron, CO, March 15-21, 1996

The Division continues to participate in the Life Sciences Research Foundation post-doctoral fellowship program, with three-year fellowship support for the following individuals whose fellowships were active during all or part of FY 1995:

Dr. Zhongchi Liu (Div. of Biology, California Inst. of Technology, Pasadena)
Dr. Erica J. Pascal (Dept. Microbiology, University of Illinois, Urbana)
Dr. Paul Blount (Lab of Molecular Biology, University of Wisconsin, Madison)
Dr. David Lerner (Biology Dept., University of California, San Diego)
Dr. Peter Margolis (Dept. Biological Sci., Stanford University, Stanford, CA)
Dr. Hank Bass (University of California, Berkeley)
Dr. Krishna Niyoga (Dept. Plant Biology, Carnegie Inst. of Washington, Stanford, CA)
Dr. Jian-Kang Zhu (Lab of Plant Molecular Biology, Rockefeller U., New York)
Dr. David Weiss (Dept. Micro. & Mole. Genetics, Harvard Medical School)
Dr. S.P. Dinesh-Kumar (Plant Gene Expression Center, Albany, CA)
Dr. Andrew Millar (University of Virginia, Charlottesville)

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

The Division of Energy Biosciences was provided close to twenty nine million dollars in fiscal year 1995. The breakdown of how the resources were distributed is indicated in the following table.

	Number of Projects	FY 95 funding (in thousands)	Percent of total funds
University and Non-Profit Institutions	200	20,710	72
Michigan State University Plant Research Laboratory	11	2,650	9
National Laboratories Brookhaven National Lab Lawrence Berkeley Lab Los Alamos National Lab National Renewable Energy Lab.	11	2,316	8
Small Business Innovation Research (SBIR) contribution, and Miscellaneous		2,710	9
Conferences, Educational Activities	15	467	2
Databases (joint funding)	1	100	>1
	238	$28,953	

The Division suffered a significant loss during this fiscal year in the passing of one of the program's long time grantees, Dr. Joe Varner. Dr. Varner was a true leader in plant science research and a longstanding excellent contributor to the program, most recently in the area of plant cell wall architecture.

In March 1995, Dr. Robert Rabson retired as Director of the Division of Energy Biosciences. Dr. Rabson was Director since the program was formed in 1979. His experience and insight is shown by the quality and quantity of the work supported during his tenure as Director. Upon his retirement, the Department of Energy honored him with an award for his years of service that had the following inscription:

> "In recognition of 33 years of distinguished Federal career service. For your support of the advancement of basic knowledge in the plant and microbial sciences, and your leadership and advice to the plant science research community. Your accomplishments are greatly appreciated by the Department of Energy, its predecessors and in particular the Office of Energy Research."

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:

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

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Abstracts of Projects Supported in FY 1995

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 (2 years)

Our studies of wood cell wall structure include both a program of measurements of the range of variation of molecular composition and organization within the walls, and an effort to develop a deeper understanding of the processes of structure formation. This latter effort has led us to seek an overarching paradigm for the assembly of the secondary cell wall. Currently information concerning the biosynthesis of the three major constituents of secondary walls is organized within the frameworks of three distinct and independent paradigms. We believe the biogenesis of the secondary wall involves significant coupling between the aggregative processes of cellulose and the hemicelluloses, and between the organization of the polysaccharide matrix and the polymerization of lignin precursors, whether they be monomers or oligomers. The methods we use to characterize molecular organization in native tissues include Raman spectroscopy and the Raman microprobe, solid state ^{13}C NMR, fluorescence spectroscopy, and photoconductivity measurements. We have complemented these methods with liquid state NMR, UV-visible absorption spectroscopy, theoretical modeling of intermolecular interactions, and traditional chemical analytical procedures. Our observations point to: (1) a significant influence of hemicelluloses on the aggregation of cellulose; (2) strong associative interactions between lignin precursors and cell wall polysaccharides; (3) a high degree of organization and coherence of order in native lignin leading to the occurrence of pathways for electronic charge transport within woody tissue. These observations have led us to propose that one of the important functions of the hemicelluloses is to organize the structure of lignin, through association with the precursors prior to their polymerization.

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

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

J.D. Cohen and J.P. Slovin, Beltsville Agricultural Research Center $86,700

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 biochemistry of how auxin is made 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. 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 four fundamental problems related to how plants get their IAA: 1) the biochemistry of the non-tryptophan pathway to auxin; 2) how a plant decides to use one or the other pathway, or both, during various developmental stages or in response to environmental stresses and stimuli; 3) the regulation and control of free IAA by enzymes that form or hydrolyze conjugates; and 4) auxin turnover, i.e. the rate at which IAA is made and destroyed. Recent studies have shown that during periods of stress, such as high or low temperature, the rates of IAA turnover increases markedly even when IAA levels appear unaffected.

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, USDA/ARS and Departments of Crop Science and Botany, NCSU

$69,905 (FY 94 funds/18 months)

Studies have been continued to characterize the control of sucrose-phosphate synthase (SPS), a key enzyme of sucrose biosynthesis, by seryl phosphorylation. We have shown that in spinach, SPS can be phosphorylated/inactivated *in vitro* by two distinct protein kinases, designated as peak I and III, based on order of elution during chromatography on FPLC-ResourceQ. Peak I kinase is dependent on micromolar [Ca^{2+}] whereas peak III is strictly Ca^{2+}-independent. In maize leaves, however, there is only a single protein kinase that inactivates SPS (SPSk) and it is strictly Ca^{2+}-dependent. The maize SPSk is similar to the spinach peak I enzyme in native molecular mass (45 versus 47-kDa), and elutes at the same salt concentration during ion exchange chromatography. Evidence has been obtained which is consistent with Ser-162 as the major regulatory phosphorylation site on maize SPS. A synthetic peptide corresponding to the putative maize phosphorylation site is readily phosphorylated by maize SPSk *in vitro* in a Ca^{2+}-dependent manner. The recognition sequence for SPSk (spinach and maize) appears to be:

-Basic-Hydrophobic-X-Basic-X-X-S-X-

SPSk activity has also been detected in tobacco and tomato leaves, and like maize, the activity is strictly Ca^{2+}-dependent. Thus, spinach is unusual in having a form of SPSk that is independent of Ca^{2+}. These results suggest that there is a role for cytosolic Ca^{2+} in the regulation of SPS activity in maize, tobacco, tomato and possibly spinach. We have also obtained preliminary evidence that sucrose synthase is phosphorylated on seryl residues *in vivo* in the elongation zone of maize leaves. There appears to be a single major phosphorylation site (by 2-D peptide mapping) and the site is conserved in both isoforms of the maize enzyme (SS1 and SS2, the products of the *Sh1* and *Sus1* genes, respectively).

If the phosphorylation is found to be of regulatory significance, these results raise the possibility that both aspects of sucrose metabolism, i.e., synthesis and degradation, may be controlled by protein phosphorylation.

U.S. Department of Agriculture
Ithaca, NY 14853

4. Phytoremediation of Metal-Polluted Soils: Mechanisms of Heavy Metal Absorption, Translocation, Accumulation and Tolerance in Plants

L.V. Kochian, U.S. Plant, Soil and Nutrition Laboratory $182,000 (18 months)

Heavy metal contamination of the environment poses serious problems to both human health and agriculture. Recently, there has been increased interest in the use of higher plants to remediate heavy metal-polluted soils. A small number of plant species have been identified that can grow in soils containing high levels of heavy metals, and will also accumulate these metals to high concentrations in the shoot. Currently, there is little fundamental information in the literature concerning the mechanisms of heavy metal transport, translocation and tolerance in these fascinating hyperaccumulator species. An interdisciplinary approach integrating physiological, biochemical and biophysical methodologies will be used to elucidate the cellular aspects of root heavy metal uptake, translocation to the shoot and the genetic bases of heavy metal tolerance and transport. We propose to study and characterize the transport and translocation of the heavy metals Cd^{2+}, Zn^{2+}, Cu^{2+} and Pb^{2+} in several hyperaccumulator species we have acquired. Investigations into the mechanisms and regulation of root heavy metal uptake will include radiotracer measurements of unidirectional heavy metal influx, studies into the dynamics and spatial aspects of cellular ion uptake with vibrating Cd^{2+}, Pb^{2+} and Cu^{2+} microelectrodes, and transport experiments using root plasma membrane vesicles for the study of the role of a putative divalent cation channel in heavy metal uptake. Radiotracer flux techniques will also be used to quantify heavy metal translocation to the shoots of the hyperaccumulator and non-accumulator species, and several different analytical approaches will be used to identify potentially important metal-binding organic ligands in the xylem and phloem of the different plant species.

Arizona State University
Tempe, AZ 85287-1604

5. Antenna Organization and Regulation in Green Photosynthetic Bacteria

R.E. Blankenship, Department of Chemistry and Biochemistry
$215,340 (FY 94 funds/2 years)

All photosynthetic organisms contain chlorophyll pigments that function as an antenna, absorbing light and transferring excitations to a photochemical reaction center where energy

storage takes place by a series of chemical reactions. The green photosynthetic bacteria are characterized by large antenna complexes known as chlorosomes. The overall objective of this project is to determine the molecular organization of pigments in chlorosome antennas, 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. Coherent oscillations are also observed in the transient absorbance profiles. Green sulfur bacteria contain a redox-activated mechanism for control of energy transfer efficiency. At high redox potential, most excitations are quenched within the chlorosome, while at low redox potentials almost all are transferred to the reaction center. The quenching effect may be a control mechanism that protects the cell from damage during conditions where light and oxygen are present simultaneously. Cloning, expression and X-ray structural studies are underway for the Fenna-Matthews-Olson bacteriochlorophyll a antenna protein, which is an intermediate in the energy transfer pathway from chlorosome to reaction center.

Arizona State University
Tempe AZ 85287-1601

6. Chlorophyll Binding and Biogenesis of Photosystem II

W.F.J. Vermaas, Department of Botany $99,000

The process of photosynthesis critically depends on the interplay of pigments and proteins to form photosynthetic pigment-protein complexes that efficiently convert light energy to chemical energy; this energy is used by photosynthetic organisms to fix carbon dioxide and to produce carbohydrates. A major aim of our research is to determine how chlorophyll biosynthesis and biogenesis of chlorophyll-binding proteins is regulated. In the light, free chlorophyll in thylakoid membranes would be very damaging to the cell, as triplet chlorophyll can react with oxygen to form the highly reactive singlet oxygen species. Therefore, chlorophyll should always be in the vicinity of efficient triplet quenchers such as carotenoids. Newly synthesized chlorophyll may become part of a carotenoid-containing protein that acts as a transporter of chlorophyll to nascent photosynthetic protein complexes. In a cyanobacterial system that is very accessible to molecular-genetic approaches such as directed gene inactivation, we have now designed a method to follow concurrently chlorophyll synthesis and biogenesis of photosystem II, a major photosynthetic pigment-protein complex. By genetic deletion of photosystem I (the other major pigment-protein complex in thylakoids) and the light-independent chlorophyll biosynthesis pathway, a system is created where light triggers both chlorophyll synthesis and photosystem II biogenesis. However, biogenesis of photosystem II lags by several hours. We observed that newly synthesized chlorophyll is first associated with a protein that fluoresces at 685 nm at 77 K, and subsequently is used for

assembly of a functional photosystem II reaction center complex. The nature of the intermediate chlorophyll-binding protein is now being elucidated.

University of Arizona
Tucson, AZ 85721

7. Polyol Function in Stress Protection of Photosynthesis through Ion Partitioning

H.J. Bohnert and R.G. Jensen, Department of Biochemistry $109,000

Water stresses act on stomatal conductance and affect photosynthetic carbon assimilation and metabolism of carbon. We focus on the engineered synthesis of osmoprotective substances that are found in a number of water stress-tolerant species. These accumulating metabolites serve an osmoprotective function. A significant amount of carbon in the biosphere is stored as acyclic and cyclic polyols. Different polyols are present in virtually every plant, albeit in higher plants they are usually found in small amounts. Using gene transformations, we have diverted carbon into the production of polyols and are investigating effects of their presence on photosynthesis, biomass production and environmental stress tolerance in transgenic plants. The introduced genes lead to the production of either mannitol, sorbitol, *myo*-inositol, or ononitol (methyl-inositol) in tobacco and *Arabidopsis*. Transgenic plants are phenotypically normal unless polyol production exceeds sucrose production by orders of magnitude in which case the plants show osmotic stress symptoms, indicating that, for example, in tobacco these polyols are not transported from the site of synthesis. Polyols confer increased salt stress tolerance albeit to different degrees depending on the type of polyol that accumulates. We are now focussing on four topics: (1) measurements of carbon flux through the engineered pathways, (2) the relation of polyol accumulation to free radical detoxification, (3) analysis of polyol functions in different compartments and (4) on effects of polyols on ion partitioning.

University of Arizona
Tucson, AZ 85721

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

M.C. Hawes, Departments of Plant Pathology and Molecular & Cellular Biology
$195,200 (FY 94 funds/2 years)

Roots of many species are programmed to produce and separate thousands of living cells, root "border" cells, from their root tips daily. The controlled release of living somatic cells into the environment is unique among higher organisms, and its function is unknown. The objective of this work is to test the hypothesis that cell wall degrading enzymes in the root cap

of pea play a role in cell wall degradation leading to border cell separation. Activities of a pectinmethylesterase (PME) and a polygalacturonase (PG) are correlated with deesterification of pectin in the root, with changes in cell wall pH, and with border cell separation. A root cap expressed PME cDNA has been cloned, and its mRNA has been shown to be induced within 5 minutes of inducing renewed border cell separation. Concomitant with this induction is a holistic switch in gene expression in the root cap. Induction of numerous genes occurs sequentially and can be plotted with respect to time and to cellular location within the cap. It may be possible to exploit this phenomenon to identify new, previously unknown plant genes that play important roles in cell wall biosynthesis and degradation.

University of Arizona
Tucson, AZ 85721

9. Rhizosphere Association of the Nitrogen FIxing Bacterial Species Azotobacter paspali with the tropical grass Paspalum notatum: Specificity and Significance to Plant Nutrition

C. Kennedy, Department of Plant Pathology $142,500 (18 months)

While a large number of nitrogen fixing bacteria that associate with monocots have been identified, few have been studied in sufficient detail to know whether significant fixed N can be provided to the plant partner by bacterial nitrogen fixation. Little is also known about process by which such bacteria colonize monocot roots or the specificity of the interaction. There is some preliminary and circumstantial evidence that the rhizosphere association between *Azotobacter paspali* and *Paspalum notatum* cb. Batatais is highly specific and may be effective in providing significant fixed N for plant nutrition. We have developed methods for production of replicate sterile plantlets that will be inoculated with wild type *A. paspali* and with two types of mutant derivatives: those that are Nif$^-$ (mutated in the *nifH* gene) and unable to fix nitrogen and those that are mutated in the *nifL* gene and excrete significant amounts of ammonium into the environment. These experiments should establish whether *A. paspali* stimulates growth of *Paspalum notatum* by transfer of fixed nitrogen. Studies of colonization of *P. notatum* by *A. paspali*, other Azotobacter species, and other soil microbes will indicate whether the association between *A. paspali* and *P. notatum* is truly specific. If so, genes involved in specificity and the colonization process will be identified.

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 $138,329

The texture of maize seeds is significantly influenced by qualitative and quantitative changes in the synthesis of endosperm storage proteins, zeins. Zeins occur in four structurally-distinct forms: the alpha-, beta-, gamma- and delta-zeins. They are synthesized on rough endoplasmic reticulum membranes (RER) and aggregate within the lumen of the RER where they remain and associate into insoluble accretions called protein bodies. The beta- and gamma-zeins, which are cysteine-rich proteins cross-linked by disulfide bonds, occur primarily at the surface of the protein body, while the alpha- and delta-zeins are found mainly in the center. Mutations that reduce the synthesis of alpha-zeins result in small protein bodies and generally cause the endosperm to develop a soft, starchy texture. Mutations that reduce gamma-zein synthesis, e.g. *opaque*15, appear to affect the number, rather than the size of the protein bodies, and also cause a soft kernel. Over expression of gamma-zein genes can convert soft endosperm mutants, such as *o*2, into seeds with a normal vitreous phenotype,and this appears to result from an increased number of protein bodies. We have recently characterized the starchy endosperm mutant, *floury*2, which uniformly reduces zein synthesis. Protein bodies in *fl*2 are small like *o*2, but they grow irregularly and accumulate large amounts of a BiP-type chaperone. We have shown that the *fl*2 mutant is associated with a defect in the signal peptide of a 22-kD alpha-zein protein that prevents its cleavage and results in accumulation of the precursor protein in protein bodies. Other phenotypic effects of *fl*2, e.g., regulation by *o*2, induction of BiP, asymmetric organization of alpha-, beta-, and gamma-zeins, can be explained on the basis of such a defect. Experiments are in progress to confirm the basis of the *fl*2 phenotype by creating transgenic maize plants that produce the defective zein protein. To examine the interactions between the various types of zeins that lead to protein body formation, we have expressed genes encoding the four different types of zein proteins in transgenic tobacco plants using rice glutelin promoters. The transgenic plants expressing alpha- and gamma-zein genes produce the proteins in developing seeds, but only gamma-zein is stably accumulated in the mature seed. However, if the two plants are crossed, both alpha- and gamma-zeins accumulate in the mature seed. Using metrizamide/sucrose gradient centrifugation, we have been able to recover protein aggregates from developing transgenic seeds with the properties of protein bodies. Experiments are in progress to immunologically localize these proteins *in situ* in developing tobacco seeds. In addition, we have begun to dissect the structure of the gamma-zein protein to investigate amino acid sequences that interact with the alpha-zein and promote its accumulation in the RER.

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 $88,929

During development, plants convert physiological signals into specific growth responses. The pathway from these physiological cues to a new developmental program involves biochemical and molecular changes in the cells. In several species of moss, addition of the hormone cytokinin to cells of the proper developmental stage causes an increase in intracellular calcium which stimulates a cascade of events leading to the formation of buds. Our research is examining the biochemical and molecular properties of a calcium channel that is involved in early events in moss vegetative bud formation.

By studying calcium influx into moss protoplasts we have shown that the moss calcium channel has similar transport characteristics (voltage-dependence, antagonist and agonist sensitivity) to voltage-dependent calcium channels in animal cells. Studies of binding of a channel antagonist (azidopine) to moss plasma membranes have shown that the number of antagonist binding sites and the affinity of the receptor for azidopine are sufficiently high to make identification of the channel protein feasible. Calcium transport and ligand binding studies have shown that the channel is uniquely regulated by cytokinin. Studies are in progress to identify the channel polypeptide by covalent binding of azidopine to moss plasma membranes. In addition, several DNA fragments which may encode the channel protein have been isolated and are being characterized. Using stage-specific moss cell cultures, we are looking at the regulation and expression of the channel during bud formation. Understanding the functional properties of the channel will help us determine the molecular mechanisms underlying calcium regulation and calcium's role in plant development.

University of Arizona
Tucson, AZ 85721

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

H.D. VanEtten, Department of Plant Pathology $94,685

Isolates of the fungus *Nectria haematococca* pathogenic on pea are able to detoxify the pea phytoalexin, pisatin, via a cytochrome P-450-mediated demethylation. In order to examine the role of pisatin demethylating ability (Pda) on pathogenicity, mutants of *N. haematococca* deficient in Pda were created by transformation-mediated gene disruption. Eleven Pda$^-$ transformants were obtained as determined biochemically by their inability to demethylate pisatin. All of the Pda$^-$ mutants were more sensitive to pisatin and less virulent on pea than

the wild type parent isolates or a Pda^+ transformant. Four transformants which had markedly reduced virulence on pea were found to lack the 1.6-Mb chromosome which carries the gene (*PDA1*) for Pda in the parent isolates. Lesions from pea plants infected with Pda^- transformants contained more pisatin per gram fresh weight of tissue than did lesions incited by the wild type isolate or a Pda^+ transformant. In addition lesion tissue produced by either a Pda^- laboratory isolate or a Pda^- transformant did not appear to contain a novel pisatin degrading activity of fungal origin. These results show that while Pda has a measurable role in pathogenesis on pea, other factors are required for high virulence and that one or more of these additional factors are located on a dispensable chromosome in *N. haematococca*.

University of Arizona
Tucson, AZ 85721

13. Role of HSP100 Proteins in Plant Stress Tolerance

E. Vierling and S. Lindquist, Department of Biochemistry and The Howard Hughes Medical Institute, University of Chicago $150,009 (18 months)

High temperature stress can severely limit crop productivity. During such stress plants express heat shock proteins (HSPs) which are hypothesized to protect them from heat damage. The proposed research will investigate whether expression of a specific HSP, HSP100, can be manipulated to increase plant thermotolerance. The yeast HSP100 protein, HSP104, has been shown to be essential for the development of thermotolerance in yeast. Moreover the induction of HSP104 alone is sufficient to provide a high degree of thermotolerance. We have shown that an *Arabidopsis* homolog of yeast HSP104 will complement the thermotolerance defect of yeast HSP104 mutants. Therefore we propose that *Arabidopsis* HSP100 is a logical target for manipulation to increase plant thermotolerance. Because of the conservation of the HSPs, results obtained with the model plant *Arabidopsis* should be directly applicable to major crop species. We will characterize the HSP100 gene family and examine the expression of these genes during heat and other stresses as well as during development. Antisense HSP100 genes will be transformed into *Arabidopsis* to test the role of HSP100 in thermotolerance. The possibility of using HSP100 to increase thermotolerance will be explored by generating transformed plants which overexpress the protein. Finally we will search for mutations in yeast HSP104 that enhance or inhibit thermotolerance, and identify proteins from yeast and *Arabidopsis* that interact with HSP100.

Boston College
Chestnut Hill, MA 02167-3860

14. Osmoregulation in Methanogens
M.F. Roberts, Merkert Chemistry Center $170,481 (FY 94 funds/2 years)

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 ^{1}H NMR studies of *Methanococcus thermolithotrophicus* and *Methanohalophilus* strain FDF1 using soluble ^{13}C-labeled substrates for methanogenesis to monitor organic solute production, uptake, or loss upon alteration of external NaCl, (2) ^{39}K and ^{23}Na relaxation studies of these organisms to characterize environmental differences for ions in low and high NaCl cultures, (3) HPLC analysis of organic solutes secreted by the cells during growth, and (4) characterization of two key enzymes involved in the salt-sensitive accumulation of the osmolytes N^{Σ}-acetyl-β-lysine and cyclic-2,3-diphosphoglycerate. The first of these provides a continuous assay of how the cells alter their intracellular solutes in response to different medium conditions. The second uses multiple quantum NMR to quantify changes in macromolecule and ion interactions with altered external NaCl (this may be critical information since *in vitro* studies have indicated that K^{+} concentrations are linked to osmolyte accumulation). The third study is aimed at understanding why some solutes are secreted by these cells and also provides information on mechanisms of osmolyte regulation. Lastly, the enzyme work should provide a molecular level view of two enzymes whose *in vivo* activity is regulated by changes in external NaCl.

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

15. Differential Regulation of Plastid mRNA Stability
D.B. Stern $86,000

Chloroplast genes are regulated by light and developmental signals. This regulation is required for the coordinated assembly of the photosynthetic apparatus. RNA-binding proteins (RNPs) play an important role in plastid gene expression by regulating RNA processing, RNA stability and translation initiation. We have focused on the chloroplast petD gene, which encodes subunit IV of the cytochrome b6/f complex. We have found that two RNPs of 55 kd and 41 kd bind specifically to the 3' untranslated region (UTR) of petD mRNA *in vitro*. The 41 kd RNP has been purified, and was found to protect petD RNA against *in vitro* degradation by an *E. coli* exoribonuclease. The 41RNP recognizes an 8 nucleotide sequence downstream of a stem/loop structure in the 3' UTR, and mutations in this sequence prevent protein binding. These mutated RNAs cannot be protected from exonuclease digestion, suggesting that 41RNP binding to RNA is required for its function. The 55 kd RNP has been purified and functional assays are in progress. To define functional elements of the 3' UTR *in vivo,* we have used stable transformation of tobacco chloroplasts. Chimeric genes consisting of a uidA

reporter gene fused to different 3' UTRs were introduced into tobacco chloroplasts, and the accumulation of uidA mRNA was measured. Preliminary experiments show that a stem/loop in the 3' UTR is necessary for correct 3' end formation in higher plant chloroplasts. When a mutation that prevents protein binding *in vitro* was introduced into the 3' UTR, 3' end formation was altered *in vivo*.

Brookhaven National Laboratory
Upton, NY 11973

16. Molecular Plant Genetics
B. Burr and F.A. Burr, Biology Department $353,000

Two recombinant inbred families have been used to construct a high density map of the maize genome. The current map has over 1,600 markers. These populations are used by the maize genetics community for rapid mapping of cloned genes. We are now working on the next generation of molecular markers based on simple sequence repeats (SSRs). Forty-eight of these have been mapped to date. These loci are highly polymorphic and easy to assay. The SSRs will greatly speed up mapping in new populations and will facilitate the use of molecular markers in plant breeding.

We used the recombinant inbred populations to study the nature of genes that have quantitative rather than qualitative effects. Preliminary lessons are that regulatory genes or genes that control rate limiting steps are the most likely to be implicated in this type of inheritance. Current emphasis is on isolation and characterization of genes regulating two model systems - anthocyanin and carotenoid biosynthesis. We are completing the characterization of *in*, an apparent negative regulatory gene involved in anthocyanin biosynthesis.

Brookhaven National Laboratory
Upton, NY 11973

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

The goal of this research effort is to elucidate the molecular signal transduction pathways that lead to the irradiance-induced changes in the abundance and composition of photosynthetic proteins. Inhibitor studies with the unicellular chlorophyte alga, *Dunaliella tertiolecta*, have revealed that the redox state of the plastoquinone pool specifically has a marked effect on the expression of the chlorophyll a/b binding proteins and their message levels. The redox state of this photosynthetic electron transport element is also known to induce protein phosphorylation. Preliminary sequence data 5' to the reading frame of the *cab* genes, reveals high homology with light regulated elements in higher plants that are responsive to

phosphorylated intermediates. These elements bind specific protein in high light adapted cells. We hypothesize that the irradiance response is keyed to photosynthetic electron transport through a phosphorylation cascade which reversibly represses *cab* gene expression. The details of the cascade are being investigated with both transient expression systems and pharmacological studies. Redox sensitive control of nuclear gene expression appears to be a general signal transduction pathway for a wide variety of environmental cues, including temperature and CO_2 availability.

Brookhaven National Laboratory
Upton, NY 11973

18. Regulation of Energy Conversion in Photosynthesis
G. Hind, Biology Department $350,000

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. These enzymes are present in low concentration relative to the photosystem components. They may be extracted from the membrane using detergents and are conveniently further fractionated and investigated concurrently.

Excitation energy transfer from photosystem II to photosystem I is modulated by the redox-sensitive phosphorylation of photosystem II proteins. Two protein kinases are involved, which are being individually fractionated and purified from the thylakoid membrane. That responsible for phosphorylating light-harvesting chlorophyll *a/b* protein (LHC-II) also phosphorylates a membrane-bound precursor of polyphenoloxidase, which we have cloned and sequenced. The site and significance of this phosphorylation are under study.

The dephosphorylation of LHC-II is catalyzed by a constitutively active phosphatase. This enzyme was isolated from pea thylakoids as a catalytically active monomer having an inhibitor-sensitivity profile resembling that of mitochondrial and prokaryotic phosphoprotein phosphatases. We are trying to determine its primary sequence, and characterize post-translational modifications that provide for attachment to the membrane.

In photosystem II, the dissipation of excess excitation energy is promoted by a reversible enzymic conversion of violaxanthin to zeaxanthin. The reverse process is catalyzed by an epoxidase for which we have found a novel inhibitor. With insight from this discovery, we are attempting to isolate and characterize zeaxanthin epoxidase, and clone the corresponding cDNA from *Hordeum* and *Arabidopsis*.

Brookhaven National Laboratory
Upton, NY 11973

19. Characterization of Fatty Acid Desaturases and Related Lipid Modification Enzymes

J. Shanklin, Biology Department $270,000

The process of fatty acid desaturation is poorly understood despite its central role in lipid metabolism. The plant stearoyl-ACP desaturase is an excellent model system for understanding this process. It is the only soluble desaturase identified in any system that can be expressed in bacteria from the cloned gene, is easily purified, and yields a stable functional protein. Components of the active site of this enzyme are being defined by a combination of site-directed mutagenesis and spectroscopic techniques, and the three dimensional structure is being determined by x-ray crystallography. Regulation of the gene is being explored in the model plant system *Arabidopsis thaliana*.

The soluble and membrane bound desaturases possess common biochemical features, indicating that they probably share a common mechanism of catalysis. The active sites of representative members of the membrane bound desaturases and related hydroxylases are also being defined by applying the information and techniques developed in the study of the stearoyl-ACP desaturase.

Brown University
Providence, RI 02912

20. δ-Aminolevulinate Biosynthesis in Oxygenic Prokaryotes

S. Beale, Division of Biology and Medicine $210,000 (2 years)

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. Genetics in Methylotrophic Bacteria
M.E. Lidstrom, Environmental Engineering Science $100,000

The purpose of this project is to use genetic techniques to study the transcriptional regulation of C1 specific functions in methylotrophic bacteria. We have studied the genes that are required for transcription of methanol oxidation (Mox) functions in *Methylobacterium extorquens* AM1. Six genes are required for transcription of the mxaF promoter, for the large subunit of methanol dehydrogenase. Two of these genes, mxbDM apparently encode a sensor kinase/response regulator pair that controls a second set of regulatory genes, mxcBQE. MxcQE also appear to encode a sensor kinase/response regulator pair of a class different from MxbDM. A third required positive-regulatory gene, mxaB has not yet been sequenced. A set of genes encoding an unknown PQQ-linked dehydrogenase is negatively regulated by these six genes. Another promoter in the Mox system, for pqqD, requires mxbDM for normal transcription and also requires mxaB for normal induction by C1 compounds, but does not require mxcBQE for transcription or induction. We have also characterized the RNA polymerase from *M. extorquens* AM1, which has been shown to contain six polypeptides. These have been identified as alpha, beta, beta-prime, omega, and two polypeptides whose N-terminal sequences show homology to sigma factors. One of these shows homology to a SigA sigma factor from *Agrobacterium tumefaciens*, and may be the vegetative sigma factor for M. extorquens AM1. The other shows homology to a group 2 sigma factor from *Streptomyces coelicolor.* This RNA polymerase preparation initiates transcription correctly from the mxaF promoter, and therefore, the 40 kDa polypeptide may be the sigma factor involved in mox-specific transcription.

California Institute of Technology
Pasadena, CA 91125

22. Molecular and Genetic Analysis of LEAFY, a Gene Controlling Floral Induction and Flower Development in Arabidopsis thaliana

E. Meyerowitz, Division of Biology $249,290 (FY 94 funds/2 years)

Earlier work has shown that the LEAFY gene of *Arabidopsis* plays a key role in floral meristem specification. When LEAFY is active in meristems, the meristems develop into flowers. When LEAFY activity is absent, meristems form either shoots or flowers with many characteristics of shoots, such as internode elongation, spiral organ phyllotaxy, and indeterminate growth. LEAFY works along with other genes in specifying floral identity: a key partner is APETALA1, and when APETALA1 and LEAFY are both inactivated by mutation, plants fail to form flowers. Recent work has shown that the genes CAULIFLOWER and UNUSUAL FLORAL ORGANS also play important roles in floral specification. One major open question is that of how LEAFY and its partner genes are activated in meristems, so that a plant can flower. This genetic question is a restatement of the physiological question of what induces flowering, and we are attempting to answer it by studying the initial activation of LEAFY. In particular, we are analyzing a collection of genes, in which mutations cause an exaggeration of the shootlike aspects of flowers in plants with partial loss of LEAFY function. These are good candidates for the genes that mediate between the environment and the activation of LEAFY, and thus for genes that control the induction of flowering.

University of California
Berkeley, CA 94720

23. The Molecular Genetics of Ligule Induction

M. Freeling, Department of Plant Biology $192,060 (FY 94 funds/2 years)

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

ligule/auricle development. Our antibody that recognizes the Liguleless1 protein should be useful. In addition, we will approach important problems of ligule homology by analyzing the expression of the above mentioned genes within the entire maize plant, and by exchanging the maize and rice *liguless* genes.

University of California
Berkeley, CA 94720

24. Regulation of Tomato Fruit Growth by MVA and GTP-Binding Proteins
W. Gruissem, Department of Plant Biology $111,000

The multibranched isoprenoid pathway from mevalonic acid (MVA) is one of the most important pathways in plants. Recent discoveries in animals and yeast have established MVA synthesis and prenylation of growth-related signal transduction proteins as critical factors for cell cycle progression, and normal cell growth and differentiation. MVA synthesis is also critical for normal growth and development in plants, but it is unknown how the sterol biosynthesis pathway integrates with control of cell division and cell growth. The research project approaches this problem from different directions, using tomato fruit growth as a model system. One aspect focuses on the temporal and spatial expression of genes for hydroxymethyl glutaryl CoA reductase (HMGR) and the subcellular location of the enzymes. HMGR is encoded by four genes in tomato that are differentially expressed, indicating stringent control over the cell-autonomous synthesis of MVA in plants. A second aspect focuses on prenyltransferases which utilize farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP) for the modification and biological activation of regulatory proteins. Activities for FTase, GGTase and RabGGTase have been characterized in tomato, and the developmental regulation and function of the enzymes is under investigation. A third aspect focuses on the target proteins for prenylation enzymes. Proteins that are substrates for FTase and RabGGTase have been cloned and are being studied for their function and prenylation. Together, the experiments will establish the basis to investigate the integration of sterol synthesis in plants through prenylated proteins with the control of cell division and growth during development.

University of California
Berkeley, CA 94720

25. Determinants of Environmental Stress Tolerance by Bacteria on Leaves
S.E. Lindow, Department of Environmental Science, Policy, and Management $84,000

Bacteria that live as epiphytes on the surface of healthy plants can be involved in plant pathogenesis, in causing plant frost injury by catalyzing ice formation, or in other processes.

The objectives of this study are to determine the traits of epiphytic bacteria that allow them to survive the stress of the hostile leaf surface environment. We have applied a random mutagenesis approach to identify genes in the epiphytic bacteria *Pseudomonas syringae* that are involved in epiphytic fitness, thereby directly identifying genes which are required for survival and growth on a leaf surface, and avoiding the limitations of investigating only predetermined phenotypes exhibited by bacteria in culture. Four transposon mutants showing different patterns of reduced growth and survival during physical stresses on leaves in the greenhouse and in the field have been biologically characterized; the distinctive behaviors of these mutants suggest that several loci are involved in stress tolerance. One locus has been completely sequenced and its regulation studied. At least two of the loci defined in the remaining mutants likely are regulatory since these mutants are pleiotropic, having altered tolerance of water stresses, extracellular polysaccharide production, changes in other traits besides epiphytic fitness. The sequences of the regions flanking the Tn5 insertions of two of these pleiotropic mutants are currently being evaluated, and used to examine the likelihood that these epiphytic fitness loci regulate the expression of other genes which individually confer only partial ability to survive environmental stresses on leaves.

University of California
Berkeley, CA 94720

26. Phytochrome from Green Plants: Assay, Purification and Characterization
P.H. Quail, Department of Plant Biology $102,000

The regulatory photoreceptor phytochrome is encoded by a family of five divergent genes, *PHYA, PHYB, PHYC, PHYD,* and *PHYE* in Arabidopsis. Phytochromes A and B (phyA and phyB) have been shown to have discrete photosensory functions in early seedling development. To determine whether differential temporal or spatial expression patterns of the *PHYA* and *PHYB* genes contribute to this phenomenon we have examined the expression of PHYA-GUS and PHYB-GUS reporter genes in transgenic *Arabidopsis*. Histochemical and quantitative biochemical analyses indicate that both transgenes are expressed extensively throughout the plant, including roots, shoots and flowers, during the entire life cycle, but with strong differences between the two in expression level and photoregulation, and more limited differences in spatial expression patterns. The data indicate that the PHYA and PHYB genes are regulated in divergent fashion at the transcriptional level, both developmentally and by the spectral distribution of the prevailing light, and that this regulation may be important to the photosensory function of the two photoreceptors.

University of California
Berkeley, CA 94720

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

B.J. Staskawicz, Department of Plant Biology $211,460 (FY 94 funds/2 years)

The RPS2 disease resistance gene is a single semi-dominant gene that specifically recognizes strains of the bacteria *Pseudomonas syringae* that contain the avirulence gene, avrRpt2. During the last year we have identified a single gene that when transformed into disease susceptible plants complements for disease resistance. The molecular characterization of this gene has revealed that the encoded RPS2 protein contains several previously recognized protein domains that are involved in signal transduction. The RPS2 protein contains a leucine zipper in the N-terminus of the protein, a nucleotide binding site and thirteen leucine rich repeats. Interestingly, these motifs are also contained in several other disease resistance genes suggesting the possibility that resistance genes may have conserved functions. We are currently in the process of characterizing the events involved in signal transduction that lead to the expression of disease resistance by employing both genetic and biochemical approaches. In addition we have initiated experiments to identify and characterize the bacterial signal (elicitor) that is recognized by *Arabidopsis* plants that contain an active RPS2 gene.

University of California
Berkeley, CA 94720

28. Analysis of Genes Essential for Floral Development in Arabidopsis

P. Zambryski, Department of Plant Biology $95,000

We have continued to investigate the role of the TOUSLED protein kinase, and recently determined that TSL function is critical for gynoecium development. This result coupled with our study of the ETTIN locus, has directed our ongoing efforts towards charcterizing the gynoecium, the female reproductive structure of flowering plants. The following summarizes *Arabidopsis* gynoecium structure in the wild type, and in *ettin* mutants. This cylindrical organ is composed of three longitudinal regions arranged in an apical-basal order: stigma, style, and ovary. The transmitting (pollination) tract is positioned along the center of the gynoecium and spans stigma, style, and ovary. Histochemistry, scanning electron microscopy and a style-specific reporter gene were used to compare the wild type pattern of gynoecium cell types and regions, with patterns formed in the gynoecia of individuals homozygous for a series of allelic mutations at the ETTIN locus. *ettin* gynoecia show morphological and histological alterations that appear to result from the merging of apical and basal regions and the development of abaxial into adaxial tissues. These abnormalities result in a reduction of the ovary region, an expansion of the stylar and stigmatic regions, and the outward

proliferation of transmitting tract tissue. These alterations can be interpreted as resulting from misspecifications of position along the longitudinal and transverse axes during gynoecium development. The results suggest that early patterning events underlie wild type gynoecium development, and that ETT functions during this early programming. Double mutant combinations between *ett* and *tsl* show dramatic loss of most gynoecium structures.

University of California
Davis, CA 95616

29. Cellulose Binding Proteins of Clostridium cellulovorans Cellulase
R.H. Doi, Section of Molecular and Cellular Biology $112,000

The goal of this project is to determine the structure, function, and the assembly process of the *Clostridium cellulovorans* cellulosome (cellulase) in order to apply this information for future optimization of its activity. Our current activities are directed towards analyzing the function of the cellulose binding domain (CBD) and the multiple endoglucanase binding domains (EBDs) of the non-enzymatic scaffolding protein (CbpA) of the cellulosome. Our primary objective is to determine the minimum sequence required for the CBD and EBD functions. In this regard we have made small deletions and single site mutations of the CBD sequence and measured its binding capacity to cellulose. Interestingly, studies to date indicate that small mutations along the entire CBD sequence significantly reduce the binding ability of CBD to cellulose, suggesting the necessity of the entire sequence for its maximum binding capacity. We have reduced the binding sequence of a single EBD to a 30 amino acid long sequence that is capable of binding endoglucanase B (EngB) or D (EngD). Thus a relatively short EBD sequence in the CbpA is capable of recognizing and binding endoglucanase molecules. In contrast to studies with *C. thermocellum*, the duplicated sequence (DS) of the endoglucanase molecule is not required for its binding to EBD. Further studies on the CBD and EBD are being carried out to determine the exact amino acid sequence and amino acid residues necessary for their functions.

University of California
Davis, CA 95616-8627

30. Plant Physiological Aspects of Silicon
E. Epstein, T.W-M. Fan, M.W.K. Silk, Department of Land, Air and Water Resources, and R.M. Higashi, Bodega Marine Laboratory $133,058

Silicon, the second most abundant element in soils, is not considered generally "essential" for higher plants, one necessary to complete their life cycle, from seed to seed. The element is therefore not included in any formulation of nutrient solutions commonly used in plant physiological research. Solution cultured plants thus lack the element, whereas normal, soil-

grown plants contain it in quantities similar to those of such major nutrients as sulfur, calcium and magnesium. We now have evidence that plants grown in solution culture supplied with silicon differ in a number of physical and chemical features from plants grown in conventional solution culture not containing silicon: conventional solution cultured plants deprived of silicon are to an extent experimental artifacts. The leaf hairs (trichomes) of wheat grown in solution culture with silicon added are stiffer than those of plants grown in conventional cultures lacking silicon. The difference is due to the silica deposited in the trichome cell walls. As for cell wall biochemistry, cell wall material isolated from wheat and tomato leaves was analyzed by pyrolysis gas chromatography-mass spectrometry (GC-MS). Some striking differences, not yet fully characterized, showed up in the chromatograms of plants grown in solution cultures without and with silicon. Both physical and biochemical evidence thus bears out our hypothesis that normal (silicon-replete) plants differ significantly from the silicon-deprived plants grown in conventional solution culture. Our findings have implications for plant energetics in both physical and chemical terms.

University of California
Davis, CA 95616

31. Regulation of Embryonic Development in Higher Plants

J.J. Harada, Section of Plant Biology $182,069 (FY 94 funds/2 years)

A unique aspect of higher plant development which has conferred significant selective advantages to the angiosperms is that their life cycle is interrupted by a period of dormancy during which the mature embryo is metabolically quiescent and in a desiccated state. The processes that occur during late embryogenesis and germination, in part, underlie the seed habit because they allow the embryo to enter into and to emerge from this state of developmental arrest and desiccation. We have taken a molecular genetic approach to define the regulatory pathways that control late embryogenesis by studying the LEAFY COTYLEDON1 (LEC1) gene of *Arabidopsis thaliana*. Mutations of this gene inactivate specific aspects of late embryogenesis, cause postgerminative development to occur prematurely, and result in trichome formation, a leaf trait, on cotyledons. We have studied the interactions of the LEC1 gene with three other genes that also appear to play regulatory roles in late embryogenesis, LEAFY COTYLEDON2 (LEC2), FUSCA3 (FUS3), AND ABA INSENSITIVE3 (ABA3). Analyses of gene expression programs in mutants defective in these genes and of the phenotypes of double and triple mutants indicate that these genes regulate distinct processes, implying that several independent pathways are involved in controlling late embryonic development. We are also studying a newly identified lec-type mutation and its interactions with LEC1. To obtain mechanistic information about the role of the LEC1 gene, studies are underway to clone it from an *Arabidopsis* line mutagenized with T-DNA.

University of California
Davis, CA 95616

32. Cellular and Molecular Characterization of Vascular Plasmodesmata
W.J. Lucas, Section of Plant Biology $202,000

Integration of plant function, at the whole-plant level, requires the processing of numerous input signals, many of which may be delivered via the long-distance translocation system of the phloem. Phloem sap, collected from functioning sieve elements, contains a large number of proteins. As the sieve element (SE) is enucleate, such proteins must be synthesized in the neighboring companion cells prior to entry into the SE, and this process likely involves transport through plasmodesmata. To test this hypothesis, we are in the process of identifying and characterizing phloem proteins that have the capacity to interact with and traffic through plasmodesmata. To this end, the gene encoding rice phloem thioredoxin (PTX) was expressed in *E. coli* and its protein product was extracted and subsequently fluorescently labeled for use in microinjection experiments. These studies provided direct experimental proof that PTX has the capacity to interact with plasmodesmata to effect its own cell-to-cell movement. Furthermore, we found that PTX interacts with plasmodesmata to cause an increase in size exclusion limit in a manner that is analogous to all viral movement proteins (MPs) studied thus far. These positive findings support the hypothesis that plant plasmodesmal transport proteins and viral MP share functionally homologous domains. These results provide a solid starting point for the cloning and sequencing of other phloem proteins that have the capacity to traffic through plasmodesmata. Such information will provide the foundation for studies on the role of plasmodesmal macromolecular trafficking, via the phloem, in the integration of plant development and function.

University of California
Davis, CA 95616

33. Protein Translocation and Assembly in Chloroplasts
S.M. Theg, Section of Plant Biology $94,000

The objective of this project is to investigate a number of aspects relating to the biogenesis and operation of the oxygen-evolving complex (OEC) in chloroplasts. This enzyme complex, which is responsible for most of the oxygen in the atmosphere, is extrinsically associated with the inner surface of the chloroplast thylakoid membrane, and is formed from at least three nuclear-encoded subunits. These subunits must be translocated across the two chloroplast envelopes and the thylakoid membrane before they can be assembled into the enzyme complex. The experiments undertaken during this project are designed to elucidate the mechanisms that lead to complex formation and enzymatic activity. Experiments monitoring the assembly of the subunits into the complex after their *in vitro* import into isolated chloroplasts have revealed that membrane transport and assembly are coupled for one of the subunits. The nature of this coupling, and its implications for homeostasis of the complex are

currently under investigation. In addition, we are investigating the environment through which the subunits pass as they traverse the thylakoid membrane. Specifically, we are monitoring the electrical conductivity of the thylakoid membrane during translocation to determine whether proteins are translocated through ion-permeable, aqueous pores.

University of California
Davis, CA 95616-8515

34. Vacuole Biogenesis in Differentiating Plant Cells
T.A. Wilkins, Department of Agronomy and Range Science $110,000

Vacuole function is contingent upon the resident protein constituents that specify the biochemical properties of the compartment. Two morphologically and biochemically distinct vacuole populations were found to co-exist in trichoblasts of developing cotton seed trichomes. Biogenesis of a novel polymorphic vacuole-type that selectively accumulates unidentified electron-dense material (EDM) occurs coincident with trichome differentiation. As the primordial trichomes begin to expand, this vacuole fuses with pre-existing and *de novo* synthesized provacuoles to form a single, homogeneous large central vacuole. Following the selective staining of the tonoplast for glycoproteins, a battery of glycan-specific antibodies revealed that the novel vacuole tonoplast is heavily studded with a glycoprotein containing an O-linked oligosacchardide related to arabinogalactans. This putative tonoplast-associated arabinogalactan glycoprotein (AGP) is presumed to function in the temporal sequestering of the EDM via the formation of an inert "protective barrier" to safeguard the physical separation of the EDM for the cytosol. The advent of vacuolar enzymes following the fusion of the two vacuole compartments results in the turnover of the AGP-like moiety and the removal of the protective barrier, thereby restoring communication across the tonoplast. However, the polymorphic vacuole and its associated EDM persist in a mutant in which the primordial trichomes fail to expand. Thus, the triggered release of the EDM is proposed to be a key subcellular event in the signal transduction pathway that presages the onset of cell expansion in primordial trichomes.

University of California
Irvine, CA 92717

35. Membrane Bioenergetics of Salt Tolerant Microorganisms
J.K. Lanyi, Department of Physiology and Biophysics $156,000

The energy costs of salt tolerance in the extremely halophilic bacteria originate from generating electrochemical ion gradients across the cytoplasmic membrane that drive massive sodium extrusion and chloride accumulation. We study the molecular mechanisms of the proton and chloride transporting bacterial rhodopsins and the proton transporting ATPase in these organisms. The research on bacteriorhodopsin and halorhodopsin, respectively,

explores the thermodynamics of the transport, the chromophore and protein changes that determine the alternating connectivity of the ion-binding 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 mutations, time-resolved optical multichannel spectroscopy, and through collaborations retinal analogues, infrared spectroscopy and x-ray diffraction are methods developed for this work. Studies of the third protein, the membrane ATPase of *H. saccharovorum,* concentrate on the origins of the complex hydrolytic mechanism, the roles played by heavy metals and extraneous ligands that appear to activate or inhibit activity, and the shared features with eubacterial and eukaryotic ATPases.

University of California
La Jolla, CA 92093-0116

36. Structure, Biosynthesis and Role of Complex Protein-Bound Glycans
M.J. Chrispeels, Department of Biology

$237,160 (FY 94 & FY 95 funds/2 years)

Plant glycoproteins contain two types of asparagine-linked oligosaccharide sidechains (glycans). Both types originate as high-mannose glycans in the endoplasmic reticulum when the proteins are first synthesized. Then, as the proteins pass through the Golgi complex, some glycans are modified by enzymes in the Golgi. We are studying the biosynthesis of these glycans, as well as the function of specific vacuolar glycoproteins that carry such glycans. With respect to vacuolar proteins, we are studying the phytohemagglutinin/arcelin/α-amylase inhibitor (αAI) family of proteins found in bean seeds. We recently cloned additional members of this gene family and are in the process of expressing them in pea seeds to test their plant defense properties. Pea seeds and azuki bean seeds transformed with one such gene (αAI-1) were found to be resistant to four species of bruchids.

One objective is to determine the three-dimensional structure of the α-amylase-αAI complex. To this end, we have obtained crystals of this complex and x-ray diffraction data are now being collected. We are now starting a project to clone the α-amylase genes of bruchids so that we may fully understand the interaction of αAI variants and α-amylases from different sources.

University of California
La Jolla, CA 92093-0116

37. Molecular Structure, Function and Physiology of K^+ Uptake Channels in Plants

J.I. Schroeder, Department of Biology

$187,090 (FY 94 & FY 95 funds/2 years)

Potassium uptake by higher plant cells of central importance to plant growth, stomatal regulation, nutrition, tropisms, osmoregulation, enzyme homeostasis and plant membrane potential control. Elucidation of the molecular mechanisms by which higher plant cells selectively transport K^+, while diminishing block by or transport of toxic metals such as Na^+, Cs^+ or Al^{3+} is of increasing environmental and agricultural concern. Patch clamp studies on guard cells and other plant cells show that K^+ uptake channels provide a molecular pathway for membrane potential control and for low-affinity proton pump-drive K^+ uptake. These "inward-rectifying" K^+ channels interact with metals which are known to show physiological or toxic effects on cation transport, growth and membrane potentials. *Arabidopsis* ion channel cDNAs have recently been cloned, and we have functionally characterized one of these as an inward-rectifying K^+ channel gene.

The long-term goal of the proposed research is to attain a quantitative description of physiological K^+ channel function and of structural domains of K^+ uptake channel clones responsible for K^+ channel activation, metal block and ion selectivity. Furthermore we will determine whether, and to which extent K^+ uptake channel structure can influence physiological and toxic effects of metals on membrane potential and cation transport in specific plant cells. These studies will contribute to a structural dissection of cation transporter functions in higher plants which play important roles in growth, development and environmental responses of plants.

University of California
Los Angeles, CA 90095

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

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

$106,698 (FY 94 funds/2 years)

Methane formation by the *Methanosarcina* species is often a rate limiting step during anaerobic biodecomposition in natural and in man made environments. These methanogens are the most versatile with respect to the substrates they use (e.g., acetate, methanol, tri-methyl, di-methyl, and methyl-amines, and H_2/CO_2). These organisms are also versatile in their ability to adapt to habitats differing in osmolarity (ca. to 1.2 M NaCl). Using recently

developed methods to grow these methanogens as single cells, and to extract high molecular weight DNA and RNA without shearing, we are examining the molecular biology of substrate utilization and osmotic adaptation in these methanogens. We are examining how glycine betatine, N-acetyl-β-lysine and α-glutamate, compounds that are used to osmoregulate the cell in response to increased external salt, are transported into the cell. We are also examining how the latter two compounds are synthesized. These processes appear to be highly regulated by the cell. Genes in *M. thermophila* that are differentially expressed in response to change in osmolarity and to changes in carbon substrate are being identified, cloned, and characterized. The resulting studies should greatly expand our understanding of how methanogens are able to sense environmental change and adapt accordingly for growth in varying anaerobic acetotrophic habitats.

University of California
Los Angeles, CA 90024-1606

39. The Gibberellin A_{20} 3ß-hydroxylase: Isolation of the Enzyme and Its Molecular Biology

B.O. Phinney and J. MacMillan, Department of Biology $74,000

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

University of California
Los Angeles, CA 90024-1606

40. Sensory Transduction of the CO_2 Response of Guard Cells
E. Zeiger, Department of Biology $188,180 (FY 94 funds/2 years)

Stomatal response to CO_2 plays a key role in balancing the rates of water loss and photosynthetic carbon fixation, and is therefore a central factor determining plant water use efficiency. This research project aims at the characterization of guard cell mechanisms for sensing CO_2. Abaxial stomata from *Vicia faba* leaves grown in a growth chamber under constant light, temperature and humidity showed an elaborate pattern of aperture changes over the course of a light cycle. These aperture changes were tightly correlated with changes in chamber CO_2 concentration (r2=0.83). Changes in chamber [CO_2] resulted, in turn, from substantial daily fluctuations in ambient [CO_2], documented by extensive CO_2 monitoring by our laboratory at UCLA. The dominant role of the stomatal response to CO_2 in the control of aperture was confirmed by manipulation of chamber [CO_2]. Fast (15 min) increases and decreases in [CO_2] caused rapid decreases and increases in aperture while constant [CO_2] resulted in constant aperture. In contrast, aperture changes in comparable plants grown under greenhouse conditions were tightly correlated with changes in incident solar radiation (r2=0.80), and is very poorly correlated with changes in [CO_2] (r2=0.09). Greenhouse-grown plants transferred to growth chamber conditions show little apparent response to manipulations of chamber [CO_2]. These data indicate that growth chamber-grown *Vicia* leaves provide an experimental system optimally suited for the study of the stomatal response to CO_2, and suggest that acclimation to environmental conditions alters the sensitivity of stomata to CO_2.

University of California
Santa Barbara, CA 93106-9610

41. Regulation and Function of Two Cell Wall Protein Genes in Medicago Roots and Root Nodules
J.B. Cooper, Department of Biological Sciences $206,389 (18 months)

The development of nitrogen-fixing root nodules in legumes involves the controlled infection of host plant tissues by bacterial symbionts. Previous work has shown that expression of the two major root proline-rich protein genes is repressed early in the development of *Medicago* root nodules. This down-regulation is accompanied by the induction of several nodule-specific PRP genes. Nodules elicited by *Rhizobium* mutants that are unable to invade host tissues maintain high levels of root PRP gene expression. The primary objective of this project is to understand the regulation and function of these proline-rich proteins during symbiont invasion. We have cloned and characterized sequences encoding four members of the PRP gene family in *Medicago truncatula*, and these cloned sequences will be used to examine the

bacterial regulation of these cell wall protein genes. If novel "invasion signals" are implicated in this regulation, we intend to characterize these signal molecules. Domain-specific antisera recognizing each distinct PRP repetitive pentapeptide will be generated, and such antisera will be used to examine *Rhizobium*-induced alterations in host cell wall architecture. Finally, reverse genetics experiments will be used to evaluate the functional significance of the down-regulation of root PRP genes during nodule invasion. These studies will begin to elucidate the molecular mechanisms used by symbiotic microbes to invade plant root tissues.

University of California
Santa Cruz, CA 95064

42. Regulation of Vacuolar pH in Citrus limon
L. Taiz, Biology Department $96,000

The vacuolar pH of the mature juice sac cells of lemon fruits (~2.4), is considerably lower than that of typical plant tissues (~5.5). To study the mechanism of over-acidification, we have been characterizing the ATP-driven H^+-pumping activities of tonoplast-enriched membrane vesicles from fruit juice sacs and seedling epicotyls. When initial rates were normalized, juice sac vesicles generated larger pH gradients and maintained them for a longer time in the absence of MgATP than epicotyl vesicles. Although the Kms were not significantly different, the Vmax of the juice sac H^+-ATPase was twice that of the epicotyl. Based on immunoblots, the density of the enzyme on the tonoplast was ~50% lower in juice sacs than in epicotyls, indicating that a significant proportion of the epicotyl V-ATPase was inactive. H^+-pumping by the epicotyl vesicles was absolutely dependent on chloride even when DY was clamped with K^+ and valinomycin, insensitive to azide and vanadate, and strongly inhibited by ADP, nitrate, bafilomycin, NEM, DCCD and antibody to the 70 kD subunit. H^+-pumping by juice sac vesicles was inhibited by ADP, but was chloride-independent and insensitive to a broad spectrum of inhibitors. However, inhibition of juice sac V-ATPase activity by nitrate, NEM and the anti-70 kD antibody increased upon detergent solubilization. A major difference between the epicotyl and juice sac V-ATPases appears to be the absence of slip in the latter. Whereas chloride stimulated, and nitrate inhibited, H^+-pumping more than ATPase activity in epicotyl vesicles, indicative of slip, no such discrepancy was observed for juice sac vesicles. The epicotyl V-ATPase exhibited DTT-reversible oxidative inactivation during incubation at 20°C, whereas the juice sac V-ATPase was insensitive to oxidation. It was estimated that as much as 50% of the epicotyl V-ATPase may be inactive due to oxidation *in vivo*. Nucleotides and anions, including nitrate, protect against oxidation. We conclude that the factors that contribute to over-acidification by the juice sac V-ATPase include the absence of slip, insensitivity to oxidation and many inhibitors, and the low permeability of the tonoplast to protons. This hypothesis will be tested using reconstituted proteoliposomes.

Carnegie Institution of Washington
Stanford, CA 94305

43. Production of Lipophilic Materials from Plants
C.R. Somerville $614,196

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

Carnegie Institution of Washington
Stanford, CA 94305

44. Molecular Basis of Disease Resistance
S.C. Somerville, Department of Plant Biology $154,188 (FY 94 funds/2 years)

Our long term objective is to identify and characterize genes conferring resistance to powdery mildew diseases, which are caused by obligate fungal pathogens of the *Erysiphe* genus. Because of the range of molecular genetic resources and tools available for *Arabidopsis thaliana*, we chose to isolate powdery mildew resistance genes from this plant species. From a survey of 48 accessions, six powdery mildew resistant accessions were identified. We demonstrated that between five and seven distinct resistance loci occur in these accessions, suggesting that powdery mildew resistance in *Arabidopsis* is as diverse and complex as powdery mildew resistance in crop species like barley. Resistance in one accession, Wa-1, was encoded by a semi-dominant allele at the ECR2 locus. This locus was mapped to chromosome 3. We propose to isolate the ECR2 gene by positional cloning and have begun to refine the map position of this locus.

In parallel with the project to clone ECR2, resistance mechanisms operating in Wa-1 were compared with those reported in powdery mildew resistant barley lines by microscopic

analysis. In *Arabidopsis* as in barley, papillae formation appeared to be a common, nonspecific response to attempted penetration by the pathogen. However, the hypersensitive necrosis response, which is highly correlated with resistance in many powdery mildew resistant barley lines, was not associated with resistance in Wa-1 in these preliminary experiments. Near-isogenic lines that differ only at the ECR2 locus will be generated by back-crossing the ECR2 resistance allele from Wa-1 into the Columbia background. These lines will provide genetic materials for quantitative physiological and biochemical experiments to evaluate resistance mechanisms.

University of Chicago
Chicago, IL 60637

45. Organization and Regulation of the Genes for Nitrogen Fixation in Rhodobacter capsulatus
R. Haselkorn, Department of Molecular Genetics & Cell Biology $95,000

Studies in numerous laboratories suggest that the nifA genes of *Rhodobacter*, of which there are two, require a hitherto undescribed sigma factor for their transcription. The principal result pointing in this direction is the observation that mutation of nifR4, the *Rhodobacter* rpoN gene, does not prevent transcription of nifA::lacZ fusions. We screened large numbers of colonies of mutagenized cells carrying nifA fusions to counter-selectable genes and reporter genes, failing to find anything of use. If there is a special sigma factor for nifA transcription, it is probably required for an essential gene as well. At one stage, a Tn5 insert was isolated that appeared to have the required properties, but on subculturing it turned out to be Nif^+. The region around this insert was cloned and sequenced anyway, and found to encode a member of the amino acid permease family. In addition, the gene encoding the principal sigma factor was cloned and sequenced. Two start points for transcription were identified by primer extension, but lac fusions indicate that only the one closer to the translation start has a real promoter. The activity of this promoter doubles when cells are transferred to N^- medium.

The cosmid encyclopedia of the *Rhodobacter* strain SB1003 was used to group and then map cosmids from two related strains called St. Louis and 2.3.1. Comparison of the fine structure physical maps of two megabase regions of the three chromosomes revealed numerous genome-changing events: large scale inversions, translocations probably resulting from successive inversions, deletions, and relocation of small elements, probably transposons. The endpoints of several inversion events have been cloned and sequencing has begun. The fine structure maps also reveal the chromosomes to be mosaics of the following kind: the chromosome is made up of alternating regions of 80-150 kb in which the physical map is either conserved more than 80% among the three strains or is polymorphic to the extent that less than 30% of the restriction sites are the same. These results implicate horizontal gene transfer as a prominent agent of bacterial chromosome evolution. Systematic sequencing of

the SB1003 chromosome has begun in collaboration with a group in Prague, Czech Republic. In principle, the cosmid encyclopedia can be used to create a set of deletion strains, removing any 30-40 kb region of the chromosome desired. This principle was proved using GTA to transfer a Gm-resistance cartridge inserted between the ends of a 30-kb deletion in a cosmid that once carried the nifHDK genes. The resulting strain is Nif^- and also Ps^- due to absence of the hemA gene.

University of Chicago
Chicago, IL 60637

46. Role of HSP100 Proteins in Plant Stress Tolerance

S. Lindquist, Department of Molecular Genetics & Cell Biology

$198,060 (18 months)

The inability of plants to withstand high temperatures and water stress provides the single greatest limit to agricultural productivity. It has been known for many years that when organisms of all types are exposed to moderately elevated temperatures they acquire the ability to survive much higher temperatures and other environmental stresses than they would otherwise be able to survive. During such conditioning treatments a large fraction of total protein synthesis is devoted to the production of a small number of proteins known as the heat-shock proteins (HSPs). The first of these proteins demonstrated to play an important role in high temperature survival is the HSP100 protein of yeast, an organism that offers many advantages for rapid genetic analysis. The HSP100 protein is highly conserved. We have recently found that although yeast cells carrying mutations in HSP104 die at high temperature, they can be rescued by homologous HSP100 protein of the vascular land plant, *Arabidopsis thaliana*. This demonstrates that the functions of Hsp100 have been conserved in thermotolerance for over 1 billion years. Since the HSP100 protein is not toxic when over-expressed, we hope to improve the thermotolerance of plants by increasing the production of HSP100. We are also attempting to define the mechanism by which Hsp104 acts in molecular terms.

University of Chicago
Chicago, IL 60637

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

D.G. Lynn, Department of Chemistry $187,624 (FY 94 funds/2 years)

Striga asiatica is a small chlorophyll-containing angiosperm that has developed the remarkable ability to establish a vascular connection with another plant. The development

of the attachment organ, the haustorium, is one of the most rapid organogenesis events known. Since this parasitic ability is wide-spread among the plant families, it has generally been assumed that the development of the attachment organ relies on ubiquitous plant developmental programs. It was therefore quite surprising when it was discovered that relatively simple host-derived phenolic compounds were sufficient to induce haustorial development. The subsequent discovery that simple phenolic compounds had evolved as the primary signal initiating vir expression in *Agrobacterium* and nod expression in *Rhizobium* and that some of these same compounds were important in plant growth and development have raised fundamentally new questions about the role of these compounds in plants.

Subsequent work on the phenolic signals in *Striga* has provided evidence that the compounds are detected via a chemical reaction, again quite distinct from our current models of hormone/growth factor detection by membrane localized binding proteins. Evidence is presented that the recognition mechanism is a redox reaction most likely controlled by plasma membrane localized oxidoreductases. While the existence of these redox systems have been demonstrated in both plants and animals, only recently has convincing evidence connecting e^- transport with plant development emerged.

Striga therefore can teach us not only about the development of host-parasite interactions but also represents a rapid and well defined developmental transition where the chemistry of the inducing signal can be exploited to provide insights into the developmental process. Work over the past year has allowed us to reproduce the activity in a cell free system and to demonstrate the unusual nature of the chemistry that it performs. We propose to use this chemistry both to provide information about the initial detectors controlling haustorial development and to help in the exploitation of the molecular biology of *Striga*.

Clemson University
Clemson, SC 29634-1903

48. The Magnesium Chelation Step in Chlorophyll Biosynthesis
J.D. Weinstein, Department of Biological Sciences $184,010 (2 years)

In plants, photosynthetic membrane biogenesis requires the co-ordinate synthesis of prosthetic groups, proteins, and various lipids. Chlorophyll and heme, two of the major prosthetic groups, share a common biosynthetic pathway that diverges at the point of metal insertion into protoporphyrin-IX. The enzyme responsible for magnesium insertion, magnesium chelatase, has now been prepared in a completely soluble form from the stroma of developing pea chloroplasts. This preparation is highly active and demonstrates the same biphasic kinetics which is indicative of a two-stage reaction (activation followed by catalysis), and which we observed in a preparation which contains membranes. The soluble preparation can be fractionated on the pseudo-affinity matrix, Blue-agarose, into two fractions which are required for activity. When preincubated with ATP, only one of these fractions is required to

overcome the lag phase of the biphasic kinetics, suggesting that the activation step requires only a subset of the components required for catalytic activity. Other researchers have identified two structural genes which probably encode subunits of the magnesium chelatase enzyme system, *Olive* and *CH-42*. By analogy to the bacterial system which has homologues to *Olive* and *CH-42 (Bch H and Bch I*, respectively), there should be a higher plant gene which is homologous to the third bacterial gene, *Bch D*. Work is now in progress to identify and clone the "missing" higher plant gene.

Cold Spring Harbor Laboratory
Cold Spring Harbor, NY 11724

49. The Suppression of Mutations Generated by Mu Transposons in Maize
R.A. Martienssen and V. Sundaresan $92,000

Mutations caused by the insertion of *Mutator (Mu)* transposable elements are frequently dependent on the presence of active autonomous elements elsewhere in the genome. Such mutations are phenotypically suppressed when *Mu* activity is lost, resulting in wild-type plants. Recessive alleles of this class at four different loci (*hcf106, a1, Kn1, vp1*) have *Mu1* or *Mu8* elements in the promoter region of the gene. In the case of *hcf106*, phenotypic suppression is accompanied by transcript initiation within the Mu1 element that results in the production of a functional chimeric *Mu1::hcf106* message. This results in a pseudorevertant phenotype that can be used to monitor loss of *Mu* activity both germinally and in somatic sectors. Analysis of double mutant combinations of *Les28, hcf106* and *Kn1-mum2* have been used to demonstrate that co-ordinate loss of activity occurs in sectors during development. This property of suppressible alleles can be used for mosaic analysis as loss of activity results in adjacent sectors of mutant and wild-type tissue.

In order to isolate new alleles of *hcf106* that respond differently to *Mu* activity, we have developed a simple technique for site-selected transposon mutagenesis in maize. Rare individual plants carrying derivative alleles were selected by PCR amplification of specific fragments from pools of seedlings. By cross-referencing two-dimensional pools, individual plants can be rapidly identified with a single round of PCR. Three new alleles (one deletion and two insertions) were recovered among 800 gametes screened. Unlike the progenitor allele, two of these alleles no longer require *Mu* activity for their phenotypic effects. This technique could be applied to maize genes of known sequence allowing insertions and deletions to be recovered *in vivo*. In many cases, these mutations would allow the function of the gene to be determined from the mutant phenotype. This technique has potential application in genome analysis where most genes are defined by sequence information alone.

University of Connecticut
Storrs, CT 06269-3125

50. Analysis of Cell Wall Properties in Polysaccharide Mutants of Arabidopsis
W.-D. Reiter, Department of Molecular and Cell Biology $133,700 (18 months)

The mechanical properties of plant cell walls, and the synthesis of new wall material are believed to play pivotal roles for the determination of cell shapes and sizes. Accordingly, an understanding of plant growth and development requires an understanding of the mechanisms underlying the synthesis of new cell wall material, and the assembly of individual cell wall components into a matrix with both growth-regulating and structural roles.

In an effort to approach cell wall-related issues by a genetic approach, we have isolated cell wall mutants of the plant model organism *Arabidopsis* by direct screening for alterations in the amounts of cell wall-derived monosaccharides. We are using selected lines from this mutant collection to determine alterations in cell wall ultrastructure via electron microscopic procedures that permit the visualization of fibrillar elements. Furthermore, experiments are under way to determine changes in the mechanical properties of the walls, and to analyze the effects of specific cell wall mutations on cell shapes and sizes. These studies are part of an effort to combine genetic, biochemical, and cell biological procedures to elucidate the mechanisms by which the cell wall is being assembled from its individual components.

Since most of the carbon fixed by higher plants is ultimately incorporated into cell wall material, results from these studies have a long-range potential to engineer plants with altered cell wall structure that may improve the usefulness of this abundant renewable resource for industrial purposes.

University of Connecticut
Storrs, CT 06269

51. Sugar Transport and Metabolism in Thermotoga
A.H. Romano and K.M. Noll, Department of Molecular and Cell Biology
$87,000 (17.5 months)

Members of the genus *Thermotoga* are hyperthermophilic anaerobes that are among the most slowly-evolving members of the phylogenetic domain Bacteria. They ferment sugars to acetate, carbon dioxide, hydrogen, and minor products via the Embden-Meyerhof-Parnas glycolytic pathway. Nothing is known, however, of the mechanisms by which sugars are transported into their cells. The goal of this project is to elucidate mechanisms of sugar transport and carbohydrate catabolism in *Thermotoga* species. We found that arginine induces ATP synthesis in sonicated extracts and can be used to provide energy during uptake studies. Glucose, 2-deoxyglucose, and 6-deoxyglucose can be used as substrates of the

glucose transport system. Glucose and 2-deoxyglucose accumulate inside the cells and appear in both unmodified and phosphorylated forms. 6-Deoxyglucose is also accumulated, but is not phosphorylated. Uptake of these sugars was sensitive to arsenate but only slightly affected by protonophores. These results suggest that glucose transport in *T. neapolitana* cells is ATP-dependent. We detected a highly active glucokinase in cells, but found no evidence of a glucose-specific PEP-dependent sugar:phosphotransferase system (PTS). The possible presence of an inducible fructose-specific PTS was not ruled out and is currently under investigation.

We have begun cloning genes encoding components of the carbohydrate transport and catabolism systems of *T. neapolitana*. The characterization of sugar transport and metabolism in this phylogenetically important bacterium will provide insights into the evolution of cell metabolism and fundamental aspects of thermophily that promise to be useful in biotechnological applications.

Cornell University
Ithaca, NY 14853

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

J. Gibson, Section of Biochemistry, Molecular and Cell Biology $87,000

Compounds containing aromatic rings are produced both naturally and by human activities in very large quantities. Plant lignins, which are largely composed of aromatic rings, comprise the second most abundant biopolymer on earth, and are therefore an important renewable resource. In contrast, many of the man-made aromatic compounds are potentially toxic, so that their environmental fate is of serious concern. Many of these compounds are carried into anoxic environments, where they are only slowly degraded, and may contaminate groundwater supplies. The biochemical pathways used in anaerobic degradation of simple aromatic compounds, including some derived from lignin and released by industry are known only in outline, and the major thrust of this project is to define the enzymology of these pathways in the versatile anoxygenic phototrophic bacterium *Rhodopseudomonas palustris*. Anaerobic degradation pathways differ from aerobic ones, in that the aromatic ring is reduced to an alicyclic compound before the ring is opened. The first step appears to be conversion of the substrate to its coenzyme A thioester, and three enzymes that carry out this type of reaction and have narrow, but overlapping, substrate specificity have been purified and studied *in vitro*. Studies on specific dehydrogenases involved in the modification of the thioesters are underway or about to begin. Our work is being closely coordinated with that of Dr. C.S. Harwood at the University of Iowa, and we are using both molecular and biochemical tools to define the pathways and regulatory phenomena. A long-range goal is to pinpoint, and then manipulate, regulatory steps in these pathways in hopes of increasing overall degradation rates under defined conditions.

Cornell University
Ithaca, NY 14853-2703

53. Cytoplasmic Male Sterility and Mitochondrial Function During Microsporogenesis

M.R. Hanson, Division of Biological Sciences $98,000

Though male reproductive development is disrupted at many different stages in the wide variety of genera in which cytoplasmic male sterility (CMS) genotypes have been characterized, abnormalities are frequently first observed immediately before or during meiosis. Mitochondrial genes have been recently found to be regulated in a tissue-specific manner during early reproductive development in wild-type plants, and mitochondrial appearance is altered in CMS mutants. Because mutant phenotype, structural, and gene expression studies all point to a special role for mitochondria during early microsporogenesis, we propose to learn more about mitochondrial function during this developmental period. Using both two-photon fluorescence and standard confocal microscopy, we plan to analyze mitochondrial movement, replication, and respiratory activity *in vivo* in isonuclear wild-type, CMS, and CMS-restored *Petunia* lines. We will compare electron transport and the regulation of alternative oxidase activity in CMS and fertile lines. We will examine transgenic plants in which products of the CMS-associated gene are expressed in different tissues in different locations in order to probe the role of this gene in disruption of microsporogenesis.

Cornell University
Ithaca, NY 14853-5908

54. Signal Transduction in the Pollen-Stigma Interactions of Brassica

J.B. Nasrallah and M.E. Nasrallah, Section of Plant Biology, Division of Biological Sciences $211,940 (FY 94 & FY 95 funds/2 years)

Self-pollination in *Brassica* is prevented by the action of genes that reside at the highly polymorphic self-incompatibility (*S*) locus. Self/nonself recognition is attributed to the activity of identical *S* haplotypes in pollen and stigma, and results in the inhibition of self-pollen at the surface of stigmatic papillar cells. We are using the *Brassica* self-incompatibility system as an experimental model for investigating pollination responses and mechanisms of cell-cell interaction in higher plants. To date, our work has focused on the characterization of the S-locus Receptor Protein Kinase (SRK), one of two *S*-locus encoded proteins that have been identified in our laboratory. SRK is a receptor-like protein that exhibits serine/threonine kinase activity and shares a high degree of sequence similarity with the secreted S-Locus Glycoprotein (SLG) that is also encoded by the *S* locus. SRK and SLG are both required for the operation of self-incompatibility and are coordinately regulated, being expressed at highest levels in the papillar cells of the stigma surface. Continued characterization of the SRK protein by biochemical, immunochemical, and molecular methods is aimed at determining if

the protein is a cell surface receptor which, when activated by contact with self pollen, phosphorylates intracellular substrates, thus coupling the recognition event at the papillar cell-pollen interface to a chain of events that leads to the arrest of pollen tube development. It is anticipated that the deciphering of SRK-mediated signaling will contribute to the elucidation of the general principles that underlie receptor-mediated signaling and cell-cell communication in higher plants.

Cornell University
Ithaca, NY 14853-8101

55. Regulation of Denitrification in Rhodobacter sphaeroides
J.P. Shapleigh, Section of Microbiology $105,000 (18 months)

Many bacteria have the capacity to respire compounds other than oxygen. Nitrate is one of the many compounds that can serve as an alternative respiratory substrate. Nitrate respiration to nitrogen gas is referred to as denitrification. Our lab is interested in understanding how bacteria regulate those proteins required for denitrification. In particular we want to understand the coordination of gene expression and to identify trans-acting proteins used to monitor environmental conditions. The bacterium we will use for our studies is a denitrifying variant of *Rhodobacter sphaeroides*. This is an ideal organism to use because genetic systems are well developed and a great deal is known about other components of the respiratory chain.

We have isolated the genes encoding nitrite, nitric oxide and nitrous oxide reductases. We will carry out a comprehensive study of the regulation of these reductases using reporter gene fusions. Individual as well as coordinate expression of the reductases will be studied. These studies will lead to a better understanding of how individual nitrogen oxide reductases are regulated as well as how denitrification is coordinately regulated with other respiratory processes.

Both random and directed approaches will be used to identify trans-acting proteins. Random mutants will be isolated using transpositional mutagenesis. Putative regulatory mutants will be identified with the use of reporter gene fusions. Directed approaches will include directed mutagenesis and the sequencing of regions flanking the nitric oxide and nitrite reductases. Preliminary work has suggested that important trans-acting factors are encoded in these flanking regions.

Cornell University
Ithaca, NY 14853

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

P.L. Steponkus, Department of Soil, Crop and Atmospheric Sciences $106,000

More than $100 million are spent annually in attempts to minimize freezing damage to agricultural crops. The majority of these expenses are for energy-costly practices to modify the ambient microclimate. In spite of these practices, annual losses are estimated to be $1.5 billion in the United States and $14 billion worldwide. The development of genotypes with increased freezing tolerance would provide a more reliable means to minimize crop losses and greatly diminish the use of energy-costly practices to modify the microclimate. The ultimate goals of this project are to provide a mechanistic understanding of the cellular and molecular aspects of freezing injury and cold acclimation and to develop rational strategies for the improvement of freezing tolerance of crop species. The current objectives are to elucidate genotypic differences in membrane lipid composition of rye and oat, which represent the extremes in freezing tolerance of winter cereals, and to determine the mechanism(s) by which cytosolic sugars increase the cryostability of cellular membranes. Studies of the effect of sugars on the cryostability (as measured by leakage and fusion) and phase behavior of phospholipid vesicles dehydrated over a continuum of osmotic pressures have revealed that both sugars and electrolytes, such as NaCl and LiCl, minimized the dehydration- induced increase in the liquid crystalline-to-gel phase transition temperature of the lipids dehydrated over the range of 0 to 40 MPa; however, at osmotic pressures > 76 MPa, sugars were much more effective than other solutes. More important, under conditions of near-equilibrium freezing, sugars were unique in their ability to minimize freeze-induce leakage and fusion that occur during passage through the phase transition.

Cornell University
Ithaca, NY 14853

57. Genetic Control of Nitrate Assimilation in Klebsiella pneumoniae

V.J. Stewart, Section of Microbiology, Division of Biological Sciences
$181,972 (FY 94 funds/2 years)

Klebsiella pneumoniae is an enteric bacterium closely related to *Escherichia coli.* Nitrate and nitrite are important nitrogen sources not only for *K. pneumoniae*, but also for many other microorganisms and most plants. In the absence of ammonium, nitrate (or nitrite) induces the synthesis of assimilatory nitrate reductase and assmilatory nitrite reductase, which act in sequence to convert nitrate to ammonium. Our previous work has concentrated on identifying and characterizing the structural genes involved in nitrate assimilation, which are organized in the *nasFEDCBA* operon. The *nasFED* genes apparently encode a nitrate uptake system;

the *nasC* and *nasA* genes encode electron-transfer and catalytic subunits, respectively, of assimilatory nitrate reductase; and the *nasB* gene encodes assimilatory nitrite reductase. A positive regulatory gene, *nasR*, is located immediately upstream of the *nasF* operon. Our current studies are designed to explore the regulation of *nasF* operon expression. Transcription is induced by nitrate or nitrite during nitrogen-limited growth. The global Ntr regulatory system stimulates gene expression during growth in the absence of added ammonium. Combined DNA sequence, deletion and mutational analysis indicates that this stimulation of *nasF* operon expression is conventional, with upstream NtrC protein binding sites serving to activate a sigma-54-dependent promoter. Nitrate and nitrite induction appears to be mediated by a transcriptional antitermination mechanism. Again, DNA sequence, deletion and mutational analyses suggest that a factor-independent transcriptional terminator is located within the transcribed leader region upstream of the *nasF* gene. In the presence of nitrate or nitrite, the NasR protein appears to inhibit termination at this site, thereby allowing high-level *nasF* operon expression. We are currently considering alternative hypotheses to describe the mechanism of this transcriptional antitermination.

Cornell University
Ithaca, NY 14853

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

D.B. Wilson, Section of Biochemistry $89,000

The goals of this project are to determine the molecular mechanisms regulating cellulase synthesis in the soil bacterium, *Thermomonospora fusca*, and to determine the mechanism by which *T. fusca* cellulases degrade crystalline cellulose. The X-ray structure of the E2 catalytic domain is continuing to be refined to achieve maximum resolution (currently 1.0 Å). We are trying to cocrystallize E2cd with two oligosaccharides, methyl umbelliferyl xyloglucose kD ~ 2×10^{-9} and cellobiosylthiocellobioside. In addition we are trying to crystallize two mutant cds, Asp_{265} Val and Glu_{263} Gly. A study of the binding of several umbelliferyl saccharides to E2 and two E2 mutants showed that binding was extremely specific and that both of the above mutants greatly decreased the binding affinity of E2. We isolated an E2 mutant Trp16 Ile that changed the substrate specificity of E2 by reducing activity specifically on amorphous cellulose and are trying to understand how a mutation outside of the active site could cause this change. The Asp_{265} Glu mutant retains activity but is expressed at a low level in *E. coli*. We are expressing it in *Streptomyces lividans* to obtain enough to characterize its activity. We have obtained crystals of E3cd, E4cd and E5cd and are working to solve their 3-D structures by X-ray crystallography. The N-terminal sequence of the *T. fusca* protein that regulates cellulase gene induction has been determined and we plan to produce an oligonucleotide probe based on this sequence and clone its gene.

Cornell University
Ithaca, NY 14853-8101

59. Conversion of Acetic Acid to Methane by Thermophiles
S.H. Zinder, Department of Microbiology $105,000

The objective of this project is to provide an understanding of thermophilic anaerobic microorganisms capable of breaking down acetic acid, the precursor of two-thirds of the methane produced by anaerobic bioreactors. Recent results include: (1) the demonstration that thermophilic cultures of *Methanosarcina* and *Methanothrix* show minimum threshold for acetate utilization of 1-2.5 mM and 10-20 μM respectively, in agreement with ecological data indicating the *Methanothrix* is favored by low acetate concentration; (2) the demonstration that, in contrast to *Methanosarcina, Methanothrix* strain CALS-1 did not accumulate H_2 during methanogenesis from acetate, but instead accumulated CO; (3) the purification and characterization of a carbon monoxide dehydrogenase/corrinoid protein complex from cell-free extracts of *Methanothrix* strain CALS-1 which shows different subunit sizes from that of *Methanosarcina*; (4) the demonstration of methanogenesis from acetate and ATP in cell-free extracts of strain CALS-1. Methanogenesis occurred at a high rate (100-300 nmol min^{-1} [mg protein]$^{-1}$), and much lower rates were obtained with acetyl-CoA or acetyl-phosphate as substrates; (5) the demonstration that methanogenesis from acetate in *Methanothrix* was independent of H_2 and other electron donors, in contrast to *Methanosarcina*, and in agreement with results obtained in whole cells; (6) the demonstration of a role for the cell membrane in methanogenesis from acetate in extracts of gently-broken cells. Current research is centered on biochemical factors which allow thermophilic *Methanothrix* to compete with *Methanosarcina*.

University of Delaware
Lewes, DE 19958

60. Metabolic Mechanisms of Plant Growth at Low Water Potentials
J.S. Boyer, College of Marine Studies $99,500

In higher plants, growth is more frequently limited by water than by any other environmental resource. The project objective is to identify the underlying molecular mechanisms causing growth limitations by focusing on the process of cell enlargement. Studies so far have shown that, in localized growing regions of germinating soybean seedlings, turgor in most of the cells was completely maintained when water potentials were low enough to inhibit growth. However, in stems, gradients in water potential decreased between the vascular tissue and the enlarging cells and growth was inhibited. A few h later, the extensibility of the cell walls decreased and an extractable 28kD protein accumulated in the wall fraction. To determine whether the protein was present in the cell walls of the intact plants, roots were pressurized sufficiently to force exudate onto the surface of the stems. The exudate contained the

protein, which had acid phosphatase activity. There was acid phosphatase activity in the cytoplasm as well. Experiments were underway to determine whether the phosphatase plays a role in the growth response. This work has been extended to growing leaves of maize where pressure is being applied to roots in order to rapidly change the xylem water potential and thus gradients in water potential in the growing tissues. This technique allows metabolic effects to be separated from effects of potential gradients and is being done in collaboration with Dr. John Passioura.

Duke University
Durham, NC 27708

61. Molecular, Genetic and Physiological Analysis of Photoinhibition and Photosynthetic Performance

J.E. Boynton, N.W. Gillham and C.B. Osmond, DCMB Group, Departments of Botany and Zoology $110,000

A major goal of this project is to combine molecular genetics, biochemistry and physiology to understand the relationship between photosynthetic performance *in vivo* and the structure of the multifunctional D1 reaction center protein of Photosystem II (PSII). Exposure of plants and algae to full sunlight level irradiance often causes photoinhibitory damage to the D1 protein encoded by the chloroplast *psbA* gene, resulting in reduced photosynthetic efficiency. We are using the unicellular green alga *Chlamydomonas reinhardtii* as a model system for our studies on D1 because of the organism's excellent genetics and ease of chloroplast transformation. Our physiological and biochemical characterization of photosynthetic function in site directed mutations with 12 of the possible 20 amino acids inserted at the Ala_{251} residue of the D1 protein is continuing. We have also isolated suppressor mutations which partially or totally corrrect the non-photosynthetic phenotype of the Arg_{251}, Gln_{251}, Glu_{251} and His_{251} mutations or the light sensitive phenotypes of the Val_{251} and Leu_{251} mutations. Some of these suppressors result in additional amino acid substitutions at D1 residue 251, whereas others appear to be in yet unidentified nuclear gene products that affect the D1 cycle of synthesis, maturation and turnover. To date we have obtained no second site suppressor mutations elsewhere in the D1 protein by EMS mutagenesis of these non-photosynthetic or light sensitive D1 mutations. As a result, we are carrying out random *in vitro* mutagenesis of the region of the psbA gene corresponding to the quinone/herbicide binding loop of the D1 protein to ascertain whether other amino acid substitutions in this region can suppress these mutations at the Ala_{251} residue. We are also examining the molecular basis for the increased synthesis of D1 and and reduced synthesis of *Rubisco* large subunit (LSU) that occur immediately after low light grown cells are transferred to high irradiance, which results in their photoinhibition. Experiments are in progress to determine whether the differential expression of these two key photosynthetic genes in response to light results from binding of specific transacting proteins to the untranslated leader regions of their mRNAs. We expect to identify gene products involved in mediating photosynthetic performance under photoinhibitory conditions that effect the structure/function of the D1 protein directly, or modulate its

synthesis, maturation and turnover. Such results may guide future manipulations of higher plants to allow them to utilize light more efficiently during photosynthesis.

Duke University
Durham, NC 27708

62. Molecular Studies of Functional Aspects of Higher Plant Mitochondria
J.N. Siedow, Department of Botany $82,000

The cytoplasmic male sterile (*cms*) trait in maize is of potential utility in bringing about the more efficient commercial production of hybrid maize lines, aiding the use of maize as a feedstock for the production of ethanol. Cms-T lines of maize cannot be utilized commercially because they are uniquely sensitive to compounds (T-toxins) derived from the fungus *Bipolaris maydis* race T (and related fungi). The source of T-toxin sensitivity is a mitochondrially-encoded 13 kDa ligand-gated receptor protein, URF13, that interacts with T-toxin to produce hydrophilic pores in the inner mitochondrial membrane. The expression of URF13 in the bacterium *Escherichia coli* confers T-toxin sensitivity on the bacterial cells, and studies indicate that T-toxin binds to URF13 in a specific and cooperative manner. This research as also led to a structural model that puts URF13 in the membrane as an oligomeric complex, with each URF13 monomer containing three membrane-spanning α-helices, two of which are amphipathic and are postulated to line the hydrophilic pore. Topological studies have confirmed the three-helix distribution of URF13 in the membrane and chemical cross-linking has been used to establish its oligomeric nature. Chemical cross linking has also been used in conjunction with site-directed mutagenesis to characterize helix:helix interactions between adjacent monomers in the URF13 oligomer and has led to a refinement of the structural model that postulates a central four-helical core. The continued combination of chemical cross-linking and site-directed mutagenesis is being undertaken to define completely the inter- and intra-helical interactions within the URF13 oligomeric structure.

University of Florida
Gainesville, FL 32611

63. Ethanologenic Enzymes of Zymomonas mobilis
L.O. Ingram, Department of Microbiology and Cell Science $191,414

Zymomonas mobilis has been recognized as a potentially important industrial microorganism for the commercial production of fuel ethanol. This organism is capable of extremely rapid glucose metabolism (1 µmoles glucose min^{-1} mg cell $protein^{-1}$) and can produce high levels of ethanol. To accomplish this rapid glycolysis, the 13 glycolytic and fermentative enzymes comprise half of the cytoplasmic protein. Our studies focus on the characterization of the genes encoding these enzymes, the contributions of individual enzymes to glycolytic flux, the

use of these enzymes to develop recombinant catalysts for fuel ethanol production, and the development of genetic tools for further improvements of *Z. mobilis*. Increasing the levels of three glycolytic enzymes (glucose facilitator protein, glucokinase, and glucose 6-phosphate dehydrogenase) increases glycolytic flux by 5% to 15%. Increasing the levels of other glycolytic enzymes actually decreased flux in a fashion predicted by the proportion of extra protein, i.e., protein burden. This protein burden has been quantified and is a potential source of error in flux control studies with abundant enzymes. Our recently discovered DNA methylase from *Z. mobilis* will be used to construct new expression vectors and specialized vectors for integration and transposon delivery. These tools, along with the elements from the highly expressed glycolytic genes, will be useful for future genetic engineering of *Z. mobilis*. Additional studies will continue to explore the genetic engineering of other organisms using the *Z. mobilis* genes for ethanol production, and new uses for the glycolytic enzymes as a means of improving the rates or yields of microbial products.

University of Florida
Gainesville, FL 32611

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

R.A. Jensen, Department of Microbiology and Cell Science $144,000

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 Georgia
Athens, GA 30602-7229

65. The Metabolism of Hydrogen by Extremely Thermophilic Bacteria

M.W.W. Adams, Department of Biochemistry and Center for Metalloenzyme Studies

$202,000 (2 years)

Extremely thermophiles or "hyperthermophiles" are a unique group of microorganisms that have the remarkable property of growing optimally near and above 100°C. They have been isolated mainly from marine volcanic environments, including deep sea vents. We grow some of these organisms in large scale culture (600 liters) and are studying their pathways of hydrogen (H_2) metabolism. The archaea (formerly archaebacteria), *Pyrococcus furiosus* (Pf, T_{max} 105°C), *Thermococcus litoralis* (T_{max} 98°C) and "ES-4" (T_{max} 110°C) produce H_2 by the fermentation of carbohydrates and/or peptides and their growth is stimulated by tungsten (W), an element seldom used in biology. From these organisms we have purified nickel-containing hydrogenases, ferredoxins, rubredoxins, four different types (POR, KGOR, IOR and POR) of 2-ketoacid ferredoxin oxidoreductases (KAORs), together with three different types of tungsten-containing aldehyde ferredoxin oxidoreductase, in which the tungsten is coordinated by a pterin cofactor. One of the tungstoenzymes (GAPOR) is thought to couple glyceraldehyde-3-phosphate oxidation to H_2 production in a new glycolytic pathway, while the other two (AOR and FOR), like the KAORs, couple reductant generated in peptide catabolism to H_2 production. All of the enzymes have optimum temperatures for catalysis above 95°C, and the redox proteins are stable at 95°C for at least 12 hours. From the hyperthermophilic bacterium, *Thermotoga maritima* (Tm, Tmax 90C), we have purified an iron-containing hydrogenase, ferredoxin and POR. Tm lacks all of the tungstoenzymes and three of the four KAORs found in the archaea. The genes for ferredoxin, AOR, POR and VOR from Pf and POR from Tm have been cloned and sequenced. In collaborative studies with D. Rees (Caltech), the crystal structure of Pf AOR has been determined to 2.3. This is the first structure for a hyperthermophilic, a tungsten-containing, or a pterin-containing enzyme. Since molecular H_2 plays a central role in the commercial production of many chemicals, a long term objective of this research is to assess the utility of hyperthermophilic hydrogenases and oxidoreductases in industrial energy conversions.

University of Georgia
Athens, GA 30602-4712

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

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

$100,000*

The IBM PC-compatible, computerized database (The Complex Carbohydrate Structure Database, CCSD) and database management system (CarbBank) were created by scientists

at the Complex Carbohydrate Research Center (CCRC), with collaboration of scientists in Europe. Building the database and making the software available to researchers worldwide were the initial goals of the project. Towards this goal, distribution and software development licenses have been formed with several major biosequence database providers. These are the National Center for Biotechnology Information (NCBI) of the U.S. National Library of Medicine (NLM) at the U.S. National Institutes of Health (NIH), the Protein Information Resource (PIR) International, composed of the National Biomedical Research Foundation (NBRF) in the U.S., the Martinsreid Institute for Protein Sequences (MIPS) in Europe and the Japan International Protein Information Database (JIPID) in Asia. NCBI and MIPS provide the CarbBank software and the CCSD on their Internet servers, while MIPS, NBRF, and JIPID distribute CarbBank and the CCSD on their Atlas CD-ROM (updated quarterly).

Based on estimates by NCBI and PIR for release CCSD8, the database, at that time, had been sent to over 600 users worldwide. The number of records in the CCSD and the publication rate of articles containing complex carbohydrates structures is growing rapidly, currently at about 12,000 records per year. CCSD13 contains over 36,000 records of complex carbohydrate sequences; CCSD8 contains over 27,500 records of complex carbohydrate sequences and corresponding bibliographic information, about 32,000 of which have been verified for accuracy. Unfortunately, current staff levels have not allowed all pertinent articles to be abstracted; there is a current backlog of about 8,000 records.

The CarbBank staff of the CCRC is emphasizing software development rather than to make an all-out effort to keep up with data entry. The DOS version of CarbBank is being enhanced, as necessary, to accommodate the continually growing CCSD. At the same time, CarbBank is being completely re-written so that it will run on a wider range of computer operating systems, including Microsoft Windows, Apple Macintosh, and UNIX. The version for Microsoft Windows is under development, with a targeted release date of October 1995. Another major goal is to develop network-compatible software to enable authors to submit entries directly to the CCSD via the World-Wide Web. We believe that direct author submission is the only affordable way the CCSD can be maintained as a comprehensive database.

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

University of Georgia
Athens, GA 30602-4712

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

$1,110,351

The University of Georgia Complex Carbohydrate Research Center (CCRC) has a multidisciplinary faculty and staff who serve as a national resource for basic research in

complex carbohydrate science. The CCRC has ten tenured or tenure-track faculty and expects to grow to about 14 faculty over the next five years. The grant supports research, analytical services, and training in plant and microbial complex carbohydrates. Five 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. Twenty-nine undergraduate and 33 graduate students are currently pursuing research projects or graduate degrees (5 M.Sc. students, 28 Ph.D. students) in the CCRC; 23 undergraduate and 12 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; 23 scientists attended these courses in 1994. The plant and microbial carbohydrate program has provided service to 176 individuals by analyzing 728 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 140 internal or external collaborative research projects of which 37 are part of the plant and microbial carbohydrate program. The CCRC has, in one way or another, assisted more than 110 corporations during the same time period; in addition, more than 100 companies were early subscribers to the Complex Carbohydrate Structure Database (when it was distributed directly from the CCRC rather than by CD-ROM or on the Internet as it is now distributed).

University of Georgia
Athens, GA 30602-4712

68. The Structures and Functions of Oligosaccharins
P. Albersheim, Complex Carbohydrate Research Center $181,000

Oligosaccharins are a class of signal molecules involved in regulation of plant growth, development, and defense against pests. The research supported is designed to identify new oligosaccharins and to elucidate how oligosaccharins function *in planta* by studying proteins that generate and process oligosaccharins as well as protein receptors for oligosaccharins. The research projects that we are investigating include determining: (i) the role of fungal endopolygalacturonases and plant polygalacturonase inhibitor proteins in pathogenesis, (ii) the role of fungal proteins that inhibit pathogenesis-related β-1,3-glucanases and chitinases of host plants, (iii) whether plant tissues contain endogenous "nodulation" factors and the effect of nodulation factors on plant growth and development, (iv) the mechanism by which *endo*glucanases and xyloglucan oligosaccharides control growth of pea stems, (v) the role of *endo*xylanases and arabinosidases in the pathogenesis of rice by *Magnaporthe grisea,* and (vi) the role of an auxin-induced *endo*-β-1,4-glucanase in elongation growth of pea stems.

University of Georgia
Athens, GA 30602-4712

69. Structural Studies of Complex Carbohydrates of Plant Cell Walls
A. Darvill, Complex Carbohydrate Research Center $415,000

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

University of Georgia
Athens, GA 30602-7229

70. Mechanisms of Lignin Biosynthesis During Xylogenesis in Zinnia elegans
K-E.L. Eriksson and J.F.D. Dean, Department of Biochemistry $88,129

Results from this project have helped reinvigorate the hypothesis that O_2-dependent enzymes, specifically laccases, play an important role in the deposition of lignin, a major component of the secondary cell walls in plant vascular tissues. Laccase activity extracted from macerated stems of *Zinnia elegans* using high-salt buffers can be resolved by isoelectric focusing into isoform classes having either basic (pI>9.0) or acidic (pI<5.0) isoelectric points. The relative proportions of these isoform classes appear to change during development of the plant stem, and each may thus represent enzymes expressed in different lignifying cell types. A

purification protocol has been developed for the predominant laccase (basic isoform) in young stems, and the purified enzyme is currently being characterized with respect to amino acid sequence, spectral characteristics, and kinetic activity. Polyclonal antibodies raised against synthetic peptides based on the gene sequence of laccases cloned from sycamore maple and yellow-poplar trees recognize the purified *Zinnia* laccase. A partial cDNA fragment isolated from stem mRNA using PCR techniques appears to encode a laccase representing the basic isoform class, but it is not known whether the purified enzyme corresponds to this exact gene. Tissue prints of *Zinnia* stem sections indicate that the laccase gene is expressed specifically in the xylem tissues. Efforts to purify and clone laccases representing the acidic isoform class are underway.

University of Georgia
Athens, GA 30602-7271

71. Genetic Analysis of Embryo Dormancy
G.A. Galau, Department of Botany $135,000 (18 months)

Primary dormancy is the inability of mature seed to germinate until specific environmental stimuli are perceived that predict that future conditions will support plant growth and seed set. The results of physiological, molecular and genetic approaches to the study of dormancy are complex and equivocal. There is a need for new classes of mutants that test the separate nature of inhibition of embryo vivipary, which is required during embryo development in virtually all species, and primary dormancy, which is present in mature embryos of only particular species. The approach taken here is to directly isolate mutants of *Arabidopsis* that appear to be deficient only in primary dormancy. Seed of these mutant lines germinate rapidly without the dormancy-breaking, cold stratification that is required by dormant lines of *Arabidopsis*. All other aspects of plant growth and development appear normal in these lines, suggesting that these mutants are defective only in the establishment or maintenance of primary dormancy. At least 4 *RGM* loci have been identified. In addition to further genetic analysis, the physiological phenotypes of these mutants are being evaluated, in particular the stages in embryo development at which wild-type and mutant embryos diverge.

Dormancy impacts crop production in several ways. Cultivars of dormant species have been bred for rapid germination, but preharvest and postharvest sprouting and loss in viability can occur in such lines. This suggests that selection has been for weak forms of embryo vivipary rather than true nondormancy. There is also extended and variable primary dormancy in many weed species. Understanding the genetic mechanisms of vivipary inhibition, dormancy, and dormancy breaking should help in addressing these problems.

University of Georgia
Athens, GA 30602-7229

72. 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 $175,000

Biomass is degraded in nature by a consortium of anaerobic microorganisms consisting of bacteria and fungi to organic compounds, methane and CO_2 which can be used as industrial feedstock chemicals or as alternate fuels. The biomass is degraded by secreted hydrolytic enzymes, such as celluloses, xylanases and esterases and eventually converted to the organic compounds and methane. Specific projects include the structure of the cellulolytic enzyme complex, the cellulosome, of *Clostridium thermocellum*; the cellulolytic and xylanolytic enzymes and cyclophilin from the anaerobic fungus *Orpinomyces* PC-2; and electron transport coupled to energy generation in *Clostridium thermoaceticum* and in the hydrogenosome of anaerobic fungi. The cellulosome of *C. thermocellum* consists of 26 different polypeptides which have different enzymatic activities or serve as supporting (scaffolding) proteins, e.g., CipA. CipA binds several enzymatic subunits and also to cellulose. This construction makes an efficient cellulolytic enzyme complex. The binding between the CipA and the catalytic subunits is being investigated as well as some newly recognized subunits. The anaerobic fungi produce very active xylanases and esterases. The enzymes are being characterized and are subjects for production by heterologous cells (e.g., in yeast). A major problem in anaerobic microbiology is the generation of energy during autotrophic growth. It is studied with the acetogens *Clostridium thermoaceticum* and *C. thermoautotrophicum*. These studies involve electron transport, ATP-generation, and H_2-cycling. Similar work is done with hydrogenosomes that are organelles of the anaerobic fungi. They evolve H_2 and purportedly generate ATP.

University of Georgia
Athens, GA 30602

73. Why Do Plants Have Two Pathways of Polyamine Synthesis?

R.L. Malmberg, Botany Department $199,820 (FY 94 funds/2 years)

Polyamines are small positively charged compounds that have been hypothesized to be involved in a wide variety of plant physiological and developmental functions. One feature of plant polyamine synthesis is the presence of two alternative pathways to putrescine, from arginine via arginine decarboxylase (ADC) and from ornithine via ornithine decarboxylase (ODC). We are exploring regulatory and functional differences between arginine and ornithine decarboxylases in plants. We have recently isolated a large collection of mutants of *Arabidopsis* that are deficient in ADC, ODC, or in genes that regulate their activity. We are

using these mutants to identify both the relative functions of ADC and ODC, and to define the factors that regulate the pathways. Our research on ADC structure has identified a novel post-translational means of regulation involving a proteolytic clipping step. We are characterizing the unusual protease that carries out this reaction.

University of Georgia
Athens, GA 30602

74. The Determinants of RNA Turnover in Higher Plants
R.B. Meagher, Department of Genetics $198,000 (2 years)

The mechanisms and determinants regulating plant RNA processing and turnover are essentially unknown. By examining the role of plant poly(A) binding proteins (PABPs) we hope to elucidate many of the important components of RNA turnover. Recent data in yeast suggests that PABP is the central component of the post-transcriptional machinery. Plants have a large and extremely diverse PABP gene family and we hypothesize that (#1): the diverse plant poly(A) binding proteins are differentially expressed and encode proteins with distinct functions. In the past few years we have shown that Arabidopsis PAB1 was root specific, PAB2 was expressed in all organs and tissues examined including root, stem, leaf, flower, pollen and siliques, and PAB3 & PAB5 were floral specific. Expression of a PAB5 promoter/reporter fusion in transgenic Arabidopsis plants was restricted to pollen, ovules, and early embryo development and we expect it plays a unique role in gamete or embryo development. When the yeast PABP gene is replaced with PAB5 on a single copy yeast plasmid, PAB5 protein controls poly(A) tail length, mediates translational re-initiation, and interacts specifically with the yeast translational factor SIS1. This preliminary work led to hypothesis #2: specific transacting factors recognize the distinct plant PABPs and regulate post-transcriptional processes. Our first three specific aims follow on these two hypotheses: (1) to determine general and plant specific functions of PAB2 and PAB5; (2) to demonstrate that the various PABP genes or subfamilies are required for normal Arabidopsis growth and development by characterizing the phenotype of plants mutated in or suppressed for PABP expression; and (3) to identify specific transacting factors that bind to PABPs and regulate post-transcriptional processes. Preliminary work suggest that PAB2 on a high copy yeast plasmid can also complement yeast and we are now examining this complementation at the functional level. Using PAB2 as a "bait" in a yeast di-hybrid system we have begun to characterize the specificity of "prey" gene products isolated from an Arabidopsis cDNA library, candidates for proteins which bind physically to PAB2.

University of Georgia
Athens, GA 30602

75. Nitrogen Control of Chloroplast Development and Differentiation
G.W. Schmidt, Department of Botany $94,000

A major environmental factor that influences plant and algal growth and development is nitrogen availability. Deficiency of this nutrient leads to conspicuous alterations of the photosynthetic apparatus and carbon metabolism as is evident by chlorosis and reduced growth rates. Toward understanding the mechanisms that underlie adaptation to nitrogen deficiency, *Chlamydomonas reinhardtii* is grown in a nitrogen-limited continuous culture system and utilized for molecular and physiological studies. Chlorophyll deficiency is found to result from depressed expression of nuclear genes encoding light-harvesting chlorophyll-binding proteins and genes encoding enzymes in the early steps in porphyrin synthesis. A major aspect of our work is to resolve the molecular and biochemical features of N-control of nuclear gene expression. At the level of chloroplasts, chlorophyll-binding proteins of photosynthetic reaction centers are present at greatly reduced levels because newly-synthesized proteins are mostly degraded in the absence of pigment ligands. Even though components of thylakoids are diminished overall, carbon fixation persists. However, metabolism is profoundly altered such that there are pronounced increases in starch and storage lipid levels. In addition, dark respiratory activities are augmented but this is highly sensitive to illumination with even low light intensities. This phenomenon, previously attributed to a chlororespiratory pathway of photosynthetic membranes, is under investigation with a focus on the identity of the intermediary components employed in the physiological relationships of mitochondria and chloroplasts that seem to be especially intimate in nitrogen-deficient cells.

University of Georgia
Athens, GA 30602

76. Post-Transcriptional Regulation and Evolution of the R/B Gene Family in Maize and Rice
S.R. Wessler, Departments of Botany and Genetics $102,000

The *R/B* genes encode a family of transcriptional activators that control the distribution of anthocyanin pigments in the maize plant. We have demonstrated that an upstream open reading frame (uORF) composed of 38 codons and found in most *R* genes, represses *R* expression post-transcriptionally about 30-fold. The ability of uORF to repress translation is dependent on the uORF codons but independent of the identity of the downstream gene. That is, the magnitude of uORF-mediated repression was altered by site-directed mutations in uORF codons but was unchanged when the *R* gene was swapped for the luciferase reporter gene. Experiments over the past year indicate that the uORF codons mediate

repression by (i) encoding a peptide that slows the flow of ribosomes downstream by retarding the movement of the translating ribosome and (ii) encoding rare codons that act synergistically with the uORF peptide to temporarily stall translating ribosomes. There are four other examples of uORF codon-involvement in translational control. In no instance is the mechanism of repression understood.

The characterization of the *R* gene family of rice is another goal of this project. The organization of the *R/B* gene family of maize is a reflection of the evolutionary history and genome architecture of maize. That is, an ancient polyploidization event and transposable element activity have been implicated in the duplication and diversification of family members. In contrast, *O. sativa* is a true diploid with 6-fold less genomic DNA than maize. Despite these differences, we have found at least two *R* genes in rice. Furthermore, our data indicate that the *R* gene family has evolved recently and independently in maize and rice.

University of Georgia
Athens, GA 30602

77. Biochemistry and Genetics of Autotrophy in Methanococcus
W.B. Whitman, Department of Microbiology $162,000 (FY 94 funds/2 years)

Even though methanogenic bacteria catalyze the terminal step in the anaerobic oxidation of organic matter, these bacteria have a very narrow substrate specificity. From this perspective, the carbon metabolism of the methanococci has been examined. Although these bacteria are unable to utilize exogenous sugars as carbon and energy sources, they contain the enzymes of the Embden-Meyerhof pathway for the oxidation of endogenous glycogen. In addition, the biochemical properties of the pyruvate oxidoreductase are similar to those of the oxidative enzymes common in other archaebacteria. Thus, methanococci contain the basic enzymatic machinery for the oxidation of sugars to acetate. To facilitate development of a genetic system in methanogens, the cryptic plasmid pURB500 from *Methanococcus maripaludis* has been sequenced in its entirety. Two large open-reading-frames and a region of complex secondary structure typical of origins of replication were discovered. The puromycin resistance cassette (*pac*) for methanococci was cloned in pURB500, and the transfer of the plasmid between strains of methanococci was demonstrated. However, it has not been possible to clone pURB500 in a number of common *E. coli* plasmids, and it may encode a product that is toxic in that bacterium. Nevertheless, derivatives of pURB500 may be useful for genetic manipulations in methanococci.

University of Georgia
Athens, GA 30602

78. Hemicellulases from Anaerobic Thermophiles

J. Wiegel, Department of Microbiology and Center for Biological Resource Recovery $91,000

The long term goal of this research effort is to develop an anaerobic thermophile that efficiently converts various types of hemicellulose-containing biomass to ethanol within a broad pH-range. The ethanologenic thermophile *T. ethanolicus* JW200, however, utilizes xylans poorly and has barely measurable xylanase, acetyl(xylan) esterase and O-methyl glucuronidase activities, which are regarded as rate limiting steps in xylan utilization by this bacterium, as xylose is readily converted to ethanol. To alter the enzyme make-up and regulation in *T. ethanolicus* we 1) characterize hemicellulolytic enzymes to identify possible candidates for cloning into *T. ethanolicus* and 2) develop a genetic system for this and other anaerobic thermophilic (eu)bacteria. We have identified a non-ethanologenic thermophile with suitable hemicellulolytic enzyme activities, *Thermoanaerobacterium* spec. JW/SL-YS485. We purified and characterized from *T. ethanolicus* the bifunctional xylosidase/arabinosidase, and from *Thermoanaerobacterium* JW/SL-YS495 two xylosidases, two acetyl(xylan) esterases, the O-methyl glucuronidase and a large cell-bound xylanase. We sequenced the xylanase and xylosidase genes. The xylanase contains repeated S-layer-like sequences at the C-terminal and we assume that they are responsible for anchoring the xylanase to the S-layer and thus, rendering the exoenzyme to be cell bound. We continue cloning of the genes of the other above mentioned enzymes and characterizing additional xylanases with optimal activities at different pH ranges as possible donors for *T. ethanolicus*. We are continuing efforts to develop a shuttle vector for *T. ethanolicus*.

University of Georgia
Tifton, GA 31793

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

W.W. Hanna and G.W. Burton, Department of Agronomy $53,000

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. Apomictic backcross-6 plants and new backcross-4 and -5 plants were developed with seed set up to 50%. Apomictic plants closely resemble cultivated pearl millet. Over 1100 crosses on 26 unknown cytoplasms revealed the identify of cytoplasms different than the present A1, A2, A3 and A4 cytoplasms. Tift 93, an inbred developed from secondary gene pool germplasm was released to produce a cytoplasmic-nuclear male sterile F1 hybrid for commercial hybrid seed production of high quality forage hybrids.

University of Hawaii
Honolulu, HI 96822

80. Violaxanthin De-epoxidase: Biogenesis and Structure

H.Y. Yamamoto, Department of Plant Molecular Physiology
$92,700 (FY 94 funds/2 years)

Violaxanthin de-epoxidase (VDE), an enzyme of the xanthophyll cycle that is localized in the lumen of thylakoids, catalyzes the conversion of violaxanthin to antheraxanthin and zeaxanthin. The formation of zeaxanthin and antheraxanthin, in conjunction with a transthylakoid delta pH, induces a non-radiative dissipation of energy in the antennae that diverts absorb energy from PSII, decreasing the quantum yield. VDE is the key enzyme for this feed-back regulatory mechanism that serves to keep PSII traps open, functioning, and protected against photoinhibition. Following purification of VDE to a single major component detectable by 2-D SDS-PAGE, amino-acid sequences were determined for the N-terminus and peptides obtained from tryptic digests. Degenerate DNA primers were constructed from these sequences to PCR amplify a portion of vde for use as a probe. Using this probe, a full-length cDNA of VDE was isolated from a lettuce library and sequenced in both directions. Cell extracts of *E. coli* transformed with vde cDNA showed typical de-epoxidase activity, proving conclusively that the sequence encodes VDE. The mature protein has three domains of interest: a hydrophobic and cysteine rich N-terminus, a lipocalin signature near the cysteine-rich region, and a glutamic-acid rich C-terminus. Site-directed and deletion mutagenesis for functional domains, isolation of vde from tobacco, inhibition and enhancement of VDE activity by plant transformation, and transport of pre-VDE into chloroplasts are in progress or planned.

University of Illinois
Chicago, IL 60612

81. Heavy Metal-lux Sensor Fusions and Gene Regulation

S. Silver, Department of Microbiology and Immunology $105,000 (15 months)

We are studying the molecular genetics of bacterial resistances to toxic heavy metals, especially 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 will be analyzed to follow the relationship between detailed laboratory studies with new environmental isolates. The newly recognized CadC transcriptional repressor of the cadmium-resistance cadmium-efflux ATPase will be studied by protein-DNA interactions *in vitro* and *in vivo*. Mutants with altered CadC repressor will be used to test specific hypotheses concerning metal-protein binding and protein-DNA binding. The enzymatic transformations of arsenic by the enzymes arsenate reductase and arsenite oxidase hold potential for microbial bioremediation of soil and water arsenic pollution. Work on the silver-resistance system has just started and the plasmid DNA determinant, its genes and proteins will be characterized.

University of Illinois
Urbana, IL 61801

82. Photosynthesis in Intact Plants
A.R. Crofts, Department of Physiology and Biophysics $110,000

The goal of the project is to understand how photosynthesis is regulated in intact plants. Under natural conditions, plants experience large differences in light intensity over the day and within the canopy, and can adapt to extreme variations in local humidity, but are able to maintain optimal rates of photosynthesis by control mechanisms which modulate input of excitation to match availability of CO_2. The mechanisms of control, and integration with the physiology of the leaf, are poorly understood. An important component of the feed-back mechanism is the proton gradient. Previous work has demonstrated that lumenal pH exerts a control on several reactions of the photosynthetic chain, and over qE-quenching and zeaxanthin formation, which control excitation delivery. Our main effort will be to understand how these different effects of lumenal pH are coordinated. We have developed portable and laboratory based instruments and methods for studying photosynthesis in intact plants, and a substantial program of research using these instruments is underway in the lab and in the field. We are studying the partial reactions of photosystem II (donor and acceptor sides), the intermediate electron transfer chain, including the cytochrome b_6/f complex, and its interactions with the quinone pool, and the down-regulation of photosynthesis through qE-quenching and zeaxanthin formation. An important component of the project is a laboratory based program of research to establish in simpler systems the parameters through which observation of photosynthetic electron transport and energy coupling in intact plants can be measured. We will continue the development of new instruments, and extend the research programs and collaborations established around the apparatus previously constructed.

University of Illinois
Urbana, IL 61801-3707

83. Genetic Studies on Cytoplasmic Male Sterility in Maize

S. Gabay-Laughnan, Department of Plant Biology

$82,450 (FY 94 funds/2 years)

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

University of Illinois
Urbana, IL 61801

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

R.B. Gennis, Department of Biochemistry $187,000

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, also known as the cytochrome *o* complex, is a coupling site. This means that the redox reaction catalyzed by the enzyme, i.e., the 4-electron reduction of oxygen to water, is coupled to the generation of a proton and voltage gradient across the cytoplasmic membrane. Our primary interest is to learn how this is done.

The *cyo* operon encoding each of the subunits of the oxidase has been cloned and sequenced. Remarkably, three of the oxidase subunits demonstrate a strong similarity to the mitochondrially encoded subunits of the eukaryotic cytochrome *c* oxidase. Recent biophysical studies have confirmed and strengthened the relationship between these two enzymes. Each enzyme contains one low spin heme, as well as a binuclear center consisting of a high-spin heme and a copper. Our research program is currently emphasizing the use of site-directed mutagenesis in combination with spectroscopic techniques to obtain structural and functional information.

Recently, the high resolution structure of a related oxidase was solved by using X-ray crystallography, and that data confirm virtually all of our predictions made on the basis of mutagenesis. We are now in an excellent position to decipher the mechanism of how this enzyme functions.

Current efforts are directed at determining precisely the steps where specific mutants perturb the enzyme turnover and at locating the ubiquinol binding site.

University of Illinois
Urbana, IL 61801

85. Regulation of Cell Division in Higher Plants
T. Jacobs, Department of Plant Biology $94,000 (20 months)

Plant growth is the cumulative product of the expansion of cells that arise by mitosis in meristems. We are interested in understanding what regulates a plant cell's decisions to begin division, to pass from one stage of the division cycle into the next, and to withdraw from the dividing state. Cell cycle passages are controlled by heterodimeric CDK/cyclin protein kinase complexes in all eukaryotes. We have cloned cDNAs encoding these CDKs and cyclins from plants and are working toward a characterization of the constitution and cell cycle activity profiles of the complexes in plant meristems. We are especially interested in identifying upstream control factors that "regulate the regulators". We are employing protein-protein interaction screening strategies to identify proteins that interact with and affect the activity of CDK/cyclins. An important developmental passage in the lives of all eukaryotic cells is that between G1 phase of the cell cycle and S-phase, when cells chose to divide, arrest, differentiate, or die. A variety of transcription factors, tumor suppressor proteins and CDK inhibitors regulate this passage in animal cells. Since regulatory proteins active at this transition are phylogenetically less conserved than those that act at G2-M, we cannot use PCR or homology-based methods to obtain plant cell homologues of these proteins. Therefore, we are again exploiting protein-protein interaction methodologies, with segments of animal G1-S regulatory proteins as probes, to identify botanical candidates for these regulators.

University of Illinois
Urbana, IL 61801

86. Genetics of the Methanogenic Archaebacterium, Methanococcus voltae with Attention to Genetic Expression Mechanisms

J. Konisky, Department of Microbiology $192,000 (2 years)

The objective of our research program is to study the genetics, physiology and molecular biology of marine methanogens including the mesophile *Methanococcus voltae* and the extreme thermophile *Methanococcus jannaschii*. We have recently turned our attention to mechanisms of protein metabolism including studies of gene expression and protein thermostability, turnover and processing. Our initial studies focus on the S-layer protein. The S-layer is composed of a hexagonal array of approximately 400,000 protein subunits and completely covers the cell surface. The S-layer protein is synthesized with a N-terminal leader sequence that is processed at an unknown stage of protein maturation and localization. In order to begin the characterization of the 5' region of the S-layer-encoding structural gene (sla) of *Methanococcus voltae* was sequenced. The sequence information was then used to identify the *in vivo* transcription products of the gene. We observed three transcripts, and upstream of each transcription start point was a region with similarity to the Box A consensus sequence observed in archaeal promoters. In two of the three cases, two Box A sequences were present in tandem. This arrangement may play a role in the high level of gene expression expected for the sla gene. Presumptive archaeal Box B signatures were also identified. Plans are now underway to study the mechanism by which the S-layer protein is processed to remove its N-terminal leader sequence.

University of Illinois
Urbana, IL 61801

87. Exploratory Studies on the Bacterial Formation of Methane

R.S. Wolfe, Department of Microbiology $70,605

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

and characterize the red-fluorescent compound, to determine its structure, its role, and its relationship to known coenzymes which may be modified when cells are under stress. Our goal is to define how sensitive, anaerobic methanogenic-cells survive under stress in terms of biochemistry.

University of Illinois
Urbana, IL 61801

88. Anthropogenic Impacts on Photosynthetic Activity: A Multidisciplinary Context for Research Training

C.A. Wraight, D.R. Bush, J.M. Cheeseman, A.R. Crofts, P.G. Debrunner, E.H. DeLucia, Govindjee, W.L. Ogren, D.R. Ort, A.R. Portis, J. Whitmarsh, R.E. Zielinski $318,560

This is an interdisciplinary research training program for the study of factors influencing the photosynthetic performance and productivity of plants, generally. The role of anthropogenic factors is further explored through the academic curriculum and seminars. Laboratory training acquaints students with techniques available to address current problems and provides a broad knowledge base and the experimental skills required to make progress with these complex issues. Many of the most significant issues span the range from physics to agronomy and an interdisciplinary outlook and multidisciplinary training are especially important for future research. Graduate students and young postdoctoral trainees work on projects coordinated between laboratories of the training program faculty, providing conceptually different outlooks and approaches to the problem. Current projects include field measurements of photosynthetic functions at the membrane level, using sophisticated but portable measuring devices; photoprotective and heat stress responses under natural conditions; mechanistic studies of photosystem II and oxygen evolution; photoprotective roles of carotenoid and light energy quenching activities; the role of the circadian rhythm in chilling sensitivity; genetic and antisense RNA analysis of the regulation of photosynthetic enzymes *in situ*, including the thylakoid ATPase, rubisco and rubisco activase. Undergraduate students are brought into the research environment through summer fellowships for research in faculty labs. A summer workshop on Photosynthesis and Global Climate Change for high school and community college teachers is designed to help them spark interest in science, *per se*, and to provide a basis for critically assessing the flow of information on this societally important issue. Efforts are made to involve teachers from predominately minority schools, by coordinating our recruitment with similar programs already in operation at the University.

Indiana University
Bloomington, IN 47405

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

N.R. Pace, Department of Biology $73,192*

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

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

The program uses existing methods and continues to develop new ones for rapid analysis of natural communities. Additionally, 16S rRNA sequence and phylogenetic analyses are carried out for selected hyperthermophilic organisms already in culture. These sequences make possible phylogenetic analysis of the organisms from which the sequences derive and contribute to the data base to which natural populations are compared. The sequences also are used for comparative analyses of hyperthermophilic and related mesophilic 16S rRNAs, to identify primary and secondary structural elements that may contribute to extreme thermostability.

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

Iowa State University
Ames, IA 50011

90. Organic Specific Expression in Maize: The P-wr Allele

T. Peterson, Department of Zoology and Genetics $99,000

We are investigating mechanisms of organ-specific gene expression using the maize *P* gene as a model. The *P* gene encodes a transcriptional regulator of three other genes required for flavonoid pigment biosynthesis in the floral organs of the maize plant. The *P-rr* allele specifies red kernel pericarps and cob glumes, while the *P-wr* allele specifies white (colorless) pericarps and red cob glumes. The flavonoid compounds may serve a number of important functions in the maize plant, and they provide a convenient visible marker for organ specific gene expression. Our results to date indicate that *P-wr* transcripts are present in both pericarps and cob glumes; however, the *P-wr* transcripts are apparently unable to efficiently activate the structural genes for flavonoid biosynthesis in the pericarp. The *P-wr-* and *P-rr-* encoded proteins are very similar, except for two single amino acid differences near the Myb and transcriptional activation domains, and a large replacement in the COOH terminal regions. We are currently investigating whether *P-wr* gene transcripts are subject to post-transcriptional regulation, or whether the *P-wr* protein is itself differentially active in pericarp and cob glume cells. The results should further elucidate fundamental mechanisms of gene expression which regulate plant development.

Iowa State University
Ames, IA 50011-1020

91. Regulation of Carotenoid Biosynthesis: The Immutans Variegation Mutant of Arabidopsis

S. Rodermel and D. Voytas, Department of Botany and Department of Zoology and Genetics $81,000

The *immutans (im)* variegation mutant of *Arabidopsis thaliana* contains green- and white-sectored leaves due to the action of a nuclear recessive gene. The mutation is somatically unstable, and the degree of sectoring is influenced by light and temperature. The cells in the green sectors contain normal chloroplasts, but those in the white sectors are heteroplastidic for normal and abnormal, non-pigmented plastids (i.e., *im* expression is "plastid autonomous"). In contrast to other variegation mutants with heteroplastidic cells, the defect in *im* is not maternally-inherited. The white tissues of *immutans* accumulate phytoene, a C_{40} carotenoid intermediate, suggesting that *im* controls the activity of phytoene desaturase (PDS), the enzyme that converts phytoene to zeta-carotene in higher plants. However, *im* is not the structural (*pds*) gene for PDS, and since *pds* mRNA and protein levels are unaffected in the *im* white tissues, it is unlikely that *im* is a transcription factor regulating *pds* expression. Fluorescence activated cell sorting (FACS) of pure-white protoplasts from transgenic *im* plants

that express a *cab* promoter/GUS fusion have revealed that *cab* transcription is much higher in these cells than in norflurazon-treated white cells from control plants. This suggests that the *im* white tissues may not be photooxidized, and that the effects on PDS activity may be a secondary consequence of a lesion in early chloroplast development. To further characterize the lesion in *im* plants, we are cloning *immutans* by chromosome walking and functional complementation. To date, we have encompassed *im* in a 200 kb YAC fragment.

University of Iowa
Iowa City, IA 52242

92. Molecular Biology of Anaerobic Aromatic Biodegradation
C.S. Harwood, Department of Microbiology $62,750

We have been studying the molecular basis for anaerobic benzoate and 4-hydroxybenzoate degradation by the bacterium *Rhodopseudomonas palustris*. These aromatic acids are intermediates in the degradation of several environmental pollutants and are formed as breakdown products of plant-derived material. They are also the starting compounds for central pathways of anaerobic benzene ring reduction and ring fission. We have cloned a large region of the *R. palustris* chromosome that is involved in aromatic acid degradation. Included in this region are the gene for 4-hydroxybenzoate-CoA ligase, the first enzyme of 4-hydroxybenzoate degradation, and the gene for benzoate-CoA ligase, which initiates benzoate degradation. Mutagenesis and sequence analysis of DNA flanking the ligase genes indicates that many additional aromatic acid degradation genes lie nearby. Our aim is to define these genes and use them to elucidate the precise sequence of enzyme reactions in the degradation pathways. We also plan 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 specific biomass conversions or for use in bioremediation.

Johns Hopkins University
Baltimore, MD 21218

93. Bacterial Nickel Metabolism and Storage
R.J. Maier, Department of Biology $111,650

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

metabolism into hydrogenase whereas others have amplified nickel-sequestering ability, that is coordinately regulated with hydrogenase. *B. japonicum* takes up nickel with a high affinity binding system, the nickel is stored intracellularly, and putative nickel-storage components have been identified and purified. Candidate proteins involved with the nickel-dependent regulation of hydrogenase both in sensing nickel and in activating hydrogenase transcription have been identified and specific mutants in each of the genes have been generated. The genes encode domains which act as nickel-binding ligands, and at least one of the gene products binds some other divalent metal ions in addition to nickel. The long-term goal is to understand the number, nature, and function of the components involved in bacterial nickel metabolism.

Johns Hopkins University
Baltimore, MD 21218

94. Transport of Ions Across the Inner Envelope Membrane of Chloroplasts
R.E. McCarty, Department of Biology $101,000

The interior of inner envelope membrane vesicles may be loaded with fluorescent probes such as the pH indicator, pyranine of the Ca^{2+} indicator, Fura 2. The transport of H^+ or of Ca^{2+} may be followed conveniently by monitoring probe fluorescence. Vesicle loading was accomplished by a freeze-thaw-sonication method. We have used extrusion of the vesicles through a 100 nm polycarbonate filter to load vesicles. Extruded vesicles appear to be mostly right-side out, whereas freeze-thaw vesicles are mostly inside-out. For plasma membrane vesicles (from red beet storage tissue), extruded vesicles were 80% right-side out and freeze-thaw vesicles, 33%. The orientation of inner envelope membrane vesicles is also likely to be similarly affected by extrusion. Partial purification and reconstitution of an inner envelope H^+-ATPase was accomplished and further evidence for Ca^{2+} transport by the vesicles obtained.

KAIROS Scientific Inc.
Santa Clara, CA 95054

95. Macromolecular Scaffolds for Energy Studies
D.C. Youvan $127,000

The green fluorescent protein (GFP) is the source of fluorescent light emission in some jelly fish. Aequorea victoria GFP absorbs blue light at 395 nm; its activation mechanism has been proposed to occur by energy transfer from the photoprotein aequorin. Wild-type GFP has a fluorescence emission maximum of 509 nm. The protein is easily isolated, very stable, and diffracts to < 2 = C5 resolution. GFP has been cloned, leading to the widespread use as a marker for gene expression and as a protein tag, both in cell culture and in multicellular organisms. Using optimized combinatorial mutagenesis techniques and Digital Imaging

Spectroscopy (DIS), we have isolated mutants of GFP showing > 100 nm shifts in the fluorescence excitation and/or emission spectra. One of these mutants, RSGFP4, has a greatly reduced Stokes shift as compared to wild-type, i.e., 20 nm versus 105 nm, respectively. A variety of other spectrally shifted derivatives is under study. Derivatives with overlapping emission and absorption spectra are of particular interest for this project. By covalently coupling derivatives with cyan emission to derivatives with cyan excitation, we can demonstrate fluorescence resonance energy transfer (FRET) using long wave UV excitation of the donor while recording green emission from the acceptor. With a Forster Ro of 50 = C5, this also provides a means for detecting GFP-tagged molecules that are in close proximity in real-time imaging experiments. Mechanistic studies of these derivatives using time-resolved spectroscopic techniques is also underway.

University of Kentucky
Lexington, KY 40536-0084

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

E. DeMoll, Department of Microbiology & Immunology; D.A. Grahame, Uniformed Services University of the Health Sciences, Bethesda, MD $92,000

This project will allow us to better understand the biochemical mechanisms involved in acetyl-CoA synthesis and cleavage in methanogens. This reversible reaction is catalyzed by the acetyl-CoA decarbonylase synthase (ACDS) enzyme complex. Also, we will characterize the role of this multienzyme complex in the regulation of growth of methanogens. We have shown that methanogens synthesize acetyl-CoA from N^5-*methyl*-tetrahydrosarcinapterin, coenzyme A, CO_2, and reduced ferredoxin (formed with H_2 by a specific ferredoxin-reducing hydrogenase). Experiments will study the kinetic mechanism of the ACDS complex. In addition, we will test our hypothesis that when the methanogens are growing with C_1 units as sole carbon source, carbon flow into anabolic pathways is regulated at the level of the ACDS complex by the available reducing equivalents. We will also investigate whether or not methanogens growing with acetate as sole carbon source regulate growth by a similar mechanism.

University of Kentucky
Lexington, KY 40546

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

R.L. Houtz, Department of Horticulture and Landscape Architecture

$88,705 (18 months)

Methylation of the ∈-amino group of lysyl residues in proteins is catalyzed by a group of highly specific protein methyltransferases (Protein Methylase III's). In several biological systems including plants, the methylation of lysyl residues has been shown to have significant effects on the activity and/or stability of the target protein substrate. In plants a prevalent and important protein which is site-specifically methylated is ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Our research is focused on providing a detailed molecular and biochemical characterization of the protein methyltransferase responsible for methylation of Rubisco (Rubisco LSMT), and a determination of the functional consequences of formation of trimethyllysine-14 in the large subunit (LS) of Rubisco. In this regard we have determined protein and DNA sequence for both pea and tobacco Rubisco LSMT. A comparison of the two protein sequences reveals a high level of similarity (75%), and the presence of five conserved imperfect copies of a leucine-rich repeat, a protein motif commonly associated with protein-protein interactions. In order to facilitate studies of the interaction between Rubisco and Rubisco LSMT, a low abundance protein *in vivo,* the cloned cDNA for pea Rubisco LSMT was expressed in *E. coli*, which resulted in the production of active recombinant Rubisco LSMT. Bacterial expression of amino- and carboxy-terminal truncations of pea Rubisco LSMT, followed by measurements of binding to spinach Rubisco as determined by ELISA, suggest that a central domain of approximately 125 amino acids is instrumental in determining the binding specificity for des(methyl) forms of Rubisco.

University of Kentucky
Lexington, KY 40546-0091

98. 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 $89,824

Fatty acids (FA's) are key components of membranes, waxes, etc., that are essential to plant growth and development. They constitute the main energy reserve for seed germination and seedling development in certain oil seeds. Oil seeds are extensively exploited commercially for their food value and as lubricants, emulsifiers, etc.

Metabolism leading to straight, long chain, even carbon number FA's in plants (catalyzed by fatty acid synthase) is well understood. Less understood is metabolism giving rise to

branched and odd carbon number FA's. We recently described a new pathway (termed alpha keto acid elongation-α KAE) that appears to utilize enzymes of branched chain amino acid biosynthesis to form short and medium chain FA's in trichome glands. We will determine if this pathway participates in medium chain (also long chain) FA biosynthesis in oil seeds of *Cuphea*, soybean, coconut and *Arabidopsis*. Since branched chain FA's are common components of wax esters, the role of α KAE in wax formation will be investigated. Key enzymes involved in α KAE will be isolated and studied. Isolation of their genes will begin in an effort to use genetic engineering to attempt modification of FA and wax ester composition. Yeast and human cDNA's for two key enzymes involved in α KAE metabolism will be introduced into the model plant tobacco to assess the feasibility of genetic engineering for modification of FA components of trichome-exudate sugar esters. Trichome exudate of certain highly-exudated, high-biomass plants represents an unexploited, renewable resource for production of medium and short chain FA's having various configurations and structures.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

99. Enzymatic Synthesis and Biomolecular Materials

M.D. Alper, D. Charych, J.F. Kirsch, D.E. Koshland, J. Nagy, P.G. Schultz, R. Stevens, F. Tropper and C.-H. Wong, Center for Advanced Materials

$149,000*

The goal of this research is the use of natural biological processes and molecules as the basis for the synthesis of new materials. One component focuses on the use of natural, engineered and "created" enzymes to synthesize new materials. The unique stereochemical control exerted by enzymes and their ability to catalyze reactions at low temperature 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 for the enzymatic synthesis of polymeric materials; engineering of enzyme structure and activity to allow the binding and polymerization of novel monomers; generation of catalytic antibodies for materials synthesis; characterization and processing of the polymer products of these reactions and understanding the structure/function relationships of this new class of materials. 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 to alter interfacial and surface properties, and to fabricate sensor devices. Thin film sensors have been developed to detect influenza virus, botulism and cholera toxins. Similar films have been used to direct the ordered crystallization of inorganic salts.

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

Lawrence Berkeley Laboratory
Berkeley, CA 94720

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

J.E. Hearst, Structural Biology Division $232,000

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

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

In *R. capsulatus* examples of this phenomenon are provided by the fact that DNA gyrase has been implicated in the induction of expression of genes for anaerobic metabolic processes such as nitrogen fixation and photosynthesis. Since DNA supercoiling is homeostatically regulated by balancing the antagonistic activities of gyrase and topoisomerase I, we are directing our efforts toward obtaining *R. capsulatus top A* mutants in order to assess the connection between supercoiling and anaerobic gene expression. We also intend to test the hypothesis that the assembly of the photosynthetic reaction center complex and the biosynthetic complexes for bacteriochlorophyll and carotenoids in the intracytoplasmic membrane are mechanistically related to DNA supercoiling.

The first step in this project is to determine the nucleic acid sequence for the gene encoding the topoisomerase I *(topA)* from *R. capsulatus*. Ultimately this gene sequence will allow for the creation of the required mutants for completion of this study.

i) Isolation of *topA* gene from a pJRD 215 cosmid library of wild-type *R. capsulatus*. The first attempt to isolate *topA* gene from a pJRD 215 cosmid library used the *E. coli* strain AS17. These efforts were unsuccessful due to the low-efficiency of transformation of the AS17 strain.

Subsequently, the *E. coli* strain IC1814 was used in our studies. This strain harbors the plasmid pIV90, whose level of resistance to ampicillin is enhanced by a decrease in the degree of plasmid DNA supercoiling. Since IC1814 is a *topA10* mutant and since the degree of plasmid DNA supercoiling of this strain is higher than the wild-type (IC1813) strain, IC1814

shows a low resistance to ampicillin. The complementation of IC1814 by a cosmid expressing the *R. capsulatus topA* gene is expected to recover the wild-type phenotype, thereby increasing the ampicillin resistance. This can be used as a selective method to search the pJRD 215 cosmid library for the *topA* gene in *R. capsulatus*.

Following transformation of IC1814 with DNA from the pJRD 215 cosmid library from wild-type *R. capsulatus*, ampicillin resistant colonies have been selected and the degree of plasmid DNA supercoiling has been analyzed and compared with the wild-type (IC1813) strain. Results have shown that the selected transformants recovered the degree of DNA supercoiling present in the wild-type strain (IC1813).

ii) *R. capsulatus* topoisomerase I isolation is being carried out using a procedure that takes advantages of the binding properties of this enzyme to phosphocellulose and heparin Sepharose. Highly purified enzyme, which retains topoisomerase activity, is obtained using gel filtration chromatography and two successive affinity chromatography steps: Sepharose 4 Fast Flow and phosphocellulose and heparin Sepharose, respectively. Rabbit antiserum raised against topoisomerase I from calf thymus glands recognizes a 45kDa protein and a polypeptide of lower molecular mass (35 kDa) in the purified isolate.

The observation has been made that there may be a relationship between growth stage in *R. capsulatus* and topoisomerase I activity, the later being higher in 4 day old cultures than in 7 day old cultures in which the pigments have disappeared. This phenomenon is not surprising since topoisomerases are presumably not required during stationary and senescent phases.

The ultimate goal of these experiments is to clone the gene encoding topoisomerase I from *R. capsulatus* so that mutations in this activity can be obtained. In this way the relationship between photosynthetic membrane assembly, pigment biosynthesis, and DNA supercoiling will be determined.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

101. Mechanism of Water Splitting and Oxygen Evolution in Photosynthesis
M.P. Klein and V.K. Yachandra, Structural Biology Division $215,000

Photosynthetic oxygen evolution in plants and cyanobacteria is catalyzed by a cluster of four manganese atoms. The Mn cluster is the site of water splitting and acts as the locus of charge accumulation that is driven by the energy of four successive photons through a cycle of five intermediate S-states. The oxidation states of the Mn in four (of the five) of these S-states have been determined from Mn K-edge X-ray absorption spectra, for samples prepared by microsecond flashes at room temperature and characterized for S-state

composition by EPR. Oxidation of Mn accompanies the first flash from the dark stable S_1 to the S_2 state, little or no change in oxidation of Mn is induced by the second flash that induces the S_3 state and sizable reduction follows the third flash leading to the S_4-S_0 states. It is proposed that the oxidizing equivalent is stored on a ligand of Mn in the S_3 state. The structure of this cluster in the S_1 and S_2 states, as determined by EXAFS, is consistent with a pair of di-μ-oxo bridged binuclear Mn clusters with Mn - Mn distances of 2.72Å linked by a mono-μ-oxo bridge with Mn - Mn distance of 3.3 Å. Replacement of Ca with Sr establishes that Sr(Ca) is within 3.5 Å of the Mn cluster. One of the 2.72 Å vectors is lengthened to 2.87Å when native samples are treated with inhibitors of oxygen evolution, F^-, or NH_3, a water analog. From polarized EXAFS the orientation of the 2.72, 2.87 and 3.3 Å Mn-Mn vectors was determined to be 55, 67 and 43°, respectively, relative to the membrane normal.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

102. Photosynthetic Pigment Proteins and Photosynthetic Light Reactions
K. Sauer, Structural Biology Division $250,000

Excitation transfer and trapping in reaction centers of photosynthetic membranes occurs in less than one nanosecond following the absorption of visible light photons. Using fast time-resolved fluorescence measurements, we have investigated the role of the organization of phycobilisomes that transfer energy to the thylakoid membranes of photosynthetic cyanobacteria. Understanding the basic physics underlying this rapid and extensive excitation transfer has been explored using the pigment-protein C-phycocyanin (C-PC). X-ray crystallographic structural information for C-PC has enabled us to obtain excellent agreement between theoretical predictions and our time-resolved fluorescence relaxation measurements for C-PC monomers and trimers, showing that the Förster mechanism is sufficient to explain the observed behavior. We have now begun an investigation of the excitation transfer dynamics in the LH-2 bacteriochlorophyll (BChl)-protein complex from purple photosynthetic bacteria in collaboration with colleagues from the University of Glasgow, who published the structure of this pigment-protein based on X-ray crystallography in 1995. By contrast with the situation for C-PC, the dominant interactions between BChl molecules in LH2 result in excitonic interactions that are large compared with absorption bandwidths.

Photosynthetic reaction centers convert absorbed photon energy into charge separation between electron donors and acceptors. We have investigated the effects of applied trans-membrane electric fields on the kinetics and the efficiency of this charge separation process in photosynthetic bacterial systems, where detailed structural information on the reaction centers is available, to attempt to account for the observed behavior using appropriate theoretical modelling of the influence of the applied electric fields on the reaction centers. This information should be useful for the design of biomimetic synthetic devices for solar energy conversion.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

103. Center for the Analysis of Plant Signal Transduction
D. Schmitt, W. Gruissem, and S.-H. Kim, Structural Biology Division of LBL and Department of Plant Biology, University of California, Berkeley $160,000

Several mammalian and yeast proteins involved in signal transduction and cell trafficking contain a C-terminal CAAX motif that is modified by farnesyl (FPP) or geranylgeranyl (GGPP) intermediates from the sterol biosynthesis pathway. It appears that prenylation of these proteins increases their affinity to the membrane, but it may also serve as a mechanism to modulate signaling and growth as a function of the metabolic state of the cell. We are studying the role of protein prenylation in tomato during cell division, growth, and specific signaling processes. In mammals and yeast the CAAX motif is prenylated by two hetero dimer enzymes called FPTase and GGPTase type 1. We have partial purified and characterized a FPTase from tomato. In its properties the enzyme shows high homology to its mammalian and yeast counterparts. Furthermore we cloned a gene from tomato that has high homology to the FPTbeta-subunit gene from mammals and yeast and established using immuno characterization that the protein encoded by this gene is one subunit of tomato FPTase. Our study also revealed that the enzyme is differentially expressed, specifically required in stem and root tissue, in the early stages of fruit development and during fruit ripening. We have also partial purified a GGPTase type 1 from tomato. Evidence indicates that this enzyme as well is conserved between mammals, yeast and plants.

Los Alamos National Laboratory
Los Alamos, NM 87545

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

The purpose of this project is to understand the unique aspects of the metabolism and biochemistry of methylotrophic bacteria which grow on reduced one carbon compounds such as methane, methanol, or methylamine. Methylotrophs possess a unique system for oxidizing alcohols which involves the PQQ-dependent methanol dehydrogenase (MDH). The MDH oxidation of alcohols is coupled directly to the electron transport chain, and is used to drive the net synthesis of ATP. When growing on methane or methanol, methylotrophs derive all of their energy from this process. The MDH cofactor PQQ (pyrroloquinoline quinone) is one of the three o-quinone enzyme cofactors that have been identified. PQQ is unique among this group in that it must be biosynthesized as distinct molecule. Using ^{13}C labeling and NMR spectroscopy have shown that PQQ is biosynthesized from the amino acids glutamate and tyrosine. We are continuing to probe the biosynthesis of PQQ. Molecular genetic studies in other laboratories have identified PQQ biosynthesis genes. All three organisms studied

contain an open reading frame that encodes for a 23-29 amino acid peptide. We are testing the hypothesis that this peptide is the precursor for the biosynthesis PQQ. We are currently synthesizing the *Methylobacterium extorquens* AM1 peptide labeled with L-[3'-5'-$^{13}C_2$]tyrosine and will examine extracts for their ability to oxidize this peptide. During our PQQ biosynthesis studies we generated ^{13}C-labeled samples of PQQ which were used to probe the mechanism of MDH. Using NMR spectroscopy we demonstrated that upon formation of the enzyme-substrate complex the alcohol binds to the C-5 carbonyl of PQQ to form a hemi-ketal structure. In addition, when cyanide, a competitive inhibitor of MDH binds reversibly to PQQ as a cyanohydrine at the C-5 carbonyl. The ability of methylotrophs to grow on C_1 compounds gives them considerable industrial potential because they could be used to produce a variety of useful compounds from inexpensive and renewable precursors such as methanol or methane. Their ability to oxidize a wide variety of chemicals make them potentially important for treatment of waste streams. For the potential of these organisms to be realized, we must understand fundamental aspects of the their physiology.

University of Maryland
Baltimore, MD 21201

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

F.T. Robb, Center of Marine Biotechnology $94,157

Our research focuses on the molecular basis for protein thermostability and the regulation of gene expression in hyperthermophiles. We have isolated and characterized glutamate dehydrogenases (GDHs) from several hyperthermophilic Archaea. These enzymes represent the most thermostable dehydrogenases described so far, with half lives of several hours at 100°C. The *gdhA* genes from these organisms were cloned and sequenced. GDHs from *Pyrococcus furiosus* and *Thermococcus litoralis* have been overexpressed in *E. coli*. Heat treatment of the *E. coli* extracts triggered the assembly of the inactive monomers into active hexamers, resulting in fully active recombinant enzymes. Site directed mutagenesis of *Pyrococcus* and *Thermococcus* GDHs is now in progress. The mutagenesis targets were selected by comparison of thermophilic GDH sequences with the sequence and molecular structure of a mesophilic GDH, from *Clostridium symbiosum*. Several potentially significant features emerged from this modeling; (i) insertions and deletions between the mesophilic and thermophilic sequences are located at the junctions of the secondary structure elements; (ii) the most frequent amino acid exchanges involve substitutions which increase the hydrophobicity and side chain branching in the more thermostable enzyme, the most frequent substitution being valine to isoleucine; (iii) these sequence changes are consistent with an increase in the packing of the core of the thermophilic proteins. Crystallization of GDHs from *P. furiosus* and *Pyrococcus endeavori* occurs readily, and the diffraction data from *T. litoralis* GDH are at 3.2Å resolution.

We have established that in *P. furiosus*, GDH is regulated at the level of transcription by maltose and that pyruvate dikinase is induced by maltose in the growth medium. The promoters of these genes are under investigation using *in vivo* and *in vitro* mapping techniques. A subtractive library was constructed to identify other genes which are repressed by maltose. The resulting clones are currently being sequenced and will be submitted to the same transcription analysis as the *gdhA* gene.

University of Maryland
Baltimore, MD 21202

106. Mechanisms of Transcriptional Gene Regulation in the Methanogenic Archaea

K. Sowers, Center of Marine Biology $87,000

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 methanogenesis. 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 large-scale harvesting technique was developed to rapidly chill 20 liter cultures of *M. thermophila* and maintain *cdh* promoter activity. An *in vitro* transcription assay has been developed using a template that contains the promoter and 300 base pairs of the 5' end of *cdhA*, and the *lac* terminator sequence. The *in vitro* assay has been optimized and we have shown >20-fold expression of *cdh*' mRNA expression with cell-free extracts of acetate-grown cells compared with methanol grown cells. Cell-free extracts with *cdh*-specific transcription activity have been partially purified by PEG precipitation and we are presently fractionating extracts by FPLC to isolate transcription factors. Concurrently, a plasmid expression vector is being constructed that will enable us to measure *cdh* transcription activity *in vivo*. 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

107. Identifying Calcium Channels and Porters in Plant Membranes
H. Sze, Department of Plant Biology $92,000

The regulation of cellular Ca level is one of the most significant functions of plant cells. Changes in cytosolic Ca is an important intracellular signal, and Ca in endomembrane compartments is required for the proper functioning of the secretory system. Cytosolic Ca levels is regulated in large part by Ca pumps which export excess cytosolic Ca into the extracellular space and into internal stores. Multiple Ca-pumping ATPases exist in plants, however, it is unclear how many distinct Ca pumps there are and how they are regulated. Our current goal is to identify selected Ca pumps with biochemical and molecular methods as a step to determine their primary structures, function, their regulation, and subcellular location. Biochemical evidence indicate plants have at least two distinct types of Ca pumps: (i) a calmodulin-binding Ca-ATPase of 120 kD from endomembranes of carrots has been partially purified using a calmodulin-affinity chromatography; and (ii) another Ca pump identified also as a 120 kD phosphoenzyme that did not bind to calmodulin. To identify these pumps at the molecular level, genes encoding Ca-ATPase homologs have been isolated from *Arabidopsis.* Two genes shared similarity to animal SERCa pumps, and their functional activity will be tested by expression in a heterologous system. Studying the structure and regulation of plant Ca pumps is an important step towards understanding how plants grow, develop and adapt.

University of Massachusetts
Amherst, MA 01003

108. Cellulose Fermentation by Nitrogen-Fixing Anaerobic Bacteria
S. Leschine, Department of Microbiology $184,300 (FY 94 funds/2 years)

In anaerobic environments rich in decaying plant material, the decomposition of cellulose 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, particularly under conditions of combined nitrogen deprivation. A second aspect of our research involves investigations of the multicomplex cellulase-xylanase enzyme system which is produced by the mesophilic, N^2-fixing, ethanol-producing bacterium, *Clostridium papyrosolvens* C7. This enzyme system catalyzes the depolymerization of crystalline cellulose and other plant cell wall polysaccharides and consists of at least seven distinct extracellular, high-molecular-weight multiprotein complexes, each with different enzymatic and structural properties. We are exploring the possibility that a 125,000-molecular weight glycoprotein, which is found in all complexes and apparently lacks enzyme activity, functions as a scaffolding and/or cellulose-binding protein in the multicomplex

cellulase-xylanase system. In addition, we are examining this enzyme system for activities associated with the degradation of the hemicellulosic portion of biomass. The research will provide fundamental information on the physiology and ecology of cellulose-fermenting, N^2-fixing bacteria, and on the intricate processes involved in carbon and nitrogen cycling in anaerobic environments. Also, it will advance understanding of the enzymology of cellulose hydrolysis by anaerobic bacteria. Furthermore, the information obtained will be valuable for the development of practical applications, such as the bioconversion of plant biomass (e.g., agricultural, forestry, and municipal waste materials) to fuels such as ethanol.

University of Massachusetts
Amherst, MA 01003

109. Isolation of Genes Involved in a Novel Auxin Biosynthetic Pathway
J. Normanly, Department of Biochemistry and Molecular Biology
$174,653 (2 years)

Biosynthesis of the primary plant auxin, indole-3-acetic acid (IAA), has been the subject of intense study for decades, yet a definitive biosynthetic pathway remains to be proven. A long-held theory has been that tryptophan is the primary precursor to IAA, and several pathways have been postulated in which IAA is derived from tryptophan. Recent *in vivo* experiments along with the isolation of tryptophan auxotrophic mutants in both maize and *Arabidopsis* have revealed a novel IAA biosynthetic pathway that does not involve tryptophan. We refer to this pathway as the tryptophan-*independent* pathway. Towards our long term goal of identifying the enzymes and intermediates involved in this pathway, we are employing a molecular approach involving a TLC-based screen for *Arabidopsis* genes that synthesize IAA in a tryptophan-independent manner. We will examine the *in vivo* function of these genes via reverse genetics and GC-SIM-MS analysis, and initiate a study of the temporal and spatial regulation of their expression.

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

110. Molecular Basis of Symbiotic Plant-Microbe Interactions
F.J. de Bruijn $250,455

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

111. Molecular Mechanisms That Regulate the Expression of Genes in Plants
P. Green $245,455

The steady state level of an mRNA depends both on its rate of synthesis (transcription) and its rate of degradation. Rapidly accumulating data indicate that degradation rates of mRNAs in eukaryotes vary over a wide range and can be regulated by a variety of stimuli. However, in contrast to transcription, the mechanisms that control mRNA stability are largely unknown. A major objective of our work is to identify and characterize the molecular components that control the rates of mRNA degradation in plants (e.g., RNases and the mRNA sequences that they recognize) and determine how they interact. We have recently developed a system to measure mRNA decay rates in stably transformed tobacco cells grown in suspension cultures. Using this system we have shown that DST sequences, highly conserved among plant SAUR genes, target reporter transcripts for rapid decay in tobacco. Studies are now underway to elucidate the mechanisms by which DST sequences and other instability determinants mediate selective mRNA decay. To determine if results obtained with cultured cells hold true for regenerated plants, we are also exploring ways to measure rates of mRNA degradation in transgenic tobacco. In addition, we have begun to characterize the RNases of *Arabidopsis* as a first step towards differentiating between the RNases that play a role in mRNA degradation and those that have other roles in RNA metabolism.

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

112. Molecular Biology of Plant-Bacterial Interactions
S.Y. He $185,450

This project investigates the molecular basis of signalling during pathogenic interactions between plants and bacteria. We have previously characterized a cluster of bacterial genes,

known as *hrp* genes, that control the ability of plant pathogenic bacteria to initiate many important interactions with higher plants, including pathogenesis on susceptible plants and elicitation of the hypersensitive defense response (HR) on resistant plants. Most *hrp* gene products have been shown to be involved in the assembly of a novel protein secretion apparatus (the Hrp secretion apparatus) in the bacterial envelope. Understanding the functions of and plant responses to the proteins that traverse the HRP apparatus will have significant ramifications in the elucidation of molecular basis of diverse plant-bacterial interactions. We have identified five *Pseudomonas syringae* pv. tomato (a virulent pathogen of tomato and *Arabidopsis thaliana*) extracellular proteins (EXPs) whose secretion is controlled by the Hrp secretion apparatus. Several *A. thaliana* mutants that fail to respond to these Hrp-controlled EXPs have been isolated. These mutants exhibited either reduced HR to an HR-eliciting bacterium or reduced susceptibility to the pathogen, *P.s.* pv. tomato. In addition, two plant genes, hi7 and hi11, whose induction by bacteria is dependent on functional *hrp* genes, have been cloned. Our planned experiments for the remainder of the year will characterize these isolated bacterial EXPs and plant mutants and genes in order to have a better understanding of the role of Hrp-controlled EXPs in molecular signalling between plants and pathogenic bacteria.

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

113. Biogenesis of Plant-specific Cell Organelles
K. Keegstra $260,455

We are continuing studies on the biogenesis of plastids and plant cell walls. The aspect of plastid biogenesis currently under investigation is the transport of cytoplasmically synthesized precursors into chloroplasts. In particular, we are focusing on identifying and characterizing the components of the transport apparatus responsible for mediating this translocation process. Outer envelope membrane polypeptides of 34-kD, 75-kD and 86-kD have been identified as putative transport components. cDNA clones and antibodies for all three proteins are available. We are employing these tools to investigate the function of each protein. Additional cross-linking efforts are continuing with the goal of identifying other components of the transport apparatus, especially those located in the inner envelope membrane.

Our studies of cell wall biogenesis are focused on the glycosyltransferases involved in synthesizing the complex carbohydrates present in cell walls. We are currently purifying the fucosyltransferase involved in the synthesis of xyloglucan, a major hemicellulosic polysaccharide present in the primary walls of dicots. In addition, we have isolated and characterized cDNA clones encoding a protein thought to be involved as a primer or an intermediate during the synthesis of xyloglucan. Antibodies against this protein are being prepared and will be used to investigate the role of this protein during wall biosynthesis.

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

114. Action and Synthesis of Plant Hormones
H. Kende $260,455

The objective of this project is to gain knowledge on the synthesis and mode of action of the plant hormones ethylene and cytokinin. Our research on ethylene biosynthesis and action combines physiological, biochemical and molecular approaches. We are interested in two problems that receive relatively little attention, namely the mechanisms of positive and negative feedback regulation of ethylene biosynthesis and the role of ethylene in vegetative growth. We are investigating the effect of ethylene on the expression of multiple genes encoding both enzymes of the ethylene biosynthetic pathway, 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase and are localizing the expression of these genes. Our work on growth of deepwater rice deals with the control cell wall extension and the regulation of cell cycle genes in the intercalary meristem. In addition, we are identifying genes in the intercalary meristem whose expression is regulated by gibberellin, the plant hormone that is ultimately responsible for rapid growth of rice stems. This is being done using differential display of mRNA. We have resumed work on a hormonal response that we have studied twenty years ago, namely the control of nitrate reductase (NR) activity by cytokinins in *Agrostemma githago*. Progress in research on cytokinins has been slow relative to that with other plant hormones. After having described the system at the physiological and biochemical levels, we have cloned cDNA probes for three NR genes of *Agrostemma* and are investigating whether nitrate and cytokinins regulate the same or different NR genes. We are also localizing the expression of NR genes in embryos of *Agrostemma* by *in situ* hybridization.

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

115. Interaction of Nuclear and Organelle Genomes
L. McIntosh $255,455

Our approach to understanding the regulation of energy balance - and thus growth and yield - in plants to use molecular genetics to create specifically engineered organisms, organisms altered in either photosynthesis or respiration. There are many genetic and functional differences between plant and animal mitochondria and one of most striking is the presence of a second terminal oxidase in higher plants. Plants contain an "alternative" oxidase which was first characterized by its resistance to cyanide. The electrons flowing through this alternative pathway are not linked to the production of a transmembrane potential and thus are lost, or "wasted", for the production of ATP. This alternative oxidase is induced by many environmental stresses and thus it is important to understand how plant respiration is

controlled and, in particular, how the distribution of electron flow between the two terminal oxidases is affected by the environment and is reflected in altered carbon utilization, or yield.

The cyanobacterium *Synechocystis* sp. PCC 6803 is used to study the reaction centers of Photosystem (PS)I and PSII. Site-specific mutagenesis is used to identify specific amino acid residues responsible for the "spitting" of water and generation of oxygen by PSII. The reaction center of PSI is also being investigated through alteration of specific amino acid residues. Recently, the amino acids ligands for the core [4Fe-4S] center F_x along with both the Fe-S centers FA and FB have been confirmed using this approach.

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

116. Sensory Transduction in Plants
K.L. Poff $220,455

The primary objective of this project is to understand the mechanisms for the acquisition of environmental information via light reception. In particular, we are studying phototropism in flowering plants. This response is under the control of the blue light photoreceptor pigment system(s) which control(s) numerous light responses. For these studies, we have developed a genetic system with which we can dissect the initial steps in the transduction sequences. Screening procedures have been devised and used to identify mutants of *Arabidopsis* with altered phototropism. Based on analysis of such mutants, it has become evident that a major component of phototropism is phototropic adaptation. Under conditions of long term irradiation, plants have the capacity to adapt to the irradiation, such that their responses at the beginning and end of a long irradiation are not equal.

Therefore, an understanding of this process of adaptation is necessary to understand the plant's response to the long term irradiations that are important for the plant under field conditions. Our approach to the study of the pathways for phototropism and phototropic adaptation includes genetic, physiological and biophysical characterization of mutants and ecotypes that differ in their phototropic response. This genetic/biophysical approach should permit positive identification of the receptors, access into the transduction sequence, and eventual understanding at the molecular level of the events from reception to the bending responses.

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

117. Molecular Mechanisms of Trafficking in the Plant Cell
N.V. Raikhel $250,455

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. We are now concentrating our efforts on the identification and isolation of receptors which recognize these sorting sequences and subsequently mediate protein transport to the vacuole and nucleus.

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

118. Biochemical and Molecular Aspects of Plant Pathogenesis
J.D. Walton $245,455

The objective of this project is to understand the biochemical events that are important to the interaction between fungal plant pathogens and their host plants. We are studying examples of factors that control specificity as well as factors that are necessary for basic compatibility. As our model organism we are using *Cochliobolus (Helminthosporium) carbonum,* which causes leaf spot disease of maize. *C. carbonum* can be transformed with exogenous DNA and particular genes disrupted. It also has a sexual stage with two mating types. Race 1 of *C. carbonum* is highly virulent on maize that is homozygous recessive at the nuclear *Hm* locus due to its ability to produce HC-toxin, a host-selective toxin. We have cloned the *Tox2* gene cluster that controls HC-toxin production and are studying its structure and relation to the enzymology of HC-toxin biosynthesis. In order to study the specificity of HC-toxin against maize we have prepared radiolabelled HC-toxin. Maize leaves can metabolize HC-toxin to the inactive 8-alcohol. Susceptible plants appear to be lacking the enzyme, and NADPH-dependent reductase, that catalyzes this reaction. We are studying, as examples of basic compatibility factors, cell wall-degrading enzymes made by *C. carbonum.* These enzymes have been proposed, but never shown, to have an important role in penetration, nutrient assimilation, and tissue invasion, as well as in triggering host defenses. We have cloned the single endopolygalacturonase genes and used it to make a mutant of *C. carbonum* with a disrupted polygalacturonase gene. The mutant was as pathogenic as the wild-type

fungus. We have also isolated xylanase and laminarinase and are cloning the corresponding genes.

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

119. Developmental Biology of Nitrogen-Fixing Cyanobacteria
C.P. Wolk $240,455

Anabaena and related filamentous cyanobacteria utilize solar energy to fix nitrogen gas within spaced cells called heterocysts. We are characterizing the genes, and their roles, that allow *Anabaena* to fix nitrogen in the presence of oxygen, the Fox^+ phenotype. Genes *hetC*, required for heterocyst formation; *devA*, required pleiotropically for heterocyst maturation and *hepA*, required for synthesis or stabilization of the heterocyst envelope polysaccharide are all activated extensively between 4 and 8 h after nitrogen stepdown, and all encode ATP-binding cassette transporters. Close to, and 3' from, each of these genes is an extensively conserved, ca. 31-bp sequence that we denote a Fox Box, and that may have regulatory significance for the differentiation process. Gel retardation experiments indicate that a protein binds specifically to this sequence. The protein HU of *Escherichia coli* can help to bend, or stabilize loops in, DNA; we have found that an Anabaena gene that encodes a protein very similar to HU is required for heterocyst formation. These observations may be related; perhaps the HU of *Anabaena* helps to maintain contact between the Fox Box-associated protein and 5' regulatory regions of genes that are required for progression of heterocyst differentiation. 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

120. Environmental Control of Plant Development and Its Relation to Plant Hormones
J.A.D. Zeevaart $235,455

Plant growth and development are affected by environmental factors such as daylength, temperature, and moisture. Hormones often function as chemical messengers between the perception of an environmental factor and the morphological expression. The objectives are to identify the hormones involved, how their synthesis and catabolism are regulated by the environment, and how they act. 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? As a first step towards answering that question, GA 20-oxidase genes have been cloned from

Arabidopsis and spinach, and functionally expressed as fusion proteins in *E. coli*. GA 20-oxidase is a multifunctional enzyme that oxidizes and eliminates C-20 of the GA skeleton (GA53 GA44 GA19 GA20). Effects of the photoperiod on GA 20-oxidase expression are now under investigation.

Abscisic acid (ABA) is a plant hormone whose synthesis is greatly stimulated when plants experience a water deficit. Conversely, ABA is rapidly inactivated to phaseic acid (PA) when plants are rehydrated. The objective of our studies is to find out how the metabolism of ABA is regulated by the water status of the tissue. Two new mutants from *Arabidopsis* impaired in two separate steps late in the ABA biosynthetic pathway have been obtained. These mutants are now being characterized biochemically.

Michigan State University
East Lansing, MI 48824-1101

121. Xylan-Degrading Enzymes of Cytophaga xylanolytica
J.A. Breznak, Department of Microbiology $95,500

Xylan degradation by the anaerobic gliding bacterium, *Cytophaga xylanolytica*, is initiated by a repertoire of hydrolytic enzymes (the "xylanase system") that is: almost entirely cell-associated; stable to long term storage; and devoid of activity against cellulose or carboxymethylcellulose. We are studying the physicochemical and catalytic properties of individual components of the xylanase system, as well as the genes encoding them. The α-L-arabinofuranosidase (ARAF) component, purified 150-fold from Triton X-100 extracts of whole cells, exhibited an Mr = 210 kDa with subunits of 56 kDa and a pI of 6.1. The enzyme displayed a Km = 0.504 mM and a Vmax = 319 μmol per min per mg protein, when assayed with p-nitrophenylarabinoside as substrate at its optimum pH (5.8) and temperature (45-50° C). ARAF also hydrolyzed methylumbelliferyl arabinoside and liberated arabinose from natural substrates (rye, wheat, birchwood, corn, and oat arabinoxylans, and sugar beet arabinan). Work is in progress to deduce the amino acid sequence of ARAF from the nucleotide sequence of a 4 kb fragment of *C. xylanolytica* DNA that was cloned into *E. coli* on pUC 18, and which also encodes an endoxylanase and a β-xylosidase. Future efforts will include a characterization of endoxylanase, β-xylosidase, and the recently discovered acetyl esterase components and an examination of the extent of synergy that occurs between them during arabinoxylan hydrolysis.

Michigan State University
East Lansing, MI 48824-1319

122. The Role of Bacterial Surface Glycoconjugates in the Rhizobium/Legume Symbiosis

R.I. Hollingsworth, Department of Biochemistry $98,000

In our laboratory, we are interested in determining the role(s) that the cell surface and membranes of bacteria play in determining the outcome of the interaction between *Rhizobium* and legumes that ultimately leads to the symbiotic fixation of nitrogen. One of the central ideas between our research effort is that there must be some compatibility or overlap between the plant cell surface chemistry and that of the bacterium. This compatibility or overlap contributes to host range. It is clear that this must be true because defects in the structures of bacterial molecules that are known to be cell surface associated or membrane associated (such as capsular and lipopolysaccharides and nod factors) lead to the loss of certain facets of the symbiotic program. The primary direction our research takes is to determine the chemical structures of all the potential membrane lipid, glycolipid and carbohydrate components in the bacteria. We then determine whether there is any species specificity associated with any of these structures. Another task is to determine whether there is any overlap between any of these structures either spatially, functionally or biosynthetically. This is done by careful membrane separations followed by isolation and characterization of lipid and glycolipid components, the use of mutants with known impairments in their ability to fix nitrogen symbiotically and by immunochemical methods. We are especially interested in cell surface or membrane components that might overlap biosynthetically with bacterial or plant lipid or carbohydrate structures. Thus far we have demonstrated that there is considerable overlap between the lipid and glycolipid chemistry of *Rhizobium* and plants.

Michigan State University
East Lansing, MI 48824-1312

123. Control of Triacylglycerol Biosynthesis in Plants

J. Ohlrogge, Department of Botany and Plant Pathology $92,000

Triacylglycerol (TAG) is the major form of carbon storage in seeds of many important crops and the oils extracted from these plant seeds represent a $20 billion dollar commodity with a wide variety of uses in the food and chemical industries. The overall objective of this research project is to understand how triacylglycerol biosynthesis is controlled in plants. Whereas leaves, roots and other tissues usually contain less than a few percent of their total lipid in the form of TAG, seed lipids frequently contain over 95% TAG. A metabolic or biochemical explanation for this dramatic difference in lipid composition has not yet been established. In order to begin to provide such an explanation we will examine the relative importance of possible alternative control mechanisms by examining the *in vivo* pool sizes of

key intermediates in the pathway for TAG biosynthesis. In particular, methods will be developed to measure the plastid pools of acetyl-CoA and malonyl-CoA. In addition, the size and fatty acid composition of the acyl-CoA and diacylglycerol pools in plants which produce different fatty acids will be determined. Leaf and seed tissue will be compared to establish correlations between the different lipid metabolism of these tissues and the pools of the key pathway intermediates. The role of diacylglycerol acyltransferase will be evaluated by examining its expression and kinetic properties in seeds and leaves and the specificity of the CDP:choline phosphotransferase will be examined to determine its ability to provide specific partitioning of unusual fatty acids into triacylglycerol and their exclusion from phospholipids.

Michigan State University
East Lansing, MI 48824

124. A National Cooperative for Genetic Engineering of Plant Lipids
J. Ohlrogge, Department of Botany and Plant Pathology $58,000

In order to explore the wide range of potential applications of genetic engineering techniques to the production of useful new plant lipids, it will first be necessary to develop a detailed mechanistic understanding of most aspects of plant lipid metabolism and to acquire genes for the key enzymes and structural proteins. Progress toward these general goals will be greatly facilitated by the creation of a National Plant Lipid Cooperative (NPLC). The NPLC will provide an efficient mechanism for facilitating exchange of ideas, information and research materials among all members of the North American community. Exchange of ideas will occur through the development of an electronic newsgroup and sponsorship of participation of young scientists in regular meetings and workshops devoted to plant lipid metabolism. A major objective, in this regard, will be to encourage young scientists to initiate research programs on ignored topics of strategic importance to the whole field by allocating resources to the development of these areas. Exchange of information will be stimulated by the development and distribution of several databases containing all published information about plant lipid metabolism and the chemical composition of lipids from different plant species. Finally, the NPLC will commission the production and distribution of essential research materials which are not commercially available but which are required in order to pursue new avenues of research.

Michigan State University
East Lansing, MI 48824-1319

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

J. Preiss, Department of Biochemistry $84,000

Structure-function relationships of the ADPglucose pyrophosphorylase from higher plants (potato tuber and spinach leaf) will be studied. This research entails the use of amino acid residue chemical modifying reagents (e.g., pyridoxal-5-P, phenylglyoxal, 8-azido-ATP and 8-azido-ADPglucose) in experiments designed to determine the nature of involvement and the location of the various amino acids at the catalytic and regulatory (allosteric) sites. The cDNA clones of the large and small subunits of the potato ADPglucose pyrophosphorylase have been expressed in *E. coli*. The expression of these genes and prior chemical modification studies on the purified spinach leaf ADPglucose pyrophosphorylase enables us to do site-directed mutagenesis at various regions of the cyanobacterial and plant enzymes to gain more insight on the nature of the catalytic and effector sites and the 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) requires two different subunits for high activity in contrast to the bacterial homotetramer enzyme. Attempts will be made to construct "mutant", active plant enzymes that may either be less sensitive to allosteric inhibition by phosphate or that may not require the allosteric activator, 3-phosphoglycerate, for activity. These could 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 Ligninolytic Enzyme Systems in Selected Wood-rotting Fungi

C.A. Reddy, Department of Microbiology $85,999

Lignin is the second most abundant renewable organic polymer in the biosphere. Lignin biodegradation research has important applications in biopulping, decolorization and detoxification of pulp and paper mill effluents, and bioremediation of toxic recalcitrant environmental pollutants. Laccases, lignin peroxidases (LIPs), and manganese-dependent peroxidases (MNPs) are three classes of lignin-modifying enzymes that are believed to be important in lignin-degradation by white-rot fungi. Some white-rot fungi contain all three classes of the lignin-modifying enzymes while others contain only one or two classes of these enzymes. Further studies indicated that laccases are much more widely distributed among

white-rot fungi as compared to the other two. Moreover, laccases have potential applications in the brewing industry, preparation of biosensors, and organic syntheses. Hence, we have initiated investigations on the laccases of selected white-rot fungi. Very recently we showed the presence of laccase gene-specific sequences in several genera of white-rot fungi. Our initial studies also showed for the first time the presence of laccase activity and laccase gene-specific sequences in the brown-rot fungi *Gloeophyllum trabeum* and *Poria placenta*. In-depth studies on the laccases of selected wood-rotting fungi, including the temporal production, isozyme profiles, molecular weights, and gene sequencing will be the focus of further study. We will also continue studies on characterizing lip and mnp gene expression in wood-grown cultures of *Phanerochaete chrysosporium*, the most widely studied white-rot fungus. Specifically, we will be studying the inter-play of carbon and nitrogen levels on regulation of lip and mnp gene expression in cultures grown on poplar wood.

Michigan State University
East Lansing, MI 48824-1319

127. One Carbon Metabolism in Anaerobic Bacteria: Regulation of Carbon and Electron Flow During Succinic Acid Fermentation

J.G. Zeikus, Department of Biochemistry $108,999

The overall objective of this project is to understand the fundamental biochemical mechanisms that control and regulate carbon and electron flow in anaerobic chemosynthetic bacteria that couple CO_2 fixation to the production of succinic acid. Enzymes, genes, and electron carriers in *Escherichia coli* and *Anaerobiospirillum succiniciproducens* are used for metabolic engineering a homosuccinate fermentation. A key enzyme in catabolic succinate production, the *A. succiniciproducens* phosphoenolpyruvate (PEP) carboxykinase gene (*pckA*) has been cloned, sequenced, and expressed in *E. coli*. In the past few years, several bacterial PEP carboxykinase genes have been cloned, but little is known about the structure of these enzymes and their function in CO_2 fixation. The purified recombinant enzyme will be used for X-ray crystallographic studies, and its biochemical and kinetic characterization will help understand the role of this enzyme in *A. succiniciproducens* CO_2 fixation and succinate production. *E. coli* will be metabolically engineered to direct the fermentation processes toward succinate production. Succinate production will be monitored in *E. coli* expressing *A. succiniciproducens* PEP carboxykinase, in the presence of plasmid-born fumarate reductase and/or malate dehydrogenase. We have already demonstrated an increase in succinate production by *E. coli* expressing *A. succiniciproducens* PEP carboxykinase. The aim of the research is to control and engineer a fermentation balance of 1 glucose+$2CO_2$ + 2"H"$_2$, converted into 2 succinic acid and $2H_2O$. Succinic acid is a potential biochemical replacement for petrochemical derived maleic anhydride, an energy intensive commodity chemical.

University of Michigan
Ann Arbor, MI 48109-1048

128. Molecular Genetics of Myosin Motors in Arabidopsis
J. Schiefelbein, Department of Biology $79,540 (FY 94 funds/2 years)

The normal development and metabolism of plant cells depends on the appropriate organization and movement of the cell contents. At present, little is known about the molecules involved in organizing or transporting the components of the plant cell, such as the nucleus, endoplasmic reticulum, or secretory vesicles. Based largely on studies of animal and fungal cells, one of the molecules thought to be involved in intracellular trafficking in plants is the actin-based motor protein myosin. To examine the role of myosin in plants, we are isolating and analyzing the genes that encode myosin proteins in the flowering plant *Arabidopsis thaliana*. The polymerase chain reaction technique was used to clone five different myosin-like gene fragments, and using these as hybridization probes, we identified at least nine different myosin genes in *Arabidopsis*. Sequence comparisons of the motor domain of the predicted proteins to non-plant myosins shows that the *Arabidopsis* myosins are present in two major classes, which are distinct from the other classes of eukaryotic myosins. Our current efforts are centered on defining the function of specific myosin isoforms in plant cells. Two major lines of investigation are being pursued: (1) Analysis of the expression of myosin isoforms. We are characterizing the tissue-specific expression of particular myosin genes by in situ RNA hybridization studies and promoter-GUS reporter gene fusion assays. (2) Alteration of myosin gene expression in transgenic plants. We are currently examining transgenic plants which possess constructs designed to inhibit the expression of specific myosin isoforms or to overexpress particular myosin isoforms. These studies should lead to a greater understanding of the role of molecular motors in plant growth and development.

University of Minnesota
Minneapolis, MN 55455

129. Genetics of Bacteria that Utilize One-Carbon Compounds
R.S. Hanson, Department of Microbiology $86,011

Methylotrophic bacteria are bacteria that grow on one carbon compounds including methane, methanol, methylamines and some halomethanes. Some methylotrophs synthesize up to 20% of their soluble protein as the periplasmic quinoprotein methanol dehydrogenase (MDH). At least 20 genes are known to be required for the expression of MDH in the facultative methylotroph, *Methylobacterium organophilum* XX. Eight of these genes are required for transcription of the MDH structural genes, *m x a*F and *m x a*l. Two genes, *mxc* Q and E encode members of a two-component, positively acting, regulatory system uniquely required for the induction of MDH synthesis. The binding site for the *mxc* E gene product has been

identified upstream of the *m x aF* gene. The proteins of this two component regulatory system are being purified and their modifications in response to inducers of MDH synthesis will be studied. DNA binding proteins encoded by other regulatory genes will be identified and the role of other regulatory genes in transcription m x aF will be examined.

Synthesis of a soluble methane monooxygenase (sMMO) occurs in response to copper deprivation in some methane-utilizing bacteria. Mutants unable to transport copper properly synthesize this enzyme constitutively. Copper binding compounds that may play a role in copper uptake and DNA binding proteins that respond to the presence or absence of copper will be identified and their role in the expression of the sMMO structural genes will be examined.

University of Minnesota
Minneapolis, MN 55455-0312

130. The Mechanism of Switching from an Acidogenic to a Butanol-Acetone Fermentation by Clostridium acetobutylicum

P. Rogers, Department of Microbiology $188,180 (FY 94 funds/2 years)

The overall objective of this project is to elucidate the detailed mechanism by which solvent-forming bacteria such as *Clostridium acetobutylicum* regulate the well know shift in fermentation pathway between alcohol-acetone and organic acid production. We eventually want to isolate and describe: (1) the regulatory genes and protein elements that determine induction of synthesis of the solvent-pathway enzymes; and (2) how this regulation system interacts with the sporulation and exopolysaccharide formation in clostridia. Thus, we are searching for regulatory elements that may involve environmental sensors and also DNA binding proteins that act as enhancers or positive control proteins.

We have produced 40 pleiotrophic Tn 916 transposon-induced mutants of *C. acetobutylicum* that fail to switch to solvent formation, do not form granulose bodies, and do not sporulate. We are studying complementation of these mutants with a library of 1 to 3.5 Kb DNA fragments from wild-type *C. acetobutylicum* carried on an *E. coli/C. acetobutylicum* shuttle vector, pKR13, produced in our laboratory. With electroporative transformation followed by screening for granulose-positive revertants, we have isolated a number of *C. acetobutylicum* clones. Three of these clones, transferred back into *E. coli*, reverted more than one Tn916-mutant of *C. acetobutylicum* to granulose-positive (Gra^+). Sequencing of clones is in progress in order to identify the regulatory genes. New clones as well as the Gra^+-clones will be screened for reversion of Tn916 mutants to form solvents and/or sporulate. We will attempt to identify the function and protein products of cloned regulatory genes of *C. acetobutylicum*.

University of Minnesota
St. Paul, MN 55108-1095

131. Isolation and Characterization of Ammonia Monoxygenase of Nitrosomonas
A.B. Hooper, Department of Genetics and Cell Biology $112,000

Oxidation of NH_3 provides energy for growth in this autotrophic bacterium. Ammonia monoxygenase (AMO) catalyzes $NH_3 + 2e^- + 2H^+ + O_2 \rightarrow NH_2OH + H^2O$. Alternate substrates include CO, methane and halogenated aliphatic and aromatic compounds. Electrons are passed to AMO by unknown carriers from hydroxylamine oxidoreductase and cytochrome *c*554. Acetylenic substrates derivatize a protein subunit of AMO allowing us to isolate two putative subunits of the enzyme (AMO-A and -B) and show that they contain iron and copper. We haved cloned and sequenced genes for the two proteins and generated a model of membrane orientation and possible metal binding sites.

Work in progress designed to isolate and analyze the structure and mode of action of AMO includes improvement of the assay and stabilization of the enzyme system. The latter may include proteins in the electron donor pathway, AMO-reductase and AMO-hydroxylase components. If Ubiquinone is in the electron donor pathway we predict that it is reduced by a novel proton translocating cytochrome c554 oxidoreductase. The structure of the active site and electron-transfer redox centers will be analyzed by optical, EPR, Mossbauer and other types of spectroscopy. We will test the model of membrane topology by protein chemistry. We will determine the sequence of binding of dioxygen, substrate and addition of electrons; identify the form of activated oxygen generated by the enzyme during catalysis; and test a hypothesized radical rebound mechanism with the use of specifically deuterated ethylbenzenes.

University of Missouri
Columbia, MO 65211

132. Cellulose Synthesis and Morphogenesis
T.I. Baskin, Division of Biological Sciences $160,050 (FY 94 funds/2 years)

The goal of this research is to understand plant morphogenesis. The project will quantify the relationship between morphogenesis and alignment of cellulose microfibrils. Experiments will use roots of *Zea mays*, because we have a physiological treatment, water stress, that profoundly alters root morphology, and will use single cells growing in culture. We have measured the spatial distribution of expansion, in length and radius, for well watered and water-stressed roots, and found differences between the root stele and cortex. Measured roots have been fixed and sectioned; and alignment of cellulose microfibrils quantified with electron microscopy of metal/carbon replicas of the inner-most wall layer. Similar experiments will be done with tissue culture cells, allowing superior preservation with cryofixation.

Additionally, polarized light microscopy will be used as a complementary method to quantify cellulose alignment. Comparison of microfibril alignments among cells of different shapes will reveal the relationship between cell shape and cellulose alignment. Comparison of data from polarized light and electron microscopy will show the relative importance of microfibrils in the inner-most layer. Finally, the degree of organization of cellulose in different tissues and positions of the roots will be related to their morphology. The work will deepen understanding of how microfibrils shape the cells and tissues of higher plants. This research will uncover basic mechanisms of plant morphogenesis and can thus be expected to have a positive impact on applied efforts to optimize plant growth for human benefit.

University of Missouri
Columbia, MO 65211

133. Dosage Analysis of Gene Expression in Maize
J. Birchler, Division of Biological Sciences $202,000 (2 years)

The goal of this project is to understand further the basis of dosage sensitive regulatory effects on gene expression in maize. These effects act directly or inversely on the quantitative level of gene products when the dosage of specific chromosomal segments is varied. 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

134. Position Effect as a Determinant of Variegated Pigmentation in Maize
K.C. Cone, Division of Biological Sciences $64,000

The aim of this project is to understand the mechanisms that control epigenetic variation in gene expression. We are studying *Pl-Bh,* a gene that controls the synthesis of purple anthocyanin pigments in the maize plant. Plants carrying *Pl-Bh* exhibit an unusual pattern of pigmentation characterized by variegation, rather than the usual uniform pattern of pigmentation. In addition, *Pl-Bh* exhibits altered tissue-specificity, relative to other Pl alleles. The nucleotide sequence of *Pl-Bh* DNA is virtually identical to that of wild-type *Pl* DNA; however, *Pl-Bh* DNA is more heavily methylated. Our hypothesis is that the variegated pattern of *Pl-Bh* expression and its altered tissue-specificity are due to an epigenetic position effect. To address this idea, we taking both molecular and genetic approaches. On the molecular front, we are trying to document differences in chromosomal organization/architecture between *Pl-Bh* and the normal *Pl* gene. From the genetic angle, we are looking for *cis-* and trans-acting factors that modify *Pl-Bh* expression. So far, we have identified genes that act in trans to suppress *Pl-Bh* expression in the kernel. One of these genes has been mapped genetically, and experiments are underway to determine whether this gene has an effect on DNA methylation levels of *Pl-Bh.*

University of Missouri
Columbia, MO 65211

135. Molecular Analyses of Nuclear-Cytoplasmic Interactions in the Genus Zea
K.J. Newton, Division of Biological Sciences $142,500 (18 months)

We are exploiting evolutionary divergence to identify cooperating nuclear and mitochondrial elements involved in mitochondrial gene expression. Cytoplasmic genomes from three teosintes have been introgressed into different maize nuclear backgrounds. The major objective of the current project is to use the maize nuclear/teosinte cytoplasmic combinations to identify regulators of mitochondrial transcription. We have discovered an unusual mitochondrial promoter of a mitochondrial cytochrome oxidase gene (*Zp-cox2*) which requires a specific dominant allele of a nuclear gene (*Mct*) to be active. We are testing whether the same nuclear factor is responsible for the quantitative differences we have observed in

transcripts for the two other mitochondrial cytochrome oxidase genes and whether the quantitative differences reflect differences in transcription initiation. The results from this study should indicate if there is a multiplicity of transcription factors required for plant mitochondrial gene activity. We will also investigate whether we can use this system to detect additional transcription factors -- influencing genes that code for other mitochondrial components. We are especially interested in identifying any factors that might be involved in the coordinate regulation of mitochondrial gene expression. Other goals include investigating the possible developmental regulation of the unusual *Zp-cox2* promoter by *Mct*, as well as identifying the source of the *Zp-cox2* promoter.

University of Missouri
Columbia, MO 65211

136. Targeting and Processing of the Thiol Protease Aleurain
J.C. Rogers, Biochemistry Department $154,412 (18 months)

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, if not all, cells in barley; its functional importance to the plant is unknown. Plant thiol proteases in general are important in processes such as degradation of seed storage proteins during germination, and degradation of proteins in senescing leaves to mobilize amino acids for use elsewhere. An understanding of mechanisms regulating their targeting to sites where they function may provide new approaches towards more effective mobilization of protein reserves. An understanding of mechanisms that control the activation of their proenzymes may enhance our ability to protect or mobilize those reserves at specific times during plant development. The efficiency with which numerous hydrolytic enzymes are directed to the vacuole will determine not only their activity in that compartment, but also whether they are secreted and thereby potentially able to affect the structure of the cell wall. We have identified and cloned a potential receptor protein that binds proaleurain and directs it into a pathway to the 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 destination of proaleurain, the aleurain-containing vacuole, 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 Missouri
Columbia, MO 65211

137. Genetics of the Sulfate-Reducing Bacteria
J.D. Wall and B.J. Rapp-Giles, Biochemistry Department $89,000

The accessibility of the sulfate-reducing bacteria to genetic manipulation has been expanded as a result of our recent efforts. Transposon mutagenesis has been demonstrated. A random mutagenesis transposon has been created from Tn*7* and improvements in the efficiency of this mutagenic vector are an immediate goal. A promoter probe library has been constructed. DNA fragments from *Desulfovibrio* containing sequences promoting transcription in *E. coli* also act as promoters with the same relative strength in *Dv. desulfuricans*. These tools are now being applied to obtain a better understanding of the energy metabolism of *Desulfovibrio*. Genes for cytochrome c3 and rubredoxin have been isolated and will be targeted for interruption to determine the physiological roles.

Mount Sinai School of Medicine
New York, NY 10029

138. The Respiratory Chain of Alkaliphilic Bacteria
T.A. Krulwich, Department of Biochemistry $110,000

The special energetic demands of life at high pH are correlated with the presence of high concentrations of respiratory chain components in membranes of aerobic alkaliphiles. This laboratory is engaged in the dissection of the structure, function, and physiological roles of these components. The work for the current year will focus on: (a) production of mutants that carry a deletion in the caa_3-type terminal oxidase that has been implicated as having a special role in alkaliphily in *Bacillus firmus* OF4. Are there compensatory changes in expression of an alternate oxidase that allows growth at pH 7.5 and, if so, is there also growth at pH 10.5? (b) the putative gene locus encoding a variety of stress-related proteins, including alkaliphile hemoglobin-like proteins, will be further characterized. The sequence of the locus will be completed, the specific hemoglobin-like protein that complements oxidase-deficient mutants of *Escherichia coli* will be determined, and a deletion strain of the alkaliphile will be constructed to assess the function of the hemoglobin-like proteins in *B. firmus* OF4; and (c) studies of the genes encoding a *d*-type cytochrome and of a distinct locus that apparently can lead to induction of a new *d*-type cytochrome in *E. coli* will be directed towards their further characterization.

National Renewable Energy Laboratory
Golden, CO 80401

139. The Water-Splitting Apparatus of Photosynthesis

M. Seibert, Photoconversion Branch $135,000

The focus of our work is on understanding structural and functional aspects of the reaction center (RC)/water-oxidizing system of photosystem II (PSII). We have used Scanning Tunneling Microscopy to image the lumenal surface of the PSII membrane at <10 Å resolution and have visualized dimeric protrusions rising 50 Å above the plane of the membrane surface. These protrusions are associated with the intrinsic proteins and Mn required for PSII function. We have also used steady-state approaches to examine noncompetitive Mn inhibition of diphenylcarbazide (DPC) photooxidation by Mn-depleted PSII preparations. Past work has identified four ligands, two carboxyls and two histidyls, including His 337 on the RC D1 protein, that bind Mn with high affinity in the presence of DPC. Recent site-directed mutant studies from another laboratory are now consistent with the His 337 result. Coordinated steady-state and single-turnover flash studies have identified two types of ligands that bind Mn with high affinity in the presence of DPC--at least one that is specific for Mn and others that bind divalent cations in addition to Mn. The Mn-specific ligand, a carboxyl residue, binds a Mn that is photooxidized once by Y_Z^+ when DPC is present and that donates electrons to PSII under steady-state conditions in the absence of DPC. Thus, the Mn-specific ligand probably binds the initial Mn bound during the photoactivation process. For the first time we can now relate high affinity, Mn-binding ligands identified by Mn/DPC interactions to ligands important for binding Mn associated with the water-splitting process.

University of Nebraska
Lincoln, NE 68588-0118

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

T.J. Morris, School of Biological Sciences and A.O. Jackson, University of California, Berkeley $246,376 (FY 94 funds/2 years)

Our research emphasizes analysis of tomato bushy stunt virus (TBSV) determinants that are important in replication, pathogenicity and recombination. TBSV is a small RNA virus encoding five genes. It has a broad dicot host range and causes serious diseases of plants. Different TBSV strains vary in virulence by generating linear deletion mutants called defective interfering RNAs (DI RNAs) which compete with parental virus and reduce the severity of disease symptoms. We have sequenced TBSV and a number of distinct DIs and have analyzed biologically active transcripts generated *in vitro* from clones of both the parental virus and several of the DIs. We have used protoplasts and several host plants to evaluate the biological properties of the viruses, chimeric viral genomes and DI RNAs derived from the viruses. The availability of these cloned derivatives and results obtained in previous grant periods will permit implementation of the

following objectives for the renewal period: 1) To complete characterization of structures important in replication of the DI RNAs by isolating important *cis* acting elements; 2) To continue to investigate *de novo* generation and evolution of DI RNAs by refining our existing RNA recombination based model; 3) To evaluate genes and elements important in viral RNA replication and movement through mutagenesis; and 4) To apply yeast as a genetic tool for analysis of TBSV replication. Progress in the past year has included the development of this system to study RNA recombination *in vitro*, and the testing of transgenic plants expressing DIs for disease resistance.

University of Nebraska
Lincoln, NE 68583

141. Enzymology of Acetoclastic Methanogenesis
S.W. Ragsdale, Department of Biochemistry $110,000

Acetic acid is the major source of methane in nature. We have initiated mechanistic studies of key enzymes involved in fermenting acetic acid to methane. Spectroscopic, electrochemical, and kinetic methods will be used to identify kinetically competent intermediates in the pathway of methanogenesis. Intermediates in the cleavage of the C-C and C-S bonds of acetyl-CoA by CO dehydrogenase and a corrinoid/iron-sulfur protein will be detected and characterized. After acetyl-CoA is disassembled to its component methyl, carbonyl, and CoA adducts, the bound carbonyl group is oxidized to CO_2. The mechanism of CO oxidation and associated proton transfer will be defined. CO oxidation is also catalyzed by the CODH from photosynthetic organisms. This CODH will be studied as a model for the CO oxidation site of the methanogenic and acetogenic enzymes. The final step of methanogenesis is the reduction of methyl-coenzyme M to methane by methyl-CoM reductase. A current major mechanistic bottleneck is the relative inactivity of the reductase. Future plans include defining why the reductase undergoes inactivation and developing strategies to obtain an active and stable enzyme. Intermediates and the rate limiting steps in the reaction will be characterized. The proposed studies are hoped to contribute significantly to our understanding of how nature makes methane.

New York University
New York, NY 10003

142. Asparagine Synthetase Gene Regulation and Plant Nitrogen Metabolism
G. Coruzzi, Department of Biology $105,000

This project explores the regulation of asparagine synthesis and catabolism in plants, using a molecular-genetic approach. Asparagine is a major nitrogen storage compound transported to sinks where it is catabolized to provide nitrogen for biosynthetic reactions. Our studies on the regulation of genes responsible for asparagine synthesis and degradation may therefore

have significance with regard to improving nitrogen use efficiency in plants. We have shown that light or sucrose each negatively regulates the expression of a gene for glutamine-dependent asparagine synthetase (ASN1) in *Arabidopsis*. The sucrose repression of ASN1 is relieved by the addition of organic nitrogen. We propose to test our "metabolite-sensing" model which predicts that ASN1 is transcriptionally induced when the ratio of C:N metabolites is low. This mechanism would divert the flux of nitrogen from anabolically "reactive" glutamine into inert, stored asparagine under conditions of organic nitrogen excess. We will also study two other genes for AS (ASN2 and ASN3) which show distinct patterns of light regulation. By contrast to asparagine synthesis, enzymological studies have shown the asparagine catabolic enzyme asparaginase (ANSase) is induced by light. We propose to isolate the ANSase gene(s) and determine whether light, metabolic-control, and/or cell-specific regulation of ANSase gene are mechanisms that plants use to temporally and spatially separate asparagine synthesis and catabolism. We have begun to screen for *Arabidopsis* mutants altered in asparagine synthesis or asparagine catabolism using substrate and end product analogs in positive-selections. The phenotypic analysis of these mutants will define how asparagine synthesis and catabolism regulates plant growth and nitrogen use.

North Carolina State University
Raleigh, NC 27695-7905

143. Bioenergetic and Physiological Studies of Hyperthermophilic Archaea

R.M. Kelly, Department of Chemical Engineering $94,080

This project focuses on physiological and bioenergetic characteristics of two representative hyperthermophilic archaea: *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. *T. litoralis* is being studied in relation to information previously developed for *P. furiosus*: effect of sulfur reduction on bioenergetics, preferred fermentation patterns, tungsten requirement etc. A defined medium has been developed for *T. litoralis* consisting of amino acids, vitamins and nucleotides. This serves as the basis for continuous culture studies probing metabolic response to media changes. *P. furiosus* and *T. litoralis* have also been found to produce a polysaccharide in the presence of high levels of maltose. The composition and chemical structure of this polysaccharide are being investigated as well as the metabolic motivation for its production. A novel and, perhaps, primitive intracellular proteolytic complex (previously designated as protease S66) in *P. furiosus* has been isolated and the gene encoding the subunit of the complex has been cloned, sequenced and expressed in active form in *Eschericia coli*. Among other issues, the role of this complex in protein turnover and stress response is being examined in the context of this organism in addition to comparing it to other complexes in eubacterial and eukaryotic cells. Biochemical characteristics of the protease have been measured in addition to examining other proteolytic species in *P. furiosus*.

North Carolina State University
Raleigh, NC 27695-8008

144. Transcription Factors in Xylem Development
R. Sederoff, M. Campbell, R. Whetten, and D. O'Malley, Department of Forestry
$102,990

Xylem is a specialized tissue found in land plants. It is critical for both mechanical support and water and solute transport in plants. Trees accumulate massive quantities of xylem and, in doing so, form wood. Therefore, xylem is not only important in plant growth and development but is also a major source of renewable biomass. Despite its importance, little is known about the regulation of xylem development. We hypothesize that xylem development is regulated, at least in part, by transcription factors. Transcription factors play key roles in the regulation of plant and animal development. For example, members of the Myb class of transcription factors have been found to regulate developmental processes in a variety of organisms, including blood formation in animals and pigmentation patterns in plants. Recently, genes encoding Myb homologues were cloned from the developing xylem of loblolly pine. These myb genes appear to be much more abundantly expressed in developing xylem than in other tissues. These results are consistent with the hypothesis that these genes encode Myb homologues which regulate transcriptional events during xylogenesis. During the course of this work, this hypothesis will be tested using a number of experimental approaches designed to clarify the function and regulation of the pine xylem Myb homologues. These experiments should therefore result in a greater understanding of the regulation of a fundamental process in plant development. Additionally, these experiments should suggest strategies for the genetic engineering of wood.

University of North Carolina
Chapel Hill, NC 27599-3280

145. Novel Control of Signal Delivery from the Pseudomonas syringae avrRPM1 Gene to Arabidopsis thaliana
J.L. Dangl, Department of Biology
$115,000

We analyze steps leading to delivery of the *avrRpm1* encoded signal from the bacterial pathogen *Pseudomonas syringae* pv. *maculicola* isolate M2 (PsmM2), to the host plant, Arabidopsis. We identified several PsmM2 mutants which no longer deliver the *avrRpm1* signal to Arabidopsis, yet are still fully capable of eliciting a hypersensitive response on tobacco. We have cloned the M517 mutation via transposon insertion, and have complemented the M498 mutation with a small piece of wild type DNA (the original mutation was not transposon linked). The M517 mutation is regulatory: all *avr* genes analyzed so far are transcriptionally turned off in this background. The mutation in M498 defines a novel gene, since its function is required for delivery of specific avirulence from two different *avr*

genes (*avrRpm1* and *avrB*), but is not required for delivery of a third *avr* gene (*avrRpt2*). Results from our experiments will add new dimensions to our understanding of how plant disease resistance is engendered by specific *avr* genes. Moreover, in combination with parallel analysis of the plant disease resistance gene, RPM1, whose activity is defined by the presence of *avrRpm1*, we hope to answer critical questions regarding the structural requirements which induce plant disease resistance.

University of North Carolina
Chapel Hill, NC 27599-3280

146. 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 $90,486

As more genes are introduced into plants by transformation, more examples of gene silencing accumulate. Gene silencing stably, but reversibly, inhibits expression of a foreign gene introduced by transformation and simultaneously inhibits expression of any strongly homologous endogenous genes. The mechanisms that lead to gene silencing remain a mystery and the role they play in normal gene regulation is unknown. One roadblock to the study of gene silencing has been the lack of genetic tools to study the phenomenon, such as suppressors or enhancers of silencing events. Recently, a locus that enhances the silencing of the *rolB* transgene in Arabidopsis has been identified by chemical mutagenesis. It is named *EGS1* for *E*nhancer of *G*ene *S*ilencing *1*. The main objective of this research is to take advantage of the wealth of mapping and cloning tools available for Arabidopsis to clone the *EGS1* locus by map based cloning. In order to better define the timing of EGS1 activity, a detailed examination of the timing and tissue specificity of *rolB* silencing and reactivation in *egs1* mutants is also proposed. In addition, we propose to search for other transcripts that may be affected in their expression by the mutations in *egs1* in order to gain an understanding of the role the functional gene product, EGS1, plays in gene regulation. The cloned *EGS1* gene and any affected genes that can be identified will serve as invaluable tools to further investigate the mechanisms and functions of this gene silencing system.

Ohio State University
Columbus, OH 43210-1292

147. Control of Sugar Transport and Metabolism in Zymomonas mobilis
T. Conway, Department of Microbiology $161,000

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

148. In Vivo Analysis of Archaeal Transcription Signal and the Regulation of Heat Shock Promoters

C.J. Daniels, Department of Microbiology

$243,935 (FY 94& FY 95 funds/2 years)

We are examining the transcription apparatus of the Archaea in the model organism, *Haloferax volcanii*. Three questions are being addressed: what sequences are required for efficient and accurate transcription initiation, what sequences and/or structures are required for transcription termination, and how is transcription regulated. A plasmid based transcriptional reporter system has been developed, and this system has been used to identify sequences of the *H. volcanii* tRNALys promoter necessary for transcription initiation. We have found that mutations in the TATA-like boxA element, and the surrounding sequences, influence initiation efficiency of this promoter. The pattern of sensitivity parallels the sensitivity of the eucaryal RNA polymerase II promoter to mutations, supporting the proposal that the archaeal and eucaryal transcription systems are closely related. An *in vivo* termination assay has also been developed and we are examining native *H. volcanii* terminators and model termination elements containing bend sequences and oligo-T tracts. As a model for regulated gene expression we are investigating the transcription control of the *H. volcanii* heat shock gene, HSP60. Fusion studies with the reporter gene system are being used to identify sequence regions responsible for transcriptional control. We have also begun studies on the transcriptional apparatus of *H. volcanii*, and have characterized genes encoding the eucaryal-like transcription factor TBP (TATA-binding protein).

Ohio State University
Columbus, OH 43210

149. Mechanisms of Microbial Adaptation
C.J. Daniels and W.R. Strohl, Department of Microbiology $44,964

Graduate programs specializing in Microbial Biology have declined in recent years, greatly reducing the opportunities for advanced training in this important research area. As one mechanism to rebuild interest in Microbial Physiology and to train new scientists, we have developed a four week summer course focusing on the theme "Mechanisms of Microbial Adaptation". The course contains both lecture and laboratory components targeted to senior level graduate students and industrial scientists who desire an indepth introduction to microbial physiology research. Current topics in the mechanisms of microbial adaptation will be presented in lectures by the faculty participants and these will be supplemented with presentations by experts in the field. Lectures will address: adaptive response of microorganisms in fermentations, global response mechanisms, regulation of microbial autotrophism, carbon metabolism in methanogens, and biodegradation and bioconversion by microorganisms. The accompanying laboratory exercises will illustrate current practical problems in the topic areas and introduce the students to modern experimental techniques and conceptual approaches. These experiments will provide the students with experience in fermentation technology, characterization and quantitation of proteins and metabolic intermediates, enzyme activity measurements and basic kinetic analyses, genetic approaches for the identification of regulated genes, and the anaerobic techniques needed to purify oxygen-sensitive proteins. Laboratory sessions will prepare the students to challenge and expand their current research and to undertake new approaches in their experimental systems.

A second inherent goal of the course is to establish a significant interactive community of research scientists in the field of Microbial Physiology. Introducing students to leading scientists in an informal but intense science setting creates a sense of excitement and community.

Ohio State University
Columbus, OH 43210

150. Biosynthesis of Hydrocarbons
P.E. Kolattukudy, Biotechnology Center $96,000

Hydrocarbons are found in a wide variety of organisms from bacteria to higher plants. However, how hydrocarbons are produced biosynthetically is not well understood. In the green alga, *Botryococcus braunii*, we found that hydrocarbons are generated by elongation of a fatty acid, reduction of the elongated acyl-CoA to the corresponding aldehyde, followed by decarbonylation of the aldehyde into an alkane. Thus, fatty acid reduction is a key step.

Fatty acyl-CoA is also reduced to fatty alcohols that are esterified to generate wax esters. Thus, there appears to be two reductases, fatty aldehyde generating reductase for hydrocarbon synthesis and fatty alcohol generating reductase for wax ester synthesis. Both types have been purified to homogeneity. The aldehyde generating enzyme shows homology to the procaryotic fatty acid reductase. RT-PCR has been used to clone a fragment of this reductase cDNA yielding the probe necessary for cloning this reductase. The decarbonylase has been purified at low levels. This enzyme is inhibited by NADPH and oxygen that were reported to be required for conversion of aldehyde to hydrocarbon by an insect microsomal system. Development of scale up of purification of the decarbonylase to generate sufficient enzyme for mechanistic studies and for cloning purposes is in progress.

Ohio State University
Columbus, OH 43210

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

J.A. Krzycki, Department of Microbiology

$240,905 (FY 94 & FY 95 funds/2 years)

The majority of the substrates of methanogenesis are methylated compounds. These pathways represent interesting examples of highly regulated enzymes in archaebacteria, as well as major routes of methane formation in natural environments. We have been elucidating the pathways of methylated substrates in *Methanosarcina barkeri*, especially those for methylamine degradation. We have found these pathways involve different proteins with two kinds of activity. They are either corrinoid containing proteins, termed methyltransferase I (MT1), which are methylated from the growth substrate; or a second type of protein, termed methyltransferase II (MT2), which then methylates CoM using the methylated corrinoid protein.

Methanogenesis from trimethylamine (TMA), dimethylamine (DMA), and monomethylamine (MMA) involve a single isozyme of methyltransferase II termed MT2-A. MT2-A was found to be specifically required for the DMA and MMA pathways. However, a second isozyme of methyltransferase II, MT2-M, is also able to function in methane formation from TMA. MT2-M had previously been demonstrated to function only in methanogenesis from methanol, while the function of MT2-A had been unknown.

We have also identified two MT1 corrinoid proteins operating in conjunction with MT2 isozymes for the methylamine dependent methylation of CoM. The MT1 protein of the MMA pathway is a 29 kDa corrinoid protein. This protein was first detected as one of several proteins methylated in cell free extract when methyl-CoM reduction to methane is inhibited. The 29 kDa corrinoid protein and MT2-A were both isolated as two proteins which stimulate methyl-CoM formation from monomethylamine in cell extracts. The MT1 protein of the TMA

pathway is a large corrinoid protein containing 2 subunits of 53 and 26 kDa. The purified TMA-MT1 protein and either MT2-A or MT2-M are sufficient to reconstitute the TMA:CoM pathway, catalyzing the methylation of coenzyme M with TMA at 3 umol/min/mg.

We have found a new isozyme of MT2 which is isolated firmly bound to a corrinoid protein. This is a dimeric 480 kDa corrinoid protein which is methylated when methyl-CoM reductase is inhibited in otherwise methanogenic cell extracts. The larger subunit has MT2 type activity, and is homologous to both MT2-A and MT2-M, as well as to another tetrapyrrole binding enzyme, uroporphyrinogen decarboxylase. The smaller subunit binds corrinoid using the B12 binding motif of methionine synthase. The characteristics of this protein indicate it participates in CoM methylation from an unelucidated pathway for a methylated substrate.

Ohio State University
Columbus, OH 43210-1096

152. The Molecular Characterization of the Lignin-Forming Peroxidase
L.M. Lagrimini, Department of Horticulture and Crop Science $100,000

The over-expression of the tobacco anionic peroxidase in transgenic plants results in increased lignificaton. Peroxidase gene expression is significant in the primary xylem parenchymacells and in the lignifying phloem fibers. Expression is to a much lesser extent in the ray parenchyma interspersed between the secondary xylem vessels. This expression pattern is matched with peroxidase activity seen in tissue blots. It can be concluded from studies of anionic peroxidase expression that this enzyme may have a role in the lignification of the primary xylem and in phloem fibers, however, there is less evidence supporting a role for this enzyme in lignification of the secondary xylem. Many of the phenotypes observed in transgenic plants with altered peroxidase expression correlate well with auxin action. Peroxidase underproducing plants grow faster and flower sooner than wild-type, while those plants overproducing peroxidase grow slower and flower later. Growth of lateral shoots and root branches are suppressed in plants overexpressing peroxidase. Reduced peroxidase expression results in excessive growth of lateral shoots. Asymmetric growth in response to gravity is generally thought to involve auxin (IAA). When seedlings were turned 90° to gravity, roots from plants overproducing peroxidase consistently turned 10-fold faster than control seedlings. We determined that the tobacco enzyme was capable of oxidizing IAA in the absence of hydrogen peroxide or other co-factors. However, the addition of trace amounts of hydrogen peroxide eliminated a characteristic lag in the consumption of oxygen. It is possible that hydrogen peroxide may activate the enzyme, thus stimulating IAA degradation.

Ohio State University
Columbus, OH 43210

153. Structure and Regulation of Methanogen Genes
J.N. Reeve, Department of Microbiology $269,660 (18 months)

The goal of this project is to determine the structure, organization and mechanisms of regulation of expression of genes responsible for methanogenesis and to relate these parameters to the functions of their encoded gene products. We have cloned and sequenced the *Methanobacterium thermoautotrophicum (M.t)* genes that encode the enzymes that catalyze the fourth, fifth, sixth and seventh steps in methane biosynthesis from CO_2 and H_2. The fourth and seventh steps have been found to be catalyzed by pairs of functionally equivalent enzymes and transcription of the genes that encode these pairs of enzymes has been shown to be growth phase regulated. The basis for this regulation appears to be the availability of H_2, and experiments are in progress to confirm this conclusion. Genetic evidence has been obtained for the presence of three different electron transport proteins, a polyferredoxin, a flavoprotein and rubredoxin that could transport electrons from H_2 into the methanogenesis pathway. The polyferredoxin and flavodoxin have been purified, with the goal of re-creating this electron transport chain *in vitro*. Several plasmids have been isolated from strains of *M. thermoformicicum* that are closely related to *M.t.* These plasmids are being used as donor DNAs to screen electroporation protocols and genetic selections to develop a transformation system for *M.t.*

Ohio State University
Columbus, OH 43210

154. Photosynthetic Electron Transport in Genetically Altered Chloroplasts
R.T. Sayre, Departments of Biochemistry and Plant Biology $139,954

Light induced charge separation by the photochemically active chlorophyll(s) of photosystem II (PS II) generates one of the most oxidizing potentials in nature. The oxidized PS II reaction center chlorophyll(s) in turn drive the oxidation of water producing molecular oxygen. Charge separation in PS II is one of the largest of all biochemical processes, however, the structure and function of the PS II complex remains to be resolved. Structural models of the photosystem II complex have been developed based on predicted and resolved structural similarities between the PS II complex and the bacterial photosynthetic reaction center crystal structure. We are testing these PS II structural models by targeting select amino acid residues in the PS II D1 and D2 proteins for site directed mutagenesis followed by phenotypic characterization of the mutants. Our objectives are to identify and characterize residues which: 1) are involved in binding and orientation of chromophores, and 2) participate in or regulate charge transfer processes. We are generating these mutations in the chloroplast genome of the single celled alga *Chlamydomonas reinhardtii*. Previous studies have

demonstrated that *Chlamydomonas* has many advantages as a genetic and biochemical system for the characterization of PSII. Furthermore, *Chlamydomonas* serves as a model system for the genetic manipulation of higher plant chloroplasts.

Ohio State University
Columbus, OH 43210

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

F.R. Tabita, Department of Microbiology

$293,600 (FY 94 and FY 95 funds/2 years)

Aside from the reductive pentose phosphate pathway, anoxygenic photosynthetic bacteria and oxygen-evolving photosynthetic eukaryotes employ diverse metabolic routes to reduce and assimilate carbon dioxide. Since CO_2 is a highly oxidized, yet ubiquitous, form of carbon, considerable energy must be expended before organisms can reduce CO_2 to organic carbon. Thus, it is particularly important to understand how diverse photosynthetic organisms regulate their ability to use CO_2 since this is often the sole source of carbon. We are interested in the molecular signals that influence switches in CO_2 metabolic paths in these organisms, with the major focus on the anoxygenic bacteria. In *Rhodospirillum rubrum* and *Rhodobacter sphaeroides*, specific deletion of the genes that encode RubisCO, the key enzyme of the reductive pentose phosphate pathway, results in distinct physiological effects. Moreover, evidence has been obtained to indicate that alternative means to reduce CO_2 and dissipate electrons are induced in these strains and it is apparent in the wild-type that discrete molecular signals control the expression of genes required for each CO_2 fixation route. To gain an understanding of the molecular basis for this regulation, a strategy was developed to isolate genes that regulate the alternative CO_2 fixation system. Eventually, genes that encode a two-component signal transduction pathway, involving a response regulator and a sensor kinase gene, were isolated and shown to mitigate regulation of this system. When the major CO_2 fixation pathway is blocked in *Rs. rubrum* and *R. sphaeroides*, discrete protein products are synthesized in the RubisCO deletion strains under conditions of active CO_2 fixation. Several of these proteins have been identified and are associated with the alternative CO_2 fixation pathway and other processes of the cell. Current work is dedicated to determining the molecular basis by which the two-component system regulates the expression of genes that encode the above proteins. In another bacterium, *Chlorobium tepidum*, which uses the reductive tricarboxylic acid cycle to fix CO_2, one of the key enzymes, ATP citrate lyase, has been purified. In addition, the gene that encodes this protein is being isolated in this genetically tractable green sulfur bacterium. Such studies will provide the basis for understanding how this CO_2 fixation pathway is regulated.

Oklahoma State University
Stillwater, OK 74078-0454

156. The Structure of Pectins from Cotton Suspension Culture Cell Walls
A.J. Mort, Department of Biochemistry and Molecular Biology $99,448

In this project we use cotton suspension culture cells as a model system to understand the structure and, subsequently, function of pectins in plant cell walls. At present we focus on structural studies. We use an hydrous liquid HF, purified enzymes, and concentrated imidazole buffer to extract each subsection of the pectins in high yields from the walls. We find four distinct types of pectin structures: 1) Rhamnogalacturonan I (RGI), 2) Rhamnogalacturonan II, 3) 50% methyl esterified homogalacturonan (HG), and 4) ~15% methyl esterified homogalacturonan. We are concentrating on defining the apparent covalent linkage between the 50% esterified HG and the RGI. Both regions of pectin co-solubilize after endopolygalacturonase (EPG) and cellulase digestion of walls, and co-chromatograph on ion exchange chromatography. The HG is resistant to EPG digestion even after alkaline deesterification. To uncover the reasons why the HG is resistant to EPG we have studied the substrate requirements of the enzyme. We have found that the enzyme needs four adjacent non-esterified GalA residues to be able to cut the polysaccharide. Thus our previous finding that the methyl esters occur on every second GalA residue could explain its resistance except that saponification still does not allow digestion. We now think that there are xylose residues attached directly to one in three of the GalA residues within the HG. Experiments with an abundant xylogalacturonan obtained from watermelon fruit cell walls have shown that it is indeed protected from EPG by the xylose. Stretches of up to 26 GalA residues are protected. During our structural studies we are developing fluorescence detected capillary electrophoresis for oligo- and polysaccharides.

University of Oklahoma
Norman, OK 73019-0245

157. Effect of Community Structure on Anaerobic Aromatic Degradation
M.J. McInerney, Department of Botany and Microbiology $87,000

Factors affecting the rate and extent of benzoate degradation by anaerobic, syntrophic consortia were studied. Cocultures of a syntrophic benzoate degrader, strain SB, with a hydrogen/formate-using sulfate reducer degraded benzoate to a threshold that depended on the amount of substrate and acetate present. The benzoate threshold was not a function of the inhibition of benzoate degradation capacity by acetate or the toxicity of the undissociated form of acetate. Rather, a critical or minimal Gibb's free energy value may exist where thermodynamic constraints preclude further benzoate degradation. A sensitive assay to detect low formate concentrations was developed to measure the formate concentration when the benzoate threshold was reached. Both hydrogen and formate levels were low when the

threshold was reached. Thus, acetate affects the extent of benzoate degradation, implicating the importance of interspecies acetate transfer. Phylogenetic analysis indicated that strain SB clusters with sulfate reducers in the delta subclass of the *Proteobacteria*. Cell-free extracts of strain SB contained benzoyl-coenzyme A (CoA) synthetase and glutaryl-CoA dehydrogenase activities and enzymes involved in β-oxidation and substrate-level phosphorylation. This suggests that the pathway for benzoate degradation in SB is similar to that found in other anaerobes. We have isolated a new iron-reducing bacterium that completely oxidizes acetate, propionate, proline, and several other organic acids to carbon dioxide and phylogenetically represents one of the earliest lines of descent in the *Bacteria*.

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

158. Biochemical Genetics of Lignin Degradation by Phanerochaete chrysosporium

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

$131,000

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 biochemical and molecular biological approaches to characterize the enzymes and genes involved in this process.

We are studying the structure and mechanism of lignin and manganese peroxidases (LiP and MnP) via spectroscopic, kinetic and bioorganic methods. We also are characterizing an intracellular quinone reductase and a dioxygenase which are involved in the further degradation of monomeric lignin degradation products. Genes encoding these peroxidases and intracellular enzymes, including enzymes involved in H_2O_2 metabolism, are being isolated and characterized.

Using our DNA transformation system for *P. chrysosporium*, we have developed an efficient homologous expression system for MnP that produces recombinant MnP in *P. chrysosporium* under the control of a primary metabolic gene promoter. This expression system has facilitated structure/function studies of MnP via site-directed mutagenesis. A similar system is being developed for LiP. We also have developed the first gene replacement system for *P. chrysosporium* and we have used this system to disrupt a gene encoding MnP isozyme I. Disruptions of genes encoding other MnP and LiP isozymes and genes encoding intracellular enzymes involved in lignin degradation are planned.

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

159. Cellobiose Dehydrogenase and β-glucosidase from Phanerochaete chrysosporium: Effect on Cellulose Hydrolysis, Cloning, and Characterization

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

$88,000 (17 months)

Cellobiose dehydrogenase (CDII) is an extracellular hemoflavoenzyme produced by cellulose-degrading cultures of fungi such as *Phanerochaete chrysosporium, Sporotrichum thermophile,* and *Coniophora puteana*. In this study, a cDNA clone of CDH from *P. chrysosporium*, isolated from an expression library, will be sequenced. The primary structure of CDH will be deduced from the cDNA sequence. A genomic clone of CDH will be isolated and sequenced. The genomic clone will help identify the intron-exon boundaries of the CDH gene and the regulatory sequences in the 5'-noncoding region. Cellulose-degrading cultures of *P. chrysosporium* produce a novel cellulose-binding β-glucosidase. We plan to purify this β-glucosidase to homogeneity and determine whether this glucosidase has a specific cellulose-binding domain. The proposed experiments will help in understanding the structure of CDH and β-glucosidase from *P. chrysosporium*.

Oregon State University
Corvallis OR 97331-2902

160. Catalytic Mechanism of Hydrogenase from Aerobic Nitrogen-Fixing Microorganisms

D.J. Arp, Laboratory for Nitrogen Fixation Research

$162,000 (FY 94 funds/2 years)

The hydrogenase from the aerobic, nitrogen fixing microorganism *Azotobacter vinelandii* recycles the hydrogen evolved by nitrogenase. Several properties of this hydrogenase (e.g., very low rate of the back reaction, hydrogen evolution, and a low Km for hydrogen) allow it to function efficiently in an environment in which all the available substrate is generated *in situ*. The metal content (Ni and Fe) and subunit composition (two subunits, 65 KDa and 35 KDa) of this hydrogenase are typical of a large group of hydrogen oxidizing hydrogenases. The primary focuses of our research are: a) The characterization of the mechanisms of specific inhibitors of this hydrogenase (acetylene, oxygen, CO, NO and HCN). Characterizations include considerations of the competitive nature of the inhibitor, the time-dependence of the inhibition, and the dependence on the redox state of the enzyme; b) Site-directed mutagenesis studies of amino acids (Cys and His) that are conserved throughout this class of hydrogenases. Studies include the characterization of the modified hydrogenases at the putative amino acids binding the metal cofactors or involved in subunit interactions; c) The study of the immediate acceptor of electrons from hydrogenase, HoxZ,

including its purification, overexpression and *in vitro* reconstitution; d) The use of EPR and UV-vis spectroscopy to confirm the redox centers in the enzyme and their role in catalysis. This system is particularly well-suited to these investigations because the enzyme is well-characterized at the biochemical level and because the bacterium is amenable to genetic transformation.

University of Oregon
Eugene, OR 97403

161. Genetic Analysis of Chloroplast Translation
A. Barkan, Institute of Molecular Biology $150,000 (18 months)

The components of the photosynthetic apparatus accumulate in the appropriate ratios and only within the correct cell types as a consequence, in part, of the regulated expression of chloroplast genes. This project pertains to the control of chloroplast protein synthesis by nuclear-encoded factors, few of which have yet been identified. The project is focused on *crp1*, a nuclear mutation in maize. Previous results established a role for the *crp1* gene in activating the translation of *petA* mRNA and in the endonucleolytic processing of *petB* and *petD* mRNAs. The absence of monocistronic *petD* mRNA correlated with a loss of *petD* translation, providing evidence that this open reading frame is inefficiently translated in a polycistronic context. Thus, the *crp1* gene activates the expression of a small set of chloroplast genes. We have recently used a transposon insertion to clone a portion of the *crp1* gene. The clone will be used to deduce the structure of the *crp1* gene product, to localize it in the plant, and to generate protein for biochemical assays. The role of *crp1* in activating translation will be investigated by defining the step at which translation is blocked in mutant chloroplasts, identifying RNA binding activities that interact with target mRNAs in a genotype-dependent fashion, determining whether the *crp1* gene product is a component of these binding activities, and assessing activities of the *crp1* protein. Understanding the mechanism by which *crp1* activates translation will further our understanding of chloroplast translation in general. Most primary transcripts in the chloroplasts of higher plants are subject to endonucleolytic processing. The role of this processing is largely unexplored. Further experiments will explore the role of endonucleolytic RNA processing in controlling the translation of chloroplast mRNAs. Finally, Mu populations will be screened for new mutants with defects in the translation of chloroplast mRNAs.

Pennsylvania State University
University Park, PA 16802

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

J.E. Brenchley, Department of Biochemistry and Molecular Biology $109,109

Our objective is to characterize psychrophilic microorganisms and to purify, study and genetically engineer their cold-active glycosidases. Cold-active glycosidases with high activities at low temperatures can be used for low temperature conversions of saccharides found in plant biomass, paper-pulping waste, whey, etc. into chemical fuels and into fermentation media for use by biotechnology companies. Comparisons of cold-active enzymes with their corresponding proteins from higher temperature organisms will yield insight into structural features involved in thermal stability. Our goals are to complete the characterization of three DF-galactosidase isozymes cloned from a single Arthrobacter strain, to isolate and examine new psychrophiles producing novel cold-active glycosidases with even lower temperature optima, to clone, sequence and analyze the genes for selected enzymes, and to purify the most cold-active glycosidases for biochemical studies and comparisons with other enzymes. In addition to information on the characteristics of several cold-active glycosidases, our work will provide insight into the types of psychrophilic microorganisms inhabiting the understudied cold ecosystem that dominates our biosphere.

Pennsylvania State University
University Park, PA 16802-4500

163. Light-Energy Transduction in Green Sulfur Bacteria

D.A. Bryant, Department of Biochemistry and Molecular Biology
$150,350 (FY 94 funds/two years)

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. Although it is generally accepted that green bacteria contain a ferredoxin-$NADP^+$ oxidoreductase as in cyanobacteria and green plants, exhaustive attempts to identify and purify such an enzyme in *Chlorobium tepidum* were unsuccessful. This finding suggests that "textbook" views of the electron transport chaih in these organisms may be incorrect. Genes encoding five chlorosome proteins (CsmA, CsmB, CsmC, CsmD, and CsmE) have been cloned, sequenced, and transcriptionally characterized. Each of these proteins has been overproduced in *E. coli* and purified to homogeneity; polyclonal rabbit antisera have been raised for each protein.

We have confirmed that both *Chlorobium tepidum* and *Chlorobium vibrioforme* strain 8327D are naturally transformable and that homologous recombination can be used to target

interposons into chromosomally encoded genes. Mutants harboring an omega-cartridge interposon in the csmC gene have been obtained for both Chlorobium sp. Immunoblotting experiments demonstrate that the CsmC protein is missing as expected, although the mutants still assemble chlorosomes. These experiments establish the feasibility of mutational analyses by transformation and interposon mutagenesis in Chlorobium sp. Constructions have also been made for interposon mutagenesis of the *csmA, csmB, csmD,* and *csmE* genes. Using previously determined protein sequences for CsmF, CsmH, and CsmI, degenerate oligonucleotide probes have been used to identify convenient restriction fragments for the isolation of the genes encoding these additional chlorosome proteins.

Pennsylvania State University
University Park, PA 16802

164. Mechanisms Controlling Plant Cell Wall Expansion
D.J. Cosgrove, Department of Biology $98,000

Plant cell enlargement starts with biochemical processes that modify the interactions between wall polysaccharides to induce wall stress relaxation, which gives rise to cell water uptake and wall expansion. This project is aimed at elucidating the physical, cellular and molecular mechanisms controlling wall expansion and cell enlargement. We are taking three approaches. (1) High-resolution growth rate measurements of young seedlings are combined with low-amplitude pressure sinusoids to characterize the dynamic feedback behavior of endogenous growth control systems. These studies are complemented with analyses of electrical signaling in growing seedlings in response to mechanical and hydraulic perturbations. The results indicate that mechanosensitive proton pumping is part of the feedback system for growth control. (2) The biophysics of extension in isolated walls is analyzed by creep tests and stress relaxation assays of wall samples. We are learning that the rheology of isolated walls is dependent principally and characteristically on expansin activity. Moreover, wall creep is found to be sensitive to the hydration state of the wall. This result suggests that drought inhibition of growth may be mediated in part by direct effects on wall rheology. (3) We are using reconstitution assays to assess the sensitivity of the wall to expansin action. We find that wall sensitivity to expansins is regulated developmentally and also plays a part in the growth adaptation of maize roots to water stress. The results of these studies will further our understanding of basic growth processes in plants and their control by hormones, light, drought and other agents.

Pennsylvania State University
University Park, PA 16802

165. Role of Ca^{++}/calmodulin in the Regulation of Microtubules in Higher Plants
R. Cyr, Department of Biology $87,000

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. Our working hypothesis is that CaM has two opposing effects; at low Ca^{++} concentrations it stabilizes MTs to the effects of Ca^{++}, while at high concentration it interacts with pp50 (a MAP and homolog to elongation factor 1-alpha) to unbundle MTs, thereby leading to their destabilization. This scenario, fully supported by biochemical and cell biological data, has important developmental and physiological significance because it provides a molecular explanation for how MT organization can be regulated by the cell.

Pennsylvania State University
University Park, PA 16802-4500

166. Enzymological Studies of One-Carbon Reactions in the Pathway of Acetate Utilization by Methanogenic Bacteria
J.G. Ferry, Department of Biochemistry, Microbiology and Molecular & Cell Biology $145,000

Several enzymes in the pathway of acetate conversion to CH_4 and CO_2 have been purified from *Methanosarcina thermophila* and are under investigation utilizing biochemical, biophysical and molecular genetic approaches. Acetate kinase and phosphotransacetylase catalyzes the activation of acetate to acetyl-CoA. Recent results show that the genes for these enzymes are transcribed together. The regulation of enzyme synthesis in response to the growth substrate is at the transcriptional level. Transcriptional mapping suggests that different levels of these enzymes may be accomplished by mRNA processing. Site-directed mutagenesis has revealed amino acids required for catalysis which has helped to define the catalytic mechanisms of both enzymes. For example, the glutamate has been identified in acetate kinase that is phosphorylated during catalysis. All genes transcribed in the CO

dehydrogenase operon (*cdh*) have been cloned and sequenced. Transcriptional mapping identified transcript sizes, start sites and promoter sequences which suggested processing of the *cdh*-specific mRNA. The heterologously-produced corrinoid/iron-sulfur component of the CO dehydrogenase complex has been characterized revealing that the larger 60-kDa subunit contains factor III corrinoid and iron-sulfur centers, and is the site for methyl transfer to the substrate tetrahydromethanopterin. A CO dehydrogenase:heterodisulfide reductase system has been reconstituted with purified and partially purified components. The results indicate that membranes are required and molecular hydrogen is not an obligatory intermediate.

Pennsylvania State University
University Park, PA 16802

167. Characterization of Lignin and Mn Peroxidases from Phanerochaete chrysosporium
M. Tien, Department of Biochemistry and Molecular Biology $108,000

The aromatic polymer lignin is degraded predominantly by fungi. We have been characterizing fungal degradation with the white-rot fungus *Phanerochaete chrysosporium*. This fungus secretes lignin and Mn peroxidases. These two enzymes catalyze the oxidation of lignin and lignin model compounds via a free radical mechanism. The role of these two enzymes and their isozymes in lignin biodegradation is the focus of our research. We are addressing this question by characterizing the enzymology and regulation of these isozymes in *P. chrysosporium*. We are also characterizing these isozymes in other white rot fungi. We have isolated the cDNAs encoding these isozymes and have been able to express these isozymes in active form in three different expression systems. Heterologous expression has allowed us to continue our mechanistic and structural studies. The recent determination of the 3-D structure of these two enzymes has greatly facilitated our mechanistic studies. Our mechanistic studies will ultimately determine how these enzymes interact with their substrates and what structural aspects confer them with their unique reactivity.

University of Pennsylvania
Philadelphia, PA 19104-6018

168. Structural Basis of Signal and Energy Transduction in Plants
A.R. Cashmore, Plant Science Institute, Department of Biology $125,000

This training program is aimed at training graduate students in both structural and molecular genetic studies in plants. For the first year of this program students have been accepted into the graduate programs affiliated with three different Departments: Biochemistry/Biophysics, Biology, and Chemistry. These students will take a common core curriculum in chemistry,

biophysics and plant biology. In addition they will perform a laboratory rotation outside their primary discipline: students affiliated with either the Chemistry or Biochemistry/Biophysics graduate program will perform rotations in a Plant Biology research laboratory and students affiliated with the Biology graduate program will perform rotations in either Chemistry or Biochemistry/Biophysics. The students' thesis research will be carried out in any one of the three departments and will involve a topic in either plant molecular genetics or structural studies of molecules important in plant signal and energy transduction. By this mechanism plant scientists will be trained with an appreciation of both molecular genetic as well as chemical, biochemical, and biophysical approaches.

University of Pennsylvania
Philadelphia, PA 19104-6018

169. Light Responses and Photoperiodism in Arabidopsis thaliana
A.R. Cashmore, Plant Science Institute, Department of Biology $250,000

We have further characterized a collection of *rsp* mutants we have obtained which exhibit reduced sensitivity to photoperiod. Wild-type Columbia ecotype Arabidopsis shows delay in flowering time when grown under short-day conditions of 8 hours light, 16 hours dark. Some of the mutants do not show delayed flowering under an 8-hour photoperiod but instead show delay only under a shorter, 6-hour photoperiod. In the expectation that they may be affected in the period length of the endogenous circadian rhythm, which is known to influence the photoperiodic flowering response to day-length, we have examined the period length of the circadian rhythm of CAB gene expression in these mutants. Indeed, we observe that some of these mutant lines have a shortened period in the rhythm of CAB gene expression which in wild-type Columbia cycles with a free-running period length of approximately 25 hours. Preliminary results indicate that the period lengths of the mutants is shortened by about two hours which is in good agreement with the two-hour decrease in the length of the critical photoperiod for delay of flowering and provides evidence that we may have identified lesions within elements of the circadian clock.

University of Pennsylvania
Philadelphia, PA 19104-6018

170. Membrane-attached Electron Carriers in Photosynthesis and Respiration
F. Daldal, Department of Biology $195,600 (18 months)

The overall aim of our project 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). In these studies we are using the facultative photosynthetic bacterium *Rhodobacter capsulatus* as a model system for energy transduction.

The presence of a dual electron transfer (ET) pathway between the cyt bc_1 complex and the reaction center in Ps, and the cyt *c* oxidase in Res, was first indicated by our Ps- and Res-proficient mutants lacking the soluble cyt c_2. We have then demonstrated that a novel membrane-associated cyt, cyt c_y, which is structurally distinct from, and yet functionally similar to cyt c_2, is the molecular basis of this unique, entirely membrane-confined ET pathway. We have also studied various *R. sphaeroides* strains harboring cyt c_y to determine its ET properties to its heterologous physiological partners. We are now defining the membrane topology and anchor of cyt c_y, and are assessing its role in the cyt bc_1 complex-dependent respiration using appropriate mutants. We have recently succeeded in constructing an epitope-tagged derivative of cyt c_y which is still active, and are purifying it to define its physicochemical properties. Finally, we have also initiated studies on how the membrane-bound *c*-type cytochromes are biosynthesized by searching mutants affecting cyt *c* biogenesis and maturation, an important biological process for energy transduction, about which little is known.

University of Pennsylvania
Philadelphia, PA 19104-6018

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

J.R. Ecker, Department of Biology $99,000

Differential growth within plant tissue may result from division of adjacent cells or non-uniform (differential) cell elongation. The plant hormones ethylene and auxin regulate the elongation of plant cells and act together, in dark grown seedlings, in the formation of a hypocotyl hook. The apical hook is formed by asymmetric elongation of cells on opposite sides of the hypocotyl; the rate of elongation of cells on the outside of the hook is dramatically different than cells on the inside of the hook. The goal of our research program is to apply a molecular genetic approach to develop an understanding of the processes by which the hormones ethylene and auxin coordinately regulate the elongation of plant cells. Mutational analysis will be utilized to identify genes that suppress or modify the constitutive hook phenotype of the Arabidopsis mutant constitutive triple response 1. Several classes of mutants have been identified, including those which exhibit no differential elongation in the hypocotyl, represented by hookless1 and those which show novel "coiled" phenotypes. At the same time, genes will have been identified that show spatially-restricted expression in hypocotyl hook and these will serve as molecular markers to characterize the effects of ethylene and auxin in each of the mutants. It is expected that the identification of genes that control differential cell elongation in the Arabidopsis hypocotyl will provide significant new insights into the general mechanisms of differential cell elongation in other tissues and for agronomically important plants.

University of Pennsylvania
Philadelphia, PA 19104-6018

172. Structure-Function Analysis of Vacuolar H^+-Pyrophosphatase

P.A. Rea, Department of Biology $95,000

Plant cells contain alternate metabolic pathways that utilize nucleoside triphosphates or inorganic pyrophosphate (PPi) as energy sources. The full significance of this phenomenon remains to be determined but the use of these two energy currencies is exemplified by the presence of two H^+ pumps on the membrane bounding the central vacuole of higher plant cells. These are the vacuolar H^+-ATPase (V-ATPase; EC 3.6.1.3), an enzyme common to the endomembranes of all characterized eukaryotes, and a vacuolar H^+-translocating inorganic pyrophosphatase (V-PPase; EC 3.6.1.1), which is ubiquitous in plants but otherwise known in only a few phototrophic bacteria. Both enzymes pump H^+ from the cytosol to vacuole lumen to establish a H^+ gradient that is employed to energize the transport of a broad range of solutes across the vacuolar membrane.

Our research is directed at understanding the structure and function of the V-PPase at the protein level through the application of biochemical and molecular techniques. The basis of the research underway is our recent success in cloning cDNAs encoding the V-PPase from *Arabidopsis thaliana* and its isoforms from *Beta vulgaris*, reconstituting partially purified preparations of the enzyme to generate transport-competent proteoliposomes *in vitro* and our ability to heterologously express cDNAs encoding mutated forms of the V-PPase from *Arabidopsis* in the yeast *Saccharomyces* to yield membrane fractions containing appreciable quantities of integral pump.

Having defined the C-terminal topology of the enzyme and demonstrated a specific requirement for cytosolically oriented Cys^{634} for inhibition by maleimides but the dispensability of all of the conserved Cys residues, including Cys^{634}, for V-PPase function, our studies are now aimed at determining the involvement of acidic amino acid residues located within or near transmembrane spans in substrate turnover, H^+-translocation and/or activation by monovalent cations. To date we have identified three main types of mutant enzyme; those in which PPi hydrolysis is decoupled from H^+ translocation; those that have been rendered insensitive to DCCD; and, those that have altered cation selectivity. The first class of mutations is suspected to denote a portion of the structure that participates in relaying H^+ across the phospholipid bilayer whereas the third class is considered to delineate the K^+ binding site or a portion thereof. The significance of these findings with respect to the transport mechanism of the V-PPase, specifically its mode of coupling substrate hydrolysis with H^+ pumping and its near obligate requirement for K^+ for activity, is being examined through the biochemical analysis of these and double mutants in the context of our revised model for the organization of the enzyme.

Its novel origins, membership of a new category of ion translocase and utilization of such a simple phosphoanhydride as energy source, make the V-PPase a unique system for examining the mechanistic basis of energy-dependent ion translocation across biological membranes.

Purdue University
West Lafayette, IN 47907

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

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

$180,420 (FY 94 funds/2 years)

We are working towards identifying the enzymes involved in the biosynthesis of (1→3),(1→4)-β-D-glucans (MGs). These cell-wall polysaccharides are unique to the cereal grasses and have recently gained attention for their possible role in lowering serum cholesterol. MGs play an essential role in the growth of plant primary tissues. Absent from cell walls of dividing cells, the MGs accumulate specifically during cell expansion. After expansion, they decrease markedly in proportion to other polymers. During grain development, the MG appears again as major constituents of the endosperm wall. The general structure is a polymer of cellotriosyl and cellotetraosyl units connected by single (1→3)-β-D-linkages. Contiguous (1→3) linkages and longer stretches of (1→4) linkages, which are susceptible to cleavage by specific endo-glucanohydrolases, are also built into the polymer at special points. We have successfully enriched membranes of the Golgi apparatus from maize (*Zea mays* L.) by a single-step flotation gradient and used them to synthesize *in vitro* an MG identical in structure to the native cell-wall polymer (Gibeaut, D.M., and N.C. Carpita, 1994, *Proc Natl. Acad. Sci. USA* ***90***, 3850-3854). We also observed a callose synthase activity that co-localized with the Golgi apparatus and, unlike the plasma membrane-associated callose synthase, is not activated by calcium. We are using improved procedures for isolation of the Golgi apparatus and determination of structure to investigate some of the factors involved in the synthesis of this polymer *in vitro*. We hypothesize that MG synthases are related to cellulose synthases and that grass-specific Golgi-associated callose synthase arises as a result of disruption or partial degradation of MG synthase. We have found that UDP inactivates MG synthase at mM concentrations and is a potential new probe for identification of MG synthase polypeptides. We have made an affinity label from [^{35}S]-*thio*-UDP by periodate oxidation of the ribosyl vicinal hydroxyl groups to form *thio*-UDP-dialdehyde. This new affinity label does not modify the uridine group that may be essential for specificity. In preliminary experiments, at least two polypeptides were labeled in a Mg^{2+}-dependent manner and UDPG was a competitive inhibitor of labeling. In parallel studies, intrinsic and extrinsic proteins of enriched Golgi membrane preparations have been resolved by two-phase detergent partitioning. Three major intrinsic proteins were identified, and we have obtained N-terminal sequence on two of them. We are scaling up the preparations to obtain enough protein for internal sequence determination after tryptic digestion and for antibody production. We are also designing experiments to determine affinity label distribution between the intrinsic and extrinsic proteins.

Our ultimate goal is to use the antisera and peptide sequence information to identify from expression libraries the cDNAs of maize MG and Golgi apparatus-specific callose synthases.

Purdue University
West Lafayette, IN 47907-1153

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

C.C.S. Chapple, Department of Biochemistry $285,709 (FY 94 funds/2 years)

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

We have sequenced the entire F5H cDNA as well as 4.8 kb of genomic sequence. The genomic clone has been used in experiments aimed at complementing the *fah1* mutation, but surprisingly, this fragment does not restore the wild type phenotype. These data suggest that the *fah1* gene has large regions of regulatory sequence that are required for proper gene expression. Cosmid clones carrying larger inserts spanning the *fah1* gene are currently being used in attempts to complement the mutant phenotype.

We are now beginning a detailed study of the gene's regulatory regions. We have also begun studies aimed at over expressing F5H using in wild type and mutant Arabidopsis to manipulate lignin composition in a model system and will study the impact of changes in F5H expression on the chemical nature of the lignin produced in the mutant and transgenic plants.

Purdue University
West Lafayette, IN 47907

175. A Genetic Analysis of the Lumenal Proteins of the Photosystem II O_2-evolving Complex in Cyanobacteria

L.A. Sherman, Department of Biological Sciences $199,500

The primary objective of this research will be to determine the proteins and protein domains in the cyanobacterium *Synechocystis* sp. PCC6803 that interact with the Mn-stabilizing protein

(MSP) and help form the lumenal component of the PSII O_2-evolving complex. We will use a genetic and molecular biological approach to this problem so that we can thoroughly study the lumenal side of the PSII complex. We will first clone and sequence the genes encoding two small lumenal proteins that have been implicated as potential replacements for MSP. These are *petK*, coding for cytochrome C550, and *psbU*, coding for the 12 kDa protein. We will construct the double mutants *psbO petK*, *psbO psbU* and *petK psbU* to determine if these strains are still capable of photosynthetic growth and O_2-evolution.

We will also isolate mutations in other PSII genes that affect the lumenal side of PSII. We will concentrate on MSP and the lumenal domains of CP47. The screening will use a Digital Imaging Spectrometer (DIS), which permits many colonies to be analyzed for fluorescence or absorption properties simultaneously. This screening approach will allow us to perform a variety of random and site-specific mutagenesis procedures and generate a large number of potential mutants. All of these mutants will be analyzed in regard to a modified model of the S-state mechanism that we have developed. We have shown that the *Synechocystis psbO* mutant and the diazotrophicstrain *Cyanothece* have high proportion of super-reduced S-states under physiological condition and we will study this phenomenon in the mutants.

Rice University
Houston, TX 77251

176. 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 (18 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. Current knowledge concerning the molecular genetics, cell regulation and metabolic engineering of this organism is still rather limited. The objectives are to improve our knowledge of the molecular genetics and enzymology of Clostridia in order to make genetic alterations which more effectively channel cell metabolism toward production of desired products. Two factors that limit butanol production in continuous cultures are: 1) The degeneration of the culture, with an increase in the proportion of cells which are incapable of solvent production. Currently isolated degenerate strains are being evaluated to analyze the molecular mechanism of degeneration to determine if it is due to a genetic loss of solvent related genes, loss of regulatory element or an increase in general mutagenesis. Recent studies show two general types of degenerates, one which seems to have lost essential solvent pathway genes and another which has not completely 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. These studies focus on ferredoxin and rubredoxin and their oxidoreductases.

Rice University
Houston, TX 77251

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

S.I. Gibson, Department of Biochemistry and Cell Biology $101,500

Sources (such as leaves) and sinks (such as roots and seeds) of fixed carbon 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 fixed carbon interact are poorly understood, but are likely to involve the regulation of key genes by sugar levels. One aspect of our research is focused on determining how pervasive sugar-regulated gene expression is in plants. A second aspect of our research is focused on elucidating the molecular mechanism by which sugar-regulated gene expression occurs in plants. This goal is being accomplished by identifying and characterizing mutants in the model plant *Arabidopsis thaliana* that are defective in sugar-regulated gene expression. 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 variety of plants, including important crop plants such as bean, cabbage, tomato, carrot, sunflower and corn. Our interest in this area stems from our recent discovery that fumaric acid accumulates to very high levels in at least some plants. 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 accumulation in plants, a topic of great economic importance to agriculture.

University of Rochester
Rochester, NY 14627-0166

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

J.H.D. Wu, Department of Chemical Engineering $71,505

Clostridium thermocellum produces an extracellular cellulase system highly active on crystalline cellulose. The cellulase activity resides mainly in an extremely complicated protein aggregate called cellulosome. We have proposed a novel anchor-enzyme model involving two major cellulosome subunits, CelL (M_r = 250,000) and CelS (M_r = 82,000), which degrade crystalline cellulose synergistically. The model depicts that CelL functions as an anchor on the cellulose surface for CelS, the major catalytic subunit belonging to a new cellulase family. The enzymatic properties of CelS were characterized using the recombinant CelS expressed in *Escherichia coli*.

The recombinant CelS displayed typical exoglucanase characteristics including: 1) preference for amorphous or crystalline cellulose over carboxymethylcellulose (CMC); 2) inability to reduce the viscosity of a CMC solution; and 3) production of few bound reducing ends on the solid substrate. The hydrolysis products from crystalline cellulose by rCelS were cellobiose and cellotriose at a ratio of 5:1. The rCelS activity on amorphous cellulose was optimal at 70°C and at pH 5 - 6. Its thermostability was increased by Ca^{++}. Sulfhydryl reagents had only a mild adverse effect on the rCelS activity. Cellotetraose was the smallest oligosaccharide substrate for rCelS, and the hydrolysis rate increased with the substrate chain length. Many of these properties are consistent with those of the cellulosome, indicating the key role of CelS. The demonstration of an exoglucanase associated with the cellulosome shows that, like the fungal cellulase system, the bacterial cellulase system needs an exoglucanase for effective degradation of cellulose.

Rockefeller University
New York, NY 10021

179. Characterization of the Systemic Acquired Resistance Immediate-Early Response to Salicylic Acid

N.-H. Chua, Laboratory of Plant Molecular Biology

$180,420 (FY 94 funds/2 years)

Salicylic acid (SA) has been shown to be an endogenous signal molecule in plant defense responses to pathogens and in other plant processes, yet much remains to be elucidated about the molecular mechanism of its action. Following the characterization of a viral promoter element that mediates transcription induction by SA with immediate-early kinetics, we initiated a survey to identify plant genes controlled in the same manner in an effort to gain further insight into cellular SA responses. We have now identified and characterized the first such plant gene from tobacco, by using differential mRNA display. The sequence encodes a predicted protein that shares strong homology to plant glucosyltransferases, and thus it may function in the conjugation and regulation of plant signaling compounds. Induction of the glucosyltransferase gene by SA is rapid and transient, with a peak of mRNA accumulation at approximately 3 hours. Induction occurs in the presence of the protein synthesis inhibitor, cycloheximide (CHX), and the combined treatment of SA and CHX produced a sustained superinduction, typical of primary response genes characterized in various plant and animal systems. This behavior is clearly distinct from that of other characterized SA responses, such as induction of pathogenesis-related (PR) genes, which are induced only after 6 or more hours of SA treatment and are blocked in the presence of CHX. Our analysis of this and other genes in the SA pathway may elucidate components involved in SA signal transduction mechanisms and provide a greater understanding of the plant defense response.

Rutgers University
New Brunswick, NJ 08903

180. Molecular Analysis of a Thylakoid K^+ Channel
G.A. Berkowitz, Department of Plant Science $171,100 (18 months)

Ion channels are a class of membrane proteins which are very important for the metabolic functioning of cells. Because they are extremely low abundance proteins, they are very difficult to purify; no ion channel has ever been purified (and sequenced) from a plant membrane. We have developed a novel probe which can be used to identify K^+ channel proteins. This probe is an antibody generated against the conserved pore region of K^+ channels. The research rationale of this project is to use this anti-pore antibody to facilitate the first-ever purification of an intracellular plant K^+ channel protein. The target membrane for this work will be the thylakoid membrane of the chloroplast; the most ubiquitous membrane on earth, which is also easy to purify in large quantities. A K^+ channel is present in the thylakoid membrane; the function of this protein is critical for maximal photosynthetic activity. We will use the antibody to immunoprecipitate the thylakoid K^+ channel. Partial peptide sequencing of the immunoprecipitated K^+ channel will allow for specific probes to be developed which will then be used to identify a cDNA encoding the polypeptide. Sequencing of the cDNA will lead to the molecular characterization of this K^+ channel protein. Biochemical analyses will also be undertaken to characterize the native function of the thylakoid membrane K^+ channel.

Rutgers University
New Brunswick, NJ 08903-0231

181. Respiration and Active Oxygen Species Production in Stressed Plants
I. Raskin, AgBiotech Center $87,000

Plants respond to a variety of stresses by changing their respiratory metabolism. For example, we have observed that chilling stress results in the activation of the cyanide-insensitive or alternative respiratory pathway (AP) in the leaves of a variety of plant species. We suggest that the increased "heating" of the inner mitochondrial membrane at low temperatures is accomplished by a larger electron flux through the AP, which dissipates most of the energy stored in the respiratory substrates as heat. This localized thermoregulation counteracts the deleterious effects of chilling on the fluidity of the mitochondrial membrane, which determines the activity of the respiratory enzymes vital for plants.

In addition to chilling, pathogenesis is another stress associated with increases in both cyanide-sensitive and cyanide-insensitive respiration. Increased respiration in the infected tissues is associated with a substantial increase in heat production. We will test the hypothesis that active oxygen species generated as a result of the increased respiration in

plant leaves undergoing a hypersensitive response initiate the cascade of molecular events which directly leads to the increased expression of defense-related proteins. We will also assess whether salicylic acid, a known inducer of plant respiration, is the cause or the result of the respiratory burst and active oxygen species production observed during the incompatible plant-pathogen interaction. The involvement of respiration in the generation of active oxygen species will also be investigated.

Rutgers University
Piscataway, NJ 08855-0759

182. Corn Storage Protein: A Molecular Genetic Model
J. Messing, Waksman Institute $175,500 (18 months)

Our studies are focused on the agricultural production of amino acids, in particular methionine, the only one that needs to be added to corn/soybean-based animal feed. Methionine accumulates in the seed during synthesis of storage proteins. In commercial varieties of corn and soybeans, storage proteins containing methionine do not rise to a level high enough to facilitate the formulation of a balanced diet. We can find levels of methionine in corn inbred lines, sufficient to eliminate any feed supplement, if the δ-zeins of 10 and 18-kDa are overexpressed because one out of four of their amino acid residues is methionine. However, inbreds with different levels of these proteins lack a visible phenotype, thereby preventing a traditional breeding approach for a high-methionine variety. Interestingly, determinants of the high-methionine trait have still been present in teosinte, but seem to have escaped selection during early domestication of corn. The two δ-zein genes were likely derived from unequal crossing over prior to the tetraploidization of the genome and have been very conserved in all inbreds tested. Therefore, the accumulation of their products is due to the genetic background of different varieties. We have characterized one factor that exists in different alleles, explaining the differential accumulation of the 10-kDa zein. Using fine structure mapping this factor has been located within a small interval suitable for a map-based cloning approach.

Salk Institute for Biological Studies
San Diego, CA 91286-5800

183. Signal Transduction Pathways that Regulate CAB Gene Expression
J. Chory, Plant Biology Laboratory $105,000

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). Several regulatory photoreceptors are involved in the perception of light signals;

however, the exact mechanisms by which light and other signals are perceived by plant cells and converted into physiological responses are not understood.

The proposed research program focuses on the genetic, biochemical, and molecular characterization of new *Arabidopsis thaliana* mutants that have been previously isolated in our laboratory. We have utilized a molecular genetic approach to select for Arabidopsis 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 a minimum of 17 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 (5 genes). Experiments are underway to clone several of these positive and negative regulatory genes. The proposed experiments will further our knowledge of light-mediated signal transduction pathways, and will be 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

184. Genetic Engineering with a Gene Encoding a Soybean Storage Protein
R.N. Beachy, Department of Cell Biology $97,999

Regulation of the family of genes that encode the β-conglycinins, the seed storage protein of soybean (*Glycine max*) is primarily at the level of gene transcription, although there is evidence of post-transcriptional regulation in some members of the gene family. Our research is directed to the identification of nucleotide sequences and protein factors that are involved in regulating the expression of members of this gene family. Recently we completed the analysis of transgenic tobacco plants that contain chimeric genes comprised of sequences 5' and 3' of the genes encoding the α'- and β-subunits of β-conglycinin and the reporter gene, *uid*A. The results of these studies clearly demonstrated that temporal regulation of expression of the chimeric gene (during the development of the immature embryo) is controlled by sequences 5' of the gene, while the amount of protein product is influenced by nucleotide sequences 3' of the coding sequence.

To identify the proteins, referred to as the soybean embryo factors (SEF proteins), that bind to nucleotides 5' of the coding sequence and the core promoter (i.e., 5' of the TATA sequence), we employed a cloning vector derived from a yeast expression system. In this system nucleotide sequences proposed as important for binding of SEF proteins were ligated

upstream of a yeast core promoter ligated with a selectable marker. Cloned cDNA derived from embryo mRNAs was ligated with a plasmid to construct fusion proteins with the activator domain of the yeast promoter. Colonies were identified that contain sequences encoding a protein that binds the TATA element of the α'-promoter, and another that binds a sequence upstream of the TATA sequence previously implicated in regulating expression of the promoter. The sequence of the cloned cDNA was determined and the derived protein sequence provides information about the nature of the putative regulatory protein. We will continue our analysis of this protein by developing antibodies and relevant nucleotide probes to localize expression of the gene in developing embryos.

The Scripps Research Institute
La Jolla, CA 92037

185. Targeting Transporters to the Plasma Membrane in Plant Cells
J. Harper, Department of Cell Biology $182,360 (FY 94 funds/2 years)

Our long term goal is to engineer new solute transport systems into the plasma membrane of plant cells. This knowledge will provide the potential for new strategies in bioremediation and crop improvement. For example, plants engineered with a more active heavy metal transporter could be used in bioremediation efforts to clean up toxic metals from contaminated soils.

As a prerequisite to engineering plants with new transport systems, it is important to understand how transporters are normally targeted to the plasma membrane. Therefore, our first objective is to understand the rules which govern the targeting of integral membrane proteins. The hypothesis being tested is that targeting to the plasma membrane requires specific information. The approach is to use an *E. coli* lactose transporter as a model protein and investigate its intracellular localization when expressed in transgenic plants. The first objective is to determine the transporter's "default" targeting location, and then determine what "information" is required to (re)direct the transporter to the plasma membrane.

As part of an effort to investigate novel transport systems, we have also cloned a unique heavy metal transporter from a model plant, Arabidopsis. This transporter belongs to the super-family of P-type ATPases which includes the plasma membrane proton pump. Our short term goal is to determine the pump's ion specificity and intracellular location. This information is critical to understanding the pump's normal biological function, as well as its potential use in bioremediation.

The Scripps Research Institute
La Jolla, CA 92037

186. Nuclear Genes Regulating Translation of Organelle mRNAs
S. Mayfield, Department of Cell Biology $100,000

We have identified and purified a set of four proteins that bind with high specificity and affinity to the 5' untranslated region (UTR) of the chloroplast *psbA* mRNA in *Chlamydomonas reinhardtii*. Binding of these proteins to the *psbA* mRNA and translation of the *psbA* mRNA are light regulated. We have identified two independent biochemical mechanisms by which the binding activity of these proteins is regulated: ADP-dependent phosphorylation and thioredoxin mediated reduction. Using site-directed mutagenesis and chloroplast transformation we have identified an RNA element contained within the 5' UTR of the *psbA* mRNA that is recognized by these RNA binding proteins, and that functions as a component of *psbA* translational activation. We have mapped the secondary structure of the *psbA* UTR in solution using chemical and enzymatic probes. Finally we have characterized *psbA* RNA binding proteins in nuclear mutants deficient in *psbA* translation. In one such mutant a 47 kDa *psbA* binding protein (RB47) is absent. This mutant lacks *psbA* RNA binding activity, has greatly reduced association of the *psbA* mRNA with ribosomes, and has no detectable *psbA* translation. These data suggest that the *psbA* 5' UTR binding proteins that we have identified are the translational activators previously defined by genetic analysis in *C. reinhardtii*. The cloning and characterization of the RNA binding protein 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 29425-2501

187. Molecular Mechanisms Controlling Proton Pumping by Bacteriorhodopsin
R.K. Crouch, Associate Provost for Research and Dean, College of Graduate Studies $125,000

Bacteriorhodopsin is a small protein of the purple membrane of *Halobacterium salinarium* which on the absorption of light undergoes conformational changes resulting in the transport of protons across the cell membrane. The protein contains the aldehyde form of vitamin A, retinal, as its chromophore. This system is particularly interesting as the protons transferred can be used to make ATP.

The molecular mechanism of this proton transport is being explored by the combined use of mutants of bacteriorhodopsin and analogues of retinal. The amino acids to be mutated have been selected so as to test various aspects of the chromophore protein interaction. The

analogues have been chosen to likewise examine a specific interaction of the protein with its chromophore. By combining these two approaches, we propose to understand molecular mechanism which underlie the light driven movement of protons through bacteriorhodopsin and to use this understanding to control the proton movement.

Southern Illinois University
Carbondale, IL 62901

188. Regulation of Alcohol Fermentation by Escherichia coli
D.P. Clark, Department of Microbiology $187,986 (FY 94 funds/2 years)

The purpose of this project is to elucidate the way in which the synthesis of ethanol and related fermentation products are regulated in the facultative anaerobe *Escherichia coli*. In particular, we are investigating the roles of certain genes which are induced during fermentative growth. Structural and regulatory mutations affecting the expression of the fermentative alcohol dehydrogenase have been isolated. The *adhE* structural gene has been sequenced and the upstream sequences responsible for anaerobic induction have been partially characterized by means of gene fusions. The build up of reduced NADH during anaerobic conditions appears to play the major role in regulating the *adhE* gene. The regulation of *adhE* depends on *adhR* and we are presently attempting to clone this regulatory gene. The protein encoded by the *adhE* gene expresses both alcohol and acetaldehyde dehydrogenase activities. In addition it acts as the deactivase for pyruvate formate lyase and is thus a trifunctional enzyme. The N-terminal region is homologous to other aldehyde dehydrogenases and the C-terminus to the family of Fe activated alcohol dehydrogenases. The *adhE* gene of *Salmonella* has also been sequenced and although clearly homologous to that of *E. coli*, shows significant differences. Differences in kinetic behavior of the ADH activities are being investigated. The *Salmonella* enzyme has a broader substrate range and can use alcohols of six or more carbons in length, whereas the *E. coli* enzyme only works up to C4. The *ldhA* gene, encoding the fermentative lactate dehydrogenase has also been cloned and sequenced. We have also sequenced and subcloned the regulatory region and are constructing gene fusions between *ldh* and the *cat* and *lacZ* genes. The mechanism of ldhA induction in response to a acidic conditions will be investigated using these fusions. In strains deleted for both the *ldhA* and *pta* genes, neither lactic nor acetic acid can be produced anaerobically. Under these conditions we have found a novel fermentation product in such double mutant strains, and are presently characterizing this by NMR.

Stanford University
Stanford, CA 94305-5020

189. Nodulation Genes and Factors in Rhizobium-Legume Symbiosis
S.R. Long, Department of Biological Sciences $232,463

Our work concerns the nitrogen fixing symbiosis of *Rhizobium meliloti* and its legume host, alfalfa. Using biochemistry and genetics, we are studying signals important in early stages of the bacteria-plant interaction. This work may reveal limiting factors for establishment of symbiosis, which in turn may be useful in improving or extending symbiotic nitrogen fixation and eventually decreasing the use of energy intensive chemical nitrogen fertilizers. We are studying the bacterial signals, called Nod factors, that cause the plant host to form symbiotic root nodules, and are examining the initial period of the host response. Our work on the signals includes optimizing preparative procedures, and trying to determine biosynthetic pathways. Nod factors are modified short oligomers of N-acetyl glucosamine, with an N-acyl substitution on the non-reducing end residue and a C-6-sulfate substitution at the reducing end residue. We developed an affinity bead method for isolation of these morphogenic Nod factors from bacterial culture supernatants. We have established that some nod genes encode enzymes that synthesize Nod factor. We have recently sequenced the second of these genes (nodP_2Q_2) and cloned the third (*saa*) and a possible fourth. We have also shown *in vitro* that the NodH enzyme catalyzes transfer of sulfates to the Nod factor, and that this requires a dimeric or larger N-acetylglucosamine-containing molecule as an acceptor. We have established other *in vitro* reactions for analyzing the pathway of Nod factor synthesis. Some of the enzymes discovered in this work may have uses in industrial processes for sulfation of novel molecules. We are studying the early responses of the alfalfa host plant to Nod factors. In particular, we are examining the root hairs of plants by electrophysiology and dye and protein injection. We are currently attempting to modify these techniques to extend the length of time during which we can observe live root hair reactions.

Stanford University
Stanford, CA 94305

190. Biochemical and Molecular Characterization of Enzymes for Cell Wall Synthesis
P.M. Ray, Department of Biological Sciences $197,880 (FY 94 funds/2 years)

Our laboratory is attempting to identify polypeptides responsible for enzymatic synthesis of cell wall polysaccharides (sugar polymers), which are the source of most of the renewable fuels available to mankind. We have targeted two such enzymes: plasma membrane-associated glucan synthase-II (GS-II) and Golgi-associated GS-I. By a complex purification procedure, together with direct photolabeling and antibody evidence, we can associate GS-II activity of pea seedlings with 2 polypeptides of 55 and 70 kDa. Separately,

by affinity chromatography we have isolated in pure form a polypeptide doublet of about 40 kDa which various evidence suggests is part of the Golgi polysaccharide synthase system, which forms cell wall matrix (i.e., non-cellulosic) polysaccharides. Our overall goal is to demonstrate whether these are in fact polysaccharide synthase components and, if so, to get information on how they act and are regulated.

By immunoscreening a pea cDNA expression library using antibodies against each of these polypeptide bands we have cloned genes corresponding to the 55-kDa, 70-kDa and 40-kDa polypeptides, and have obtained sequences for all of them. The 40-kDa sequence is unique. By immunocytochemistry we have shown that the 40-kDa polypeptide is specifically and dramatically localized to the trans part of pea (and also cotton) Golgi dictyosomes, and does not occur significantly in ER, plasma membrane, or other membranous organelles. Since the trans region has been identified as the site of xyloglucan synthesis, this finding strengthens the indications that the 40 is involved in polysaccharide synthesis.

Antibodies against the 55- and 70-kDa bands each selected two different gene sequences, so each of these bands evidently contains 2 polypeptides of very similar molecular mass. One of these sequences, in the case of both the 55- and the 70-kDa bands, proved closely similar to the tonoplast ATPase (V-ATPase) subunit of similar molecular mass, which was surprising because the antigens were obtained from purified plasma membranes. The other sequence, in the case of the 70, proved to be that for calnexin, a calcium-binding protein whose gene Delmer has also recently obtained in trying to clone a GS-II gene from cotton. In the case of the 55, the second clone has an entirely unique sequence. To evaluate the status of these four polypeptides we selected IgGs directed specifically at each of them by using protein expressed (in coli) from each of the clones to immunoadsorb polypeptide-specific IgGs from our antisera. By immunocytochemistry we found that IgGs specific for either the 55- or the 70-kDa V-ATPase-like polypeptides recognized both the tonoplast and the plasma membrane, showing that the presence of the V-ATPase-like polypeptides in our preparations is not necessarily due to contamination of our plasma membrane material with tonoplast fragments. We found that all four polypeptide-specific IgGs recognize the appropriate MW band on Western blots derived from a GS-II preparation that had been highly purified by product entrapment. Therefore, all of these polypeptides may be part of a GS-II complex.

Because of a report that the yeast glucan synthase analogous to GS-II involves a polypeptide of about 220 kDa, a size large enough to have been excluded from the gels that we used to identify the 55- and 70-kDa GS-II components, we ran lower acrylamide concentration gels and found that a previously unrecognized 220 kDa polypeptide correlates closely with enzyme activity when solubilized GS-II is fractionated by density gradient centrifugation. We shall soon determine whether the antibody that we have now raised against this polypeptide will adsorb GS-II activity and, if so, whether it will pull out an entire GS-II complex containing some or all of the previously discussed, presumptive GS-II components. We are now screening to obtain a genetic clone for the 220 kDa polypeptide.

University of Tennessee
Knoxville, TN 37996-0845

191. Plant Recognition of Bradyrhizobium japonicum Nod factors
G. Stacey, Department of Microbiology $140,000 (18 months)

We are studying the nitrogen-fixing symbiosis between *Bradyrhizobium japonicum* and soybean. *B. japonicum* infects soybean roots and induces the formation of a nodule, a new organ, in which the bacterium resides. Organogenesis of the nodule is induced by substituted lipo-chitin molecules synthesized by the products of the bacterial nodulation genes. Our past work has focused on characterizing the lipo-chitin signals produced by *B. japonicum* and characterizing the synthesis of these molecules. More recently, we have turned our attention to characterizing the response of soybean roots to these molecules. We have been aided in this work by the availability of 14 chemically synthesized analogues of the naturally produced lipo-chitin signals. As a bioassay, we examined the expression of early nodulin genes, plant genes rapidly and specifically expressed by rhizobial infection. This work has shown that plant recognition is a two step process, with only the latter step showing structural specificity. An unexpected and exciting discovery is that more than one lipo-chitin molecule is required to induce some of the early nodulin genes. These results suggest an unexpected complexity in the recognition of these signal molecules by the plant. Our future work will focus on investigating the molecular details of these recognition events. Our eventual goal is to elucidate the complete signal pathway involved in the soybean nodulation response. Detailed knowledge of legume symbioses is important for possible extension of biological nitrogen fixation for energy conservation.

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

192. Role of Glycolytic Intermediates in Global Regulation and Signal Transduction
J.C. Liao, Department of Chemical Engineering $101,000

The goal of this project is to determine the role of glycolytic intermediates, particularly C3 phosphates and fructose 1,6 di-phosphate, in regulation of cell physiology. It is known that many glycolytic intermediates are involved in regulation of enzyme activities at the kinetic level. However, little is known regarding the role of these metabolites in global regulation and signal transduction. Our recent data suggest that phosphoenolpyruvate (PEP), or other glycolytic intermediates, are involved in regulation of sugar uptake, nitrogen assimilation, and growth at high temperatures. We are investigating the nature of these effects and their underlying mechanisms. In particular, we will concentrate on the effects of these metabolites on (i) the regulation of phosphotransferase system (PTS) activity, (ii) transcription of glnAP2, and (iii) growth rate control at high temperatures. In addition to elucidating the details of

these regulatory mechanisms, we wish to identify the role of metabolites in these systems, and to determine if these metabolites serve as intracellular signals to coordinate carbon, nitrogen metabolism and cell growth.

Texas A&M University
College Station, TX 77843-2118

193. Regulation of Chloroplast Number and DNA Synthesis in Higher Plants
J.E. Mullet, Department of Biochemistry and Biophysics $82,000

The long term goal of this research is to understand how chloroplast biogenesis is regulated in higher plants. This is important because leaf and chloroplast development are primary determinants of a plant's photosynthetic capacity. Research will focus on early steps biogenesis including the activation of plastid DNA synthesis and plastid replication. In most plants, leaf mesophyll cells contain over 50 chloroplasts each with 100-300 copies of the plastid genome. Mesophyll cells are derived from meristematic cells of the shoot apex which contain 10-20 small, non-photosynthetic proplastids. The development of mesophyll cells and chloroplasts involves an increase in plastid number per cell, accumulation of DNA, RNA and the photosynthetic apparatus. An early step in this process is the activation of plastid DNA synthesis and plastid replication. Activation of plastid DNA synthesis will be investigated by isolating a plastid DNA polymerase and characterizing the gene(s) which encode its subunits. Further information will be obtained through analysis of mutants which show altered DNA copy number per plastid. Plastid replication will be investigated through analysis of a mutant that contains giant chloroplasts. These studies will provide information about the *cis*- and *trans*-factors and signal transduction pathways that regulate early steps in chloroplast development.

Texas Tech University
Lubbock, TX 79409

194. A Molecular Genetic Approach to Understanding Cellulose Biogenesis
R.L. Blanton, Department of Biological Sciences $63,072

Cellulose biogenesis is the process by which cells control the polymerization of glucose into a polymer of (1→4)-β-linked glucan polymer and direct its crystallization into microfibrils of cellulose. Virtually nothing is known about the biochemistry of this process in eukaryotes; none of the relevant proteins or genes associated with the process has been identified. It is likely that cellulose synthesis in eukaryotes will be associated with mechanisms that are unique to them, such as the cytoskeleton, protein targeting and processing, and signal transduction pathways. Therefore, eukaryotic model organisms for the study of cellulose synthesis are essential to a full understanding of its cellular control. In this project, we use the cellular slime mold *Dictyostelium discoideum* to study eukaryotic cellulose synthesis. It

is used as a model to study a number of basic cellular processes, because it is amenable to laboratory cultivation and manipulation, it is haploid and has a relatively small genome size, and it has great potential for molecular genetic analysis, including DNA-mediated transformation, targeted gene disruption, and tagged insertional mutagenesis.

We have adopted two approaches for the identification of genes whose products are involved in cellulose synthesis: (1) we have used a method of tagged insertional mutagenesis to randomly disrupt *Dictyostelium* genes and have been screening for mutants that are altered in cellulose synthesis; and (2) we have been using a culture system in which cellulose synthesis is synchronously induced to pursue a substractive cDNA approach to identify cDNA clones that are specific to the cellulose-synthesizing cell population.

Texas Tech University
Lubbock, TX 79409

195. Ferredoxin-Linked Chloroplast Enzymes
D.B. Knaff, Department of Chemistry & Biochemistry $89,000

Oxidation reduction titrations have been performed on spinach thioredoxins *f* and *m* and on spinach ferredoxin:thioredoxin reductase (FTR). Identical E_m values of -210 mV (n=2) were found for the active site disulfide/dithiol couples of the two thioredoxins. E_m values of -230 mV (n=2) and +340 mV (n=1) were measured for the active site disulfide and the [4Fe-4S] cluster of FTR, respectively. Alkylation of a cysteine at the active site of FTR shifts the E_m value for the [4Fe-4S] cluster to +380 mV and alters the absorbance spectrum of the cluster, suggesting that the two prosthetic groups on the enzyme interact. Chemical modification studies using *N*-bromosuccinimide point to the participation of a single tryptophan in electron transfer from ferredoxin to the FAD prosthetic group of ferredoxin:NADP' oxidoreductase. Evidence has been obtained for the ability of bean sprout extracts to catalyze the ferredoxin-dependent carboxylation of acetyl-CoA to form pyruvate.

University of Texas
Austin, TX 78713-7640

196. Molecular, Genetic, and Biochemical Analysis of Cellulose Synthesis in Arabidopsis thaliana
R.M. Brown, Jr., and K. Sathasivan, Department of Botany
$166,840 (FY 94 funds/2 years)

Membrane proteins from actively growing *Arabidopsis* were solubilized with increasing concentrations of digitonin and fractionated under native gel electrophoresis. Activities of native protein complexes in cellulose synthesis were visualized by incubation with

UDP-glucose and activators, followed by staining with Tinopal. The products, two broad bands, correspond well with the protein bands revealed in the same gel with Commassie Blue. We developed a novel technique to remove the glucan products from the bands for TEM observations. β-1,3 and β-1,4 glucans were distinguished by CBH I-gold labeling. We are denaturing proteins from native bands with SDS to determine which polypeptides are associated with the products, to determine molecular weights, and to secure purified polypeptides for micro-sequencing and antibody preparation. We have developed a rapid assay procedure based on acetic-nitric acid treatment, using a microtiter plate to screen T-DNA tagged *Arabidopsis* mutants with altered cellulose content. Using this technique, we have identified putative mutants with reduced and increased cellulose content. These putative mutants currently are being characterized. We have designed degenerate primers based on the conserved sequences in bacterial cellulose synthases and related proteins. We are utilizing these primers to perform RT-PCR of *Arabidopsis* RNA. Products will be cloned and sequenced to identify the genes coding for cellulose synthase. Biochemical studies accompanied by cloning and characterizing the *Arabidopsis* genes involved in cellulose biosynthesis should facilitate cloning similar genes from economically important plants (cotton, trees) leading to crop improvement and better energy utilization for the future.

Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

197. Enzymology of Acetone-Butanol-Isopropanol Formation

J.-S. Chen, Department of Biochemistry and Anaerobic Microbiology

$175,807 (FY 94 funds/2 years)

Several species of *Clostridium* produce acetone, butanol, and isopropanol, which are important solvents as well as chemical feedstocks and fuel additives. The goal of the project is to understand the fundamental properties of the solvent-forming fermentation so that it will be possible to prevent loss of useful traits, to gain positive control of the solvent-forming switch, and to regulate the product ratio. Our approach is to determine the molecular properties of the solvent-forming enzymes and to genetically distinguish the solvent-producing clostridia. The biochemical and taxonomic information will lead to the development of strategies for the regulation of solvent fermentation. Based on DNA sequence similarities, we have determined that many cultures presently labelled as *Clostridium acetobutylicum* are actually *Clostridium beijerinckii* and two other species, with *C. beijerinckii* containing more distinct strains than the other three species combined. This finding explains the contradictory results reported by different investigators. We have purified and characterized most key enzymes for solvent formation by *C. beijerinckii*, a species that can produce isopropanol in addition to acetone and butanol. Solvent-producing clostridia have a multiplicity of alcohol dehydrogenase (ADH), and the content of ADH has not been defined in either *C. acetobutylicum* or *C. beijerinckii*. Our present study has an emphasis on the primary ADHs of *C. beijerinckii*. The secondary ADH activity (reducing acetone to isopropanol) of the primary/secondary ADH of *C. beijerinckii* NRRL B593 is not widespread. The structural gene

for this ADH has been transferred into and expressed in *C. acetobutylicum* ATCC 824 and *C. beijerinckii* NRRL B592. This gene should be useful as a reporter gene for the study of regulation of gene expression in these clostridia.

University of Virginia
Charlottesville, VA 22901

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

M.P. Timko, Department of Biology

$196,540 (FY 94 & FY 95 funds/2 years)

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

Washington State University
Pullman, WA 99164-6340

199. Membrane Function in Lipid Mutants of Arabidopsis
J. Browse, Institute of Biological Chemistry $265,490 (18 months)

In the three years of this award, we have completed investigations of wild-type and mutant *Arabidopsis* that have provided us with a more complete understanding of the enzymology and regulation of lipid metabolism in higher plants. More importantly, our isolation and characterization of *Arabidopsis* mutants laid the ground work for important discoveries about the role of membrane lipids in the cell biology and physiology of plants. For example, we generated a triple mutant line, *fad3 fad7 fad8* that lacks 16:3 and 18:3 fatty acids. 16:3 and 18:3 are major components (>65%) of chloroplast membranes and we expected photosynthesis to be severely compromised in the triple mutant. Surprisingly this is not the case; triple mutant plants are healthy and normal in vegetative growth and development. The totally unanticipated consequence of the lack of 18:3 and 16:3 is the fact that triple mutant plants are male sterile. Our investigations have now revealed that a signaling compound derived from 18:3, jasmonic acid controls several essential functions in pollen development.

Washington State University
Pullman, WA 99164-6340

200. Regulation of Terpene Metabolism
R. Croteau, Institute of Biological Chemistry $159,538

Terpenoid oils, resins and waxes from plants are important renewable resources with a range of pharmaceutical, food and industrial uses. The objective of this project is to understand the regulation of terpenoid production using (+)-camphor metabolism in sage and (-)-methone metabolism in mint as model systems. The pathways of biosynthesis and catabolism have been established, and the relevant enzymes have been isolated and characterized. Developmental studies relating enzyme activity levels to terpene production within, and loss from, the secretory gland sites of metabolism suggest that terpene yield is largely controlled by the balance between biosynthetic and catabolic capacity. Antibodies directed against and cDNAs coding for enzymes presumed to represent rate-limiting metabolic steps have been prepared. These tools are being employed to determine the locations and temporal levels of these key enzymes, and to examine control of metabolism at the gene level, within an overall organizational framework provided by electron microscopic evaluation of the secretory cells. A method for the isolation of viable oil glands has been combined with a radio-HPLC protocol for metabolic separation and quantification to determine flux through the early sections of the pathway and to measure intracellular concentrations of key intermediates. These studies are revealing the controls on precursor supply and distribution to the different subcellular sites of terpenoid metabolism. Results from this project will target regulatory steps

for genetic engineering to increase the yields and types of terpenoid natural products that can be made available for industrial exploitation.

Washington State University
Pullman, WA 99164

201. Carbon Metabolism in Symbiotic Nitrogen Fixation
M.L. Kahn, Institute of Biological Chemistry $112,974

Combined nitrogen is the nutrient that most often limits plant growth. For this reason, nitrogen fertilizer is an important input for increasing crop yield. However, producing nitrogen fertilizer is expensive and energy intensive and excess fertilizer can increase nitrate in groundwater and soil acidity. Some plants are able to obtain nitrogen through symbiotic associations with nitrogen-fixing bacteria. The plant exchanges photosynthetically derived carbon compounds for ammonia produced by the bacteria. Enzymes of the plant and bacterial tricarboxylic acid (TCA) cycles are at the center of this exchange, generating energy, reductant and biosynthetic intermediates from the catabolism of photosynthate. We are investigating the genetics of symbiotic carbon metabolism in both Rhizobium meliloti and its host, 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 *R. meliloti* TCA cycle mutants, including those with defects in citrate synthase, isocitrate dehydrogenase and oxoglutarate dehydrogenase, and are developing conditional TCA cycle mutants to probe the relationship between the TCA cycle and nodule development and metabolism. We are also investigating the molecular genetics of plant NADP-isocitrate dehydrogenases by studying several cDNAs we have cloned that code for various forms of the enzyme and studying the enzymes themselves using biochemical and immunological methods.

Washington State University
Pullman, WA 99164-6340

202. A Comprehensive Approach to the Elucidation of Lignification at the Plasma Membrane/Cell Wall Interface
N.G. Lewis, Institute of Biological Chemistry
$404,078 (FY 94 & FY 95 funds/2 years)

Although vascular plants provide the bulk of the world's energy, food (including dietary fiber), apparel, shelter and furnishings, the means by which plants organize and assemble their biopolymers into cell walls is poorly understood.

We are examining how phenolic coupling processes are catalysed, regulated and controlled in the assembly of lignified secondary walls of plants, since these are essential for growth, development, structural support, quality and texture (e.g. of wood) and disease resistance. We are currently defining the regioselectivity of phenolic coupling processes (catalysed by specific copper-containing oxidases, laccases and peroxidases) in order to establish definitively how the native lignin polymer is assembled *in vivo*. An integral component in lignin deposition also involves H_20_2 generation, and we have established that its destruction disables lignin synthesis proper while retaining the capacity for monolignol formation. These studies are now enabling us, for the first time, to establish the precise steps involved in lignifying cell wall assemblies *in vivo*, and how the pathway is regulated. Another facet of our studies is directed to interfacing phenylpropanoid metabolism with nitrogen metabolism, since the latter is potentially a rate-limiting step in lignifying tissues. A tightly regulated nitrogen cycle linking phenylalanine, ammonia release, glutamine synthase and GOGAT activities enables the facile regeneration of arogenic acid and hence phenylalanine synthesis.

Washington State University
Pullman, WA 99164-6340

203. Interdisciplinary Plant Biochemistry Research and Training Center
N.G. Lewis, Institute of Biological Chemistry $407,000 (FY 94 funds/2 years)

Washington State University has established a Biochemistry Research and Training Center which interfaces its biochemistry program with that of the major plant sciences. The Center has three areas of strategic importance targeted: plant cell wall formation, regulation of biochemical pathways and signal transduction mechanisms. In this first year, the Center has provided research training opportunities for 19 undergraduate internships, 8 graduate students (Ph.D. and M.S. degrees), and four post-doctorals. The Center has established a NSF-USDA- DOE sponsored Plant Biochemistry Seminar Series which has attracted distinguished speakers nationwide, and also held the 1995 Plant Biochemistry Course at Pullman.

Washington State University
Pullman, WA 99164-6340

204. Enhancement of Photoassimilate Utilization by Manipulation of ADPglucose pyrophosphorylase Genes
T.W. Okita, Institute of Biological Chemistry $146,750

The goal of this project is to increase the conversion of photoassimilate into starch via manipulation of ADPglucose pyrophosphorylase (AGP), a key regulatory enzyme of starch biosynthesis. We have completed the molecular analysis of the expression of the AGP small

subunit (sAGP) gene during potato development. Unlike other plants that possess multiple sAGP genes that are expressed in specific tissues, potato utilizes only a single sAGP gene. Based on results from our molecular studies, we conclude that distinct *cis*-regulatory sequences controls the spatial expression of the sAGP gene, that 3U flanking sequences also contain *cis*-regulatory elements which enhance, suppress, and alter cell-specific expression of the sAGP gene, and that non-identical cellular expression patterns of the sAGP and large subunit lAGP genes are responsible for the apparent post-transcriptional regulation of sAGP in leaves. Using an innovative mutagenesis-glgC complementation system we have identified many potential mutants that are defective in allosteric regulation and/or catalysis. One down-regulatory mutant has been extensively characterized and requires 45-fold greater levels of the activator, 3-phosphoglycerate, for maximal enzyme activity. This phenotype is due to a single point mutation resulting in the replacement of Pro52 by a Leu. The study of these AGP mutants will not only increase our knowledge on structure-function relationships of this enzyme but will lead to novel strategies of increasing starch production and, in turn, increasing overall productivity for many crop plants.

Washington University
St. Louis, MO 63130

205. Plant Cell Wall Architecture
J.E. Varner and Z.-H. Ye, Biology Department

$228,671 (FY 94 & FY 95 funds/2 years)

Our research focuses on the further elucidation of the biosynthesis of an important wall component--lignin. Although lignin is important for plant growth and development, it decreases digestibility in forage crops. An extraction of lignin during pulp and paper production leads to environmental pollution. Therefore, reduction and/or alteration of lignin composition could reduce the pollution from pulp and paper mills, and improve the digestibility of forage crops. Full understanding of the biosynthetic pathway of lignin will be desirable for the manipulation of lignin composition and content by genetic engineering. We are using the *Zinnia in vitro* tracheary element (TE) induction system to study the methylation pathways in lignin biosynthesis. The methylation pathway mediated by caffeic acid 3-*O*-methyltransferase (COMT) has been widely accepted as the only lignin methylation pathway in the last 20 years. We found that the induction of caffeoyl-coenzyme A 3-*O*-methyltransferase (CCoAOMT) gene is specifically associated with the process of lignification in the *in vitro* TEs induced from the isolated *Zinnia* mesophyll cells and in *Zinnia* organs. The association of the CCoAOMT with lignification is also demonstrated in a number of dicotyledonous plants. We propose that the CCoAOMT is involved in an alternative lignin methylation pathway in *Zinnia*, and that the CCoAOMT-mediated methylation pathway is likely a general one in lignin biosynthesis during normal growth and development in plants. Further comparative analysis showed that while the CCoAOMT is induced in all lignifying tissues, the COMT is preferentially accumulated in the lignifying fibers. We suggest that all the intermediates in the COMT-mediated methylation

pathway might become substrates for the CCoAOMT-mediated methylation pathway after coenzyme A ligation when these two pathways occur in the same lignifying cell types. Further study is currently underway to understand the regulation of lignin methylation pathways by the overexpression and antisense-inhibition of the *O*-methyltransferases and by the characterization of the hydroxylases involved in the pathways.

Wayne State University
Detroit, MI 48201

206. Site-directed Mutagenesis of an Energy Transducing Membrane Protein-Bacteriorhodopsin
R. Needleman, Department of Biochemistry $87,999

Our goal is the understand how the light activated proton pump bacteriorhodopsin (BR), transports protons. To achieve this we have constructed mutants in the gene coding for bacteriorhodopsin (bop) using an expression system in which mutant bacteriorhodopsins are synthesized in their natural host and not in *E. coli*. This method allows the rapid and facile production of large quantities of mutant proteins derived by site-directed mutagenesis. In a collaboration with Janos Lanyi (University of California at Irvine) we have used these mutant bacteriorhodopsins to evaluate the role of particular amino acids in proton translocation. During the past year we have concentrated on defining the domains involved in the uptake and release of the proton and on developing a model for the energy transducing step in the photocycle. The work has led to a better understanding of the roles of residues D96, T46 and R227 in the proton transfer reactions of the photocycle near the cytoplasmic surface, and in general the relationship between the reprotonation of the Schiff base and the subsequent proton uptake from the cytoplasmic side. In addition this work has allowed us to gain a better understanding of the factors that control the rates of the thermal interconversion of photointermediates, and has increased our ability to predict the phenotypes of genetically engineered bacteriorhodopsins. Our future work will try to use mutational analysis to correlate global conformational changes in bacteriorhodopsin with the photocycle. Ultimately we hope that this will provide a detailed molecular mechanism for proton transport.

University of Wisconsin
Madison, WI 53706

207. Regulation of Extracellular Polygalacturonase Production in Pseudomonas solanacearum
C. Allen, Department of Plant Pathology $68,996 (FY 94 funds/2 years)

Pseudomonas solanacearum causes bacterial wilt of many economically important crop plants including potato, banana, and peanut. The bacterium produces at least three extracellular

polygalacturonases (PGs) that contribute to plant pathogenesis. These enzymes degrade pectin, a major component of plant cells. The general objective of this research is to investigate the genetic and biochemical mechanisms regulating PG production. Bacterial PG activity is increased during growth in plant tissue, suggesting that a specific signal or condition in the plant host induces expression of the PG genes. Determining how host plants induce bacterial virulence genes is central to understanding the plant-pathogen interaction. To accomplish this, we have cloned a locus required for PG production that appears to encode a *trans*-acting positive regulator. Mutants in this locus (named *pehSR*) produce about 10% of wildtype levels of *endo*-PG and about 50% of wildtype level of *exo*-PG. They are substantially reduced in virulence. *pehR-gus* reporter gene fusions are expressed at tenfold higher levels when bacteria grow in plant tissue rather than in rich medium. The DNA sequence of *pehSR* has high homology to the 2-component regulatory family, a group of environmentally-responsive control elements found in many bacteria. Interestingly, a third ORF in the operon encodes a protein similar to AmpD, which regulates ampicillin resistance in enterobacteria. Expression of *pehSR* is repressed by *phcA*, a previously described global regulator of many virulence functions, since *pehSR* expression increases twelvefold in a *phcA* mutant background. Like *phcA* mutants, strains overexpressing *pehSR* overproduce PG and exhibit increased motility. These data suggest that regulation of PG and other virulence factors occurs through a regulatory cascade that responds to a plant signal.

University of Wisconsin
Madison, WI 53706-1381

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

A.B. Bleecker, Department of Botany $183,912 (FY 94 funds/2 years)

Signal transduction involves perception of a signal by a receptor molecule and transduction of the signal to downstream components of the pathway through a series of phosphorylation events. Membrane-associated receptors with extracellular, ligand-binding domains and intracellular kinase domains are found in both animals and plants. We are trying to discover the role of TMK1, a transmembrane receptor-like protein kinase in Arabidopsis that resembles animal growth receptors. The main approaches are analysis of antisense TMK1 transgenic Arabidopsis, immunolocalization of TMK1, biochemical characterization of phosphorylation domains and interaction cloning. Antisense plants with reduced levels of TMK1 protein are reduced in size compared to wild type plants suggesting TMK1 is involved in growth regulation. TMK1 protein has been localized to the L1 layer of vegetative apices of developing plants. TMK1 cross-phosphorylates when expressed from bacterial expression vectors. The serine/threonine-rich C-terminus is a substrate for phosphorylation, although truncated constructs lacking most of this region phosphorylate weakly. cDNAs that express weak interactors have been isolated in two-hybrid screens with both the leucine-rich region and kinase domains as bait. So far, no sequence identity for the cDNAs has been found.

TMK1 interacts with a 2A protein phosphatase (KAPP) isolated by Stone et al (U.M.-Columbia) in an interaction screen with RLK5, another receptor-like protein kinase. KAPP is a substrate for TMK1 in phosphorylation assays. Additional characterization of transgenic plants, including plants transformed with a TMK1 dominant-negative construct, and localization of TMK1 in a developmental series should contribute to our understanding of receptor-like protein kinases in plants.

University of Wisconsin
Madison, WI 53706

209. Molecular Genetics of Ligninase Expression
D. Cullen, Department of Bacteriology $105,000

In addition to playing a key role in the carbon cycle, lignin-degrading fungi have demonstrated potential in emerging technologies such as biomechanical pulping, bleaching and otherwise improving chemical and mechanical pulps, converting lignin to useful chemicals, effluent treatments, and remediation of contaminated soils. These processes involve mechanism(s) which are poorly understood, and this represents a barrier to further development. Long term objectives of this research are to elucidate the basic genetics and physiology of the degradation of lignin and related aromatic compounds.

Toward these goals, the structure, genomic organization and regulation of genes involved in lignin degradation are under investigation. Emphasis is on the genes encoding extracellular peroxidases and glyoxal oxidase of the white-rot fungus *Phanerochaete chrysosporium*. A variety of experimental techniques will be employed to construct detailed integrated maps. The role of glyoxal oxidase in lignin degradation will be determined by gene disruption techniques. Identification of specific transcripts in wood and soil samples will help to identify the key genes in the degradation of lignin and organopollutants. Efficient heterologous expression systems will be developed for the production of pure isozymes for biochemical investigations.

This research will contribute to understanding the mechanism(s) of the degradation of lignin and related aromatic compounds. These studies will also provide insight into lower eukaryote genome organization and genetic regulation. Development of commercial processes will be greatly facilitated by the identification of key genes and by the production of recombinant enzymes.

University of Wisconsin
Madison, WI 53706

210. Identification of the Primary Mechanism for Fungal Lignin Degradation
K.E. Hammel, Department of Bacteriology $145,694 (FY 94 funds/2 years)

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 hemoprotein lignin peroxidases (LiPs) that have the unusual ability to cleave the recalcitrant nonphenolic structures that predominate in lignin. LiPs have been shown to depolymerize lignin *in vitro*. 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 subvermispora*, nevertheless cleaves nonphenolic lignin structures efficiently. The results were obtained in experiments with newly designed 14C-labeled arylglycerol-2-aryl ether lignin model compounds that were covalently linked to high-molecular weight polyethylene glycol to mimic the polymeric nature of natural lignin. The data show that *C. subvermispora* cleaves these models between C1 and C2 of their propyl side chain, just as LiP-producing fungi do. Cultures that exhibit this activity produce no LiP, but do secrete manganese-dependent peroxidases (MnPs) that were previously thought not to cleave nonphenolic lignin structures. However, recent results from this project show that MnPs cleave nonphenolic lignin structures *in vitro* in a co-oxidative free radical reaction that requires unsaturated lipids. Unsaturated lipids or other peroxidizable fungal metabolites may thus act in concert with MnP to allow LiP-negative fungi to attack the most recalcitrant structures in lignin.

University of Wisconsin
Madison, WI 53706

211. Organization of the R Chromosome in Maize
J. Kermicle, Laboratory of Genetics $84,000

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

specific action, parental imprinting, and paramutation. Separate attention is being given to the pattern of recombination occurring when duplications and insertions are present.

University of Wisconsin
Madison, WI 53706-1569

212. The Biochemistry, Bioenergetics and Physiology of the CO-dependent Growth of Rhodospirillum rubrum

P.W. Ludden, Department of Biochemistry $94,000

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 Ni-Fe-S-containing enzyme that oxidizes CO to CO_2, and a CO-induced hydrogenase. The CODH contains a unique NiFeS cluster and the goals of this project are to determine the biosynthesis, structure and activities of the NiFeS cluster (called the "C cluster") and an FeS cluster (called the "B cluster") in CODH. The structure and activities of the C cluster and the B cluster will be probed by Mossbauer, EPR and UV-visible spectroscopy and will employ the normal enzyme, metal-substituted enzyme and mutant enzyme forms. CODH from *R. rubrum* can be isolated in a nickel-deficient, apo-CODH form and this apo-CODH can be activated in vitro with Ni^{2+}. Metal substituted forms of CODH can be prepared by inserting Zn^{2+}, Fe^{2+} or Co^{2+} into apo-CODH in place of Ni. Site specific mutations of the *cooS*-encoded CODH have been prepared. Mutations of cysteine and histidine residues suspected to serve as ligands to the C or B clusters have been targeted. Various forms of the enzyme will be tested for the ability to bind CO or its competitive inhibitor CN- and both Mossbauer and EPR spectroscopy will be applied to determine the metal atom of the C cluster that binds substrate. Mutant forms of CODH with defects in steps of the CODH-catalyzed CO oxidation will be studied to determine the status of the B and C clusters and the path of electrons through the enzyme. CODH has been crystallized and the crystal structure of CODH and its prosthetic groups will be determined. Three gene products involved in processing of Ni and synthesis of the novel NiFeS C cluster have been identified and designated the CooC, CooT, and CooJ proteins. CooJ has been purified and shown to bind Ni. During this grant period, CooC and CooT will also be purified, assays for their activities will be developed and their roles in processing Ni for the C cluster will be established. The results of these studies will have relevance for other Ni-dependent systems including the NiFe hydrogenases and ureases.

University of Wisconsin
Madison, WI 53706

213. Starch Synthesis in the Maize Endosperm as Affected by Starch-Synthesizing Mutants

O.E. Nelson, Department of Genetics $56,867

The *sugary 3* mutants (Stinard, MNL 66:4-5, 1992) have reduced debranching (alpha-1,6-glucosidase) activity in developing endosperms and produce a water-soluble polysaccharide (WSP) as a storage carbohydrate. This WSP differs from the WSP, phytoglycogen, present in *sugary 1* endosperms by having a lower lambda max (485) following reaction with a KI/I2 solution rather than the 510 typical of phytoglycogen. Developing *su1* endosperms have also been reported (Pan and Nelson, Plant Physiol. 74:324-28, 1984) to have reduced debranching activity. Of the three peaks of debranching activity present in nonmutant endosperm extracts, one is missing in the *su1* extracts while the other two are reduced. The *su3* extracts have full activity for the enzyme missing from *su1* while the other two are much reduced. Study of *su1; su3* double mutants should indicate whether these loci encode all the debranching enzyme activity present in developing endosperms and clarify their role in starch synthesis. The endosperm mutant that conditions the production of seeds with a pronounced dimple in the crown and that produces starch with some of the attributes of a chemically modified starch, which we had tentatively assigned to a new locus, *glt,* has proven to be an unusual hypomorphic allele (*-8132*) of the *bt2* locus. This raises the intriguing question of why a mutant allele at a locus whose product in concert with that of a second locus (*sh2*) catalyzes a step in the formation of a substrate for starch synthesis should affect the structure of the starch. The deep orange color that is present in some sublines of *bt2-8132* has resulted from transgressive segregation in a cross of the original accession of *bt2-8132* times W22N.

University of Wisconsin
Madison, WI 53706

214. Feedback Regulation of Photosynthetic Processes

T.D. Sharkey, Department of Botany $89,000

Photosynthesis can be limited by the leaf's capacity for starch and sucrose synthesis. The interactions between photosynthesis and sucrose synthesis are studied using biochemical and gas exchange analysis of both naturally occurring mutant plants and transgenic plants. We have devised a relatively simple test for whether end product synthesis has been altered. Because sucrose synthesis declines with temperature faster than photosynthesis, feedback is more likely to occur at low temperature. The temperature at which photosynthesis becomes insensitive to oxygen because of feedback (T0) is a very sensitive indicator of altered end product synthesis. We are going to study feedback effects on yield at moderately

low temperature. While T0 is always affected, often photosynthesis rates are very little affected. We are now measuring leaf level partitioning by feeding radioactive carbon dioxide to leaves. Alterations in end product synthesis alters partitioning, though not always in exactly the way that would be predicted from the genetic alteration. Potato plants expressing a maize SPS gene with no apparent change in the rate of photosynthesis was found to have consistently higher yields while a similar tomato plant had increased yields under some conditions but not all. Our working hypothesis is that the partitioning between starch and sucrose can affect signals regulating plant growth and development and that these signals, rather than effects on net photosynthesis, result in increased yields. We will next examine how feedback conditions alter the expression of genes involved in regulatory feedback mechanisms such as rubisco activase and SPS.

University of Wisconsin
Madison, WI 53706

215. Molecular Mechanism of Energy Transduction by Plant Membrane Proteins
M.R. Sussman, Department of Horticulture $205,640 (FY 94 funds/2 years)

Proton pumps are membrane-bound proteins that act as biological energy transducers. The plasma membrane of plants and fungi contains an ATP-coupled proton pump (H^+-ATPase) that converts chemical into electrical energy. This enzyme is essential for the growth of plants and fungi and provides the driving force used to catalyze the uptake and accumulation of solutes. The plant/fungal plasma membrane H^+-ATPase contains a single polypeptide of Mr=100,000. Its simple polypeptide structure makes this enzyme an attractive candidate for studying the molecular basis of energy transduction. A major aim of this project is to identify aspects of the enzymes' primary structure that are essential for converting chemical into electrical energy. DNA cloning and sequencing techniques are being used to obtain the complete animo acid sequence for ATPase structural genes present in *Arabidopsis thaliana*, a model higher plant with a small genome and a rapid generation time. Using PCR and low-stringency Southern hybridization, we have identified 12 distinct genes encoding P-type ATPases. The sequence of genomic and cDNA clones is being determined for each of these isoforms present in the nuclear genome. Expression of the genes is being studied using Northern blots and 'epitope-tagging' to produce isoform-specific polypeptide probes. These studies on cell-specific expression of the ATPase gene will help to delineate the developmental and environmental signals that regulate activity of the plasma membrane proton pump *in situ*. In addition, these studies provide data necessary for testing hypotheses concerning the biological role of ion pumps and the molecular mechanism of protein-mediated energy transduction in plants.

University of Wisconsin
Madison, WI 53706

216. Analysis of Structural Domains Required for Phytochrome Function by In Vitro Mutagenesis

R.D. Vierstra, Department of Horticulture $106,000

Phytochrome is a red/far-red photoreversible photoreceptor that has a central role in light-regulated plant development. In an effort to determine how phytochrome functions at the molecular level, we have exploited a biological assay for active chromoproteins that involves the expression of a chimeric phytochrome gene in transgenic tobacco. Such ectopic overexpression induces a striking "light exaggerated" phenotype which can be used as an *in vivo* assay of receptor function. The goal of the project is to combine this transgenic system with *in vitro* mutagenesis to identify phytochrome domains potentially important to synthesis, dimerization, chromophore attachment, Pr/Pfr phototransformation, Pfr-enhanced degradation, and biological activity. Preliminary mapping has localized several important domains in oat phytochrome A including: the minimal domains necessary for chromophore attachment (residues 70-399) and assembly of the holoprotein into a red/far-red light photoreversible photoreceptor (1-672); a site necessary for dimerization (919-1093); and two domains essential for full biological activity (7-69 and 1094-1129). Within the N-terminal domain 7-62, we have recently discovered two distinct domains, one required for the structural integrity of the chromoprotein and essential for function, and a second that modulates phytochrome activity such that its removal results in a hyperactive photoreceptor. Further refined mapping of both phytochromes A and B with respect to these biologically essential N- and C-terminal domains is in progress. Completion of this work will represent an important step in the identification of domains essential to the proper assembly and function of this essential photoreceptor and will hopefully lead to an understanding of its mode of action.

Worcester Foundation for Biomedical Research, Inc.
Shrewsbury, MA 01545

217. Novel Biomaterials: Genetically Engineered Pores

H. Bayley $176,000*

A collection of nanometer-scale pores is being constructed by remodeling α-hemolysin (αHL), a protein secreted by the bacterium *Staphylococcus aureus*. The single polypeptide chain of 293 amino acids forms heptameric pores in membrane ~11 Å in internal diameter. By analyzing the properties of mutant and chemically modified α-HLs, a working model for assembly has been devised. Monomeric αHL binds to lipid bilayers and aggregates to form a heptameric prepore complex. The open pore is formed when subunits in the complex undergo a cooperative conformational change, involving the central glycine-rich loop and the N-terminal segment of the polypeptide. The central loop lines a section of the transmembrane

channel in the fully assembled pore. These mechanistic findings 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 αHL to gain control over the opening and closing of the pores. The inputs that actuate the reengineered molecules can be biochemical in nature (activation by specific proteases), chemical (modulation of activity by covalent and non-covalent attachment of small molecules) and physical (activation by light). The new pores will now 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).

Xavier University of Louisiana
New Orleans, LA 70125

218. Molecular Characterization of Bacterial Respiration on Minerals
R. Blake II, College of Pharmacy $91,000

Aerobic respiration on reduced iron is a principal metabolic activity exhibited by certain chemolithotrophic bacteria that inhabit ore-bearing geological formations exposed to the atmosphere. Each phylogenetically distinct group of iron-oxidizing bacteria expresses one or more unique acid-stable, redox-active biomolecules in conspicuous quantities during aerobic respiration on iron. Structural and functional studies continue on two such novel biomolecules that have been purified to electrophoretic homogeneity, rusticyanin from *Thiobacillus ferrooxidans* and cytochrome$_{579}$ from *Leptospirillum ferrooxidans*. The aim of these studies is to determine the role of each protein in the iron respiratory chain of its respective organism. Other redox-active components present in cell-free extracts of iron-oxidizing organisms 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 adherence to insoluble metal sulfides. These studies exploit electrical impedance, surface tension, contact angle, surface area, and other measurements commonly employed to characterize colloidal particles. 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

219. Biochemistry of Dissimilatory Sulfur Oxidation
R. Blake II, College of Pharmacy $97,000

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 stoiciometries of these bacterial oxidation reactions. Our current focus has two major experimental goals: (1) to quantify the adherence of sulfur-oxidizing bacteria to their insoluble elemental substrate. These studies exploit field flow fractionation, laser Doppler velocimetry, electrical impedance, static and dynamic light scattering, and other measurements commonly employed to characterize colloidal particles; (2) to study the enzymology of polythionate degradation in the thiobacilli that accumulate polythionates in the culture medium. Trithionate hydrolase, thiosulfate dehydrogenase, and tetrathionate hydrolase will be identified in and purified from cell-free extracts of *Thiobacillus ferrooxidans, T. intermedius*, and *T. neapolitanus*. The project is expected to help define the actual enzymes and thereby the metabolic pathways of sulfur oxidation in different groups of the thiobacilli. It is anticipated that this information will be of value in the eventual manipulation of the thiobacilli and related organisms to benefit both the environment and the mining industry.

Yale University
New Haven, CT 06520-8104

220. Molecular Genetics of the R-r Complex of Maize
S. Dellaporta, Department of Biology $96,000

The *R-r* complex of maize is a cluster of genes on the long arm of chromosome 10. Each member of the complex encodes highly related transcription factors required for anthocyanin biosynthesis in seed or plant parts. This complex exhibits meiotic instability, with loss of seed or plant color capacity associated with unequal exchanges or other mechanisms not associated with crossing over. Past studies have focused on instability associated with unequal exchanges and the molecular organization of the complex. This year our efforts have been to understand the basis of meiotic mutations in the *R-p* gene of the *R-r* complex of maize. The majority of mutations in the *R-p* gene were shown to be insertions of a novel transposable element, termed PIF (P Instability Factor). PIF differs from other maize elements in that it shows a strong target site preference -- four out of five insertions were found in the exact same target site in the *R-p* gene. These studies complete our analysis of meiotic instability of the R complex. Our future studies will focus on the origins of *R* complexity by examining this region in races of maize and sister taxa.

Yale University
New Haven, CT 06511-7444

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

T. Nelson, Biology Department $200,000 (FY 93 funds/3 years)

The dicot genus *Flaveria (Asteraceae)* includes species utilizing C3, C4, and C3/C4 intermediate schemes of carbon fixation. In the efficient C4 scheme, neighboring photosynthetic bundle sheath (BS) and mesophyll (M) cells cooperate for carbon fixation. CO_2 is fixed initially in C4 acids in M cells, then further metabolized in BS cells, via a pathway that relies on expression of the genes for pathway enzymes in M- or BS-specific patterns. In less efficient C3 species, M cells independently fix CO_2 in a C3 compound, and the BS is not generally photosynthetic. C3/C4 intermediate species exhibit anatomical and biochemical characteristics between the C4 and C3 extremes and may represent evolutionary steps between C3 and C4 forms. In both C3 and C3/C4 species, C4 pathway genes are present, but used in different spatial patterns. The variety of *Flaveria* species provides an opportunity to compare the structure and regulation of C4 pathway genes in closely related C3 and C3/C4 species. We have isolated and characterized example genes encoding malic enzyme (ME), ribulose bisphosphate carboxylase (rbcS), and malate dehydrogenase (MDH) from C3 and C4 species of *Flaveria*. Our ongoing work includes the characterization of spatial and temporal expression patterns of these genes in C3, C4 and C3/C4 species and the expression of ME-, rbcS-, and MDH-reporter gene fusions in leaf cells of C3, C4 and C3/C4 species. Our work to date suggests that the observed cell-specific patterns are the consequence of both transcriptional and posttranscriptional regulation.

Yale University
New Haven, CT 06520-8103

222. Role of Phenolics in the Adaptation of Rhizobia to the Rhizosphere and Soil

D. Parke and L.N. Ornston, Department of Biology $136,520 (18 months)

Diverse phenolic compounds which originate from lignin and plant root exudates serve as sources of carbon and energy for the nitrogen-fixing plant symbiotic bacteria *Rhizobium* and *Bradyrhizobium*. The β-ketoadipate pathway, contributing to the dissimilation of phenolics, including some flavonoids, appears to be universally distributed in these bacterial genera. *Bradyrhizobium* species in particular appear to prosper at the center of aromatic acid action: they are nutritionally diverse when presented with phenolics, and, contrasting with inducible regulation in *Rhizobium*, most enzymes of the β-ketoadipate pathway are expressed constitutively in *Bradyrhizobium*. *Bradyrhizobium japonicum pca* genes encoding enzymes of this pathway will be cloned and mutant strains constructed in order to investigate the selective value of phenolic catabolism to rhizosphere competition and survival in the soil.

Further studies will explore the role of flavonoids and other phenolic compounds in rhizobial growth and symbiosis. Emphasis will be placed on the catabolism of particular flavonoids in rhizobia in an effort to elucidate dissimilatory pathways and their regulatory controls. Analysis using HPLC will be augmented by a plasmid construction that serves as an indicator for phenolic degradation via the β-ketoadipate pathway. This broad host range plasmid carries a *lacZ* fusion to a promoter that is activated by pathway catabolites in rhizobia.

Yale University
New Haven, CT 06510

223. Electroenzymology of Plant and Fungal Vacuoles
C.L. Slayman, Department of Cellular and Molecular Physiology $119,000

Cellular integrative mechanisms operating via plant and fungal vacuoles depend upon coordination of a variety of traffic-regulating proteins in the vacuolar membrane (tonoplast). Apart from signal-receptor and messenger proteins, there are three principal classes of transport-effector proteins: **i)** an ATP-dependent vacuolar proton pump (V-ATPase), which creates a standing electrochemical gradient for H^+ and thereby provides energy for many vacuolar concentrative processes; **ii)** numerous secondary transporters dependent upon either the tonoplast voltage or the vacuole → cytoplasm pH gradient; and **iii)** an array of *bona fide* ion channels which probably serve both for "on-demand" release of vacuolar reserve substances and for fine tuning of cytoplasmic composition. At the present time our work is focussed on one protein in category **iii**, a large (120 pS) and rather non-specific cation channel, designed **YVC1**, in the tonoplast of the yeast, *Saccharomyces cerevisiae*.

Following upon general functional characterization of the channel, and development of methods for purifying it in sufficient quantity for partial sequence determination, we are commencing to clone its structural gene via synthetic oligonucleotides and PCR amplification. Emerging studies on several plant tonoplast channels have suggested that they are closely related functionally to YVC1, so that cloning of the yeast channel gene might provide a handle on plant tonoplast channels as well. Detailed physiological studies of YVC1 are also being conducted, both to set the stage for structure-function analysis of the cloned channel, and to understand in detail its roles in vaculo-cytoplasmic integration. Aspects now of particular interest include *first*, identification of the true physiologic substrate--Ca^{++}? stored amino acids? polyamines? *second*, identification of its physiologic regulators, which certainly include Ca^{++}-calmodulin, and may include reduced pyridine nucleotides and/or glutathione; and *third*, careful examination of gating intervals over a wide scale of times (10^{-5} to 10^{2} sec), to determine whether the channel's behavior is truly Markovian or may be fractal. The latter outcome would have important physical implications for our understanding of channel behavior in general.

Parallel electrophysiological work is also being carried out on two plant cation channels, presumed to be from *plasma* membranes, which have been functionally expressed in *Saccharomyces*. These are the *Arabidopsis thaliana* channels KAT1p and AKT1p. They are abundant in plasma membranes of transformed yeast strains, which they rescue from K^+ deficiency in low-K^+ media. The two channel sequences are ~65% identical in primary amino acid sequence. Both channels are selective for K^+ over most other cations, but the selectivity spectra and inhibitor pharmacology of the two are quite different. The specific differences between these two structurally very similar proteins should provide a useful handle on factors determining specificity for many different kinds of ions which bind to these channel molecules.

Yale University
New Haven, CT 06520-8114

224. Transfer RNA Involvement in Chlorophyll Biosynthesis.
D. Söll, Department of Molecular Biophysics and Biochemistry $109,000

Chlorophyll is the major pigment in photosynthesis. One molecule of chlorophyll is synthesized from eight molecules of δ-aminolevulinic acid (ALA), the universal precursor of porphyrins. The initial metabolite for the C5 pathway is Glu-tRNA which in the presence of NADPH is converted by the action of an unusual enzyme, Glu-tRNA reductase (GluTR), to glutamate-1-semialdehyde (GSA) with the concomitant release of tRNA. Glu-tRNA is a dual-function molecule; it provides glutamate for protein synthesis and GSA for porphyrin synthesis. The regulation of the flow of Glu-tRNA into the different pathways may be determined by the relative concentrations of EF-Tu and GluTR, two proteins which compete in binding Glu-tRNA. In the second step of the pathway an aminotransferase (GSA-amino-1,2- mutase, GSA-AM) converts GSA to ALA.

To further examine ALA formation in plants, we have isolated genes of *Arabidopsis thaliana* that encode the enzymes of the C5 pathway, via functional complementation of mutations in the corresponding genes of *E. coli*. There are two genes for each of the two enzymes of the C5 pathway. The HEMA1 and HEMA2 gene products (GluTR enzymes) show 85% amino acid identity, but are expressed very differently. In contrast, the GSA1 and GSA2 genes are much more related. It is not clear at present, which role this set of genes plays in ALA synthesis. To understand the nature of the promoters in these genes, we constructed fusions of the GUS reporter gene with various deletions of the 5'-UTR. Preliminary results suggest that the promoter for HEMA1 and GSA genes resides within 1.5 kb from the 5'-end of the ATG.

Transgenic *Arabidopsis* plants which express antisense mRNA for these two enzymes were generated. These plants show varying degrees of chlorophyll deficiency, suggesting that the formation of ALA by HEMA and GSA genes is an obligatory step in the biosynthesis of chlorophyll.

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

Abs. 6 Chlorophyll-Binding and Biogenesis of Photosystem II
W.F.J. Vermaas, Arizona State University

Abs. 17 Molecular Bases of Photoadaptation in Unicellular, Eucaryotic Algae
P.G. Falkowski and J. LaRoche, Brookhaven National Laboratory

Abs. 18 Regulation of Energy Conversion in Photosynthesis
G. Hind, Brookhaven National Laboratory

Abs. 61 Molecular, Genetic and Physiological Analysis of Photoinhibition and Photosynthetic Performance
J.E. Boynton, N.W. Gillham and C.B. Osmond, Duke University

Abs. 80 Violaxanthin De-epoxidase: Biogenesis and Structure
H.Y. Yamamoto, University of Hawaii

Abs. 82 Photosynthesis in Intact Plants
A.R. Crofts, University of Illinois

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. 4 Phytoremediation of Metal-Polluted Soils: Mechanisms of Heavy Metal Absorption, Translocation, Accumulation and Tolerance in Plants
L.V. Kochian, USDA - Ithaca, NY

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. 33 Protein Translocation and Assembly in Chloroplasts
S.M. Theg, University of California - Davis

Abs. 34* Vacuole Biogenesis in Differentiating Plant Cells
T.A. Wilkins, University of California - Davis

Abs. 37 Molecular Structure, Function and Physiology of K^+ Uptake Channels in Plants
J.I. Schroeder, University of California

Abs. 42 Regulation of Vacuolar pH in *Citrus limon*
L. Taiz, University of California - Santa Cruz

Abs. 56 Effects of Freezing and Cold Acclimation on the Plasma Membrane of Isolated Cereal Protoplasts
P.L. Steponkus, Cornell University

Abs. 107 Identifying Calcium Channels and Porters in Plant Membranes
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. 172 Structure-Function Analysis of Vacuolar H^+-Pyrophosphatase
P.A. Rea, University of Pennsylvania

Abs. 180* Molecular Analysis of a Thylakoid K^+ Channel
G.A. Berkowitz, Rutgers University

3. PLANT METABOLISM AND BIOSYNTHESES

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.

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. 204* Enhancement of Photoassimilate Utilization by Manipulation of ADPglucose pyrophosphorylase Genes
T.W. Okita, Washington State University

Abs. 213 Starch Synthesis in the Maize Endosperm as Affected by Starch-Synthesizing Mutants
O.E. Nelson, University of Wisconsin

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 Characterization of Fatty Acid Desaturases and Related Lipid Modification Enzymes
J. Shanklin, Brookhaven National Laboratory

Abs. 43* Production of Lipophilic Materials from Plants
C.R. Somerville, Carnegie Institution of Washington

Abs. 98 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. 123 Control of Triacylglycerol Biosynthesis in Plants
J. Ohlrogge, Michigan State University

Abs. 124 A National Cooperative for Genetic Engineering of Plant Lipids
J. Ohlrogge, Michigan State University

Abs. 199 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 and J.P. Slovin, 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. 22 Molecular and Genetic Analysis of LEAFY, a Gene Controlling Floral Induction and Flower Development in *Arabidopsis thaliana*
E. Meyerowitz, California Institute of Technology

Abs. 23 The Molecular Genetics of Ligule Induction
M. Freeling, University of California - Berkeley

Abs. 26 Phytochrome from Green Plants: Assay, Purification and Characterization
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. 31 Regulation of Embryonic Development in Higher Plants
J.J. Harada, University of California - Davis

Abs. 32* Cellular and Molecular Characterization of Vascular Plasmodesmata
W.J. Lucas, University of California - Davis

Abs. 34* Vacuole Biogenesis in Differentiating Plant Cells
T.A. Wilkins, University of California - Davis

Abs. 36 Structure, Biosynthesis and Role of Complex Protein-Bound Glycans
M.J. Chrispeels, University of California - La Jolla

Abs. 39 The Gibberellin A_{20} 3ß-hydroxylase: Isolation of the Enzyme and Its Molecular Biology
B.O. Phinney and J. MacMillan, University of California - Los Angeles

Abs. 40 Sensory Transduction of the CO_2 Response of Guard Cells
E. Zeiger, University of California - Los Angeles

Abs. 208 Biochemical and Molecular Analysis of a Transmembrane Protein Kinase from *Arabidopsis thaliana*
A.B. Bleecker, 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. 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. 24 Regulation of Tomato Fruit Growth by MVA and GTP-Binding Proteins
W. Gruissem, University of California - Berkeley

Abs. 43* Production of Lipophilic Materials from Plants
C.R. Somerville, Carnegie Institution of Washington

Abs. 46 Role of HSP100 Proteins in Plant Stress Tolerance
S. Lindquist, University of Chicago

Abs. 49 The Suppression of Mutations Generated by *Mu* Transposons in Maize
R.A. Martienssen and V. Sundaresan, Cold Spring Harbor Laboratory

Abs. 73 Why Do Plants Have Two Pathways of Polyamine Synthesis?
R.L. Malmberg, University of Georgia

Abs. 74 The Determinants of RNA Turnover in Higher Plants
R.B. Meager, University of Georgia

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 ^{13}C 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. 50 Analysis of Cell Wall Properties in Polysaccharide Mutants of *Arabidopsis*
W.-D. Reiter, University of Connecticut

Abs. 66 CarbBank - A Structural and Bibliographic Database for Complex Carbohydrates
P. Albersheim and S. Doubet, University of Georgia

Abs. 67 The University of Georgia Complex Carbohydrate Research Center (CCRC)
P. Albersheim and A. Darvill, University of Georgia

Abs. 68 The Structures and Functions of Oligosaccharins
P. Albersheim, University of Georgia

Abs. 69 Structural Studies of Complex Carbohydrates of Plant Cell Walls
A. Darvill, University of Georgia

Abs. 70 Mechanisms of Lignin Biosynthesis During Xylogenesis in *Zinnia elegans*
K-E.L. Eriksson and J.F.D. Dean, University of Georgia

Abs. 132 Cellulose Synthesis and Morphogenesis
T.I. Baskin, University of Missouri

Abs. 144 Transcription Factors in Xylem Development
R. Sederoff, M. Campbell, R. Whetten and D. O'Malley, North Carolina State University

Abs. 152 The Molecular Characterization of the Lignin-Forming Peroxidase
L.M. Lagrimini, Ohio 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 econs, 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.

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. 201* Carbon Metabolism in Symbiotic Nitrogen Fixation
M.L. Kahn, Washington State University

Abs. 222 Role of Phenolics in the Adaptation of Rhizobia to the Rhizosphere and Soil
D. Parke and L.N. Ornston, Yale 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: Implications for the Evolution of Host Specific Traits for Pathogenicity
H.D. VanEtten, University of Arizona

Abs. 25 Determinants of Environmental Stress Tolerance by Bacteria on Leaves
S.E. Lindow, University of California - Berkeley

Abs. 27 Molecular Cloning and Characterization of the *Arabidopsis thaliana RPS2* Disease Resistance Locus
B.J. Staskawicz, University of California - Berkeley

Abs. 32* Cellular and Molecular Characterization of Vascular Plasmodesmata
W.J. Lucas, University of California - Davis

Abs. 44 Molecular Basis of Disease Resistance
S.C. Somerville, Carnegie Institution of Washington

Abs. 47 Signal Transduction in Plant Development: Chemical and Biochemical Approaches to Receptor Identification
D.G. Lynn, University of Chicago

Abs. 112 Molecular Biology of Plant-Bacterial Interactions
S.Y. He, Michigan State University DOE Plant Research Laboratory

Abs. 118 Biochemical and Molecular Aspects of Plant Pathogenesis
J.D. Walton, Michigan State University DOE Plant Research Laboratory

Abs. 140 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. 145 Novel Control of Signal Delivery from the *Pseudomonas syringae avrRPM1* Gene to *Arabidopsis thaliana*
J.L. Dangl, University of North Carolina

Abs. 179 Characterization of the Systemic Acquired Resistance Immediate-Early Response to Salicylic Acid
N.-H. Chua, Rockefeller University

Abs. 181 Respiration and Active Oxygen Species Production in Stressed Plants
I. Raskin, Rutgers University

Abs. 207 Regulation of Extracellular Polygalacturonase Production in *Pseudomonas solanacearum*
C. Allen, University of Wisconsin

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

Abs. 63 Ethanologenic Enzymes of *Zymomonas mobilis*
L.O. Ingram, University of Florida

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

Abs. 130 The Mechanism of Switching from an Acidogenic to a Butanol-Acetone Fermentation by *Clostridium acetobutylicum*
P. Rogers, University of Minnesota

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.

12. EXTREMOPHILIC MICROBES

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

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.

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.

INVESTIGATOR INDEX

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ER-17