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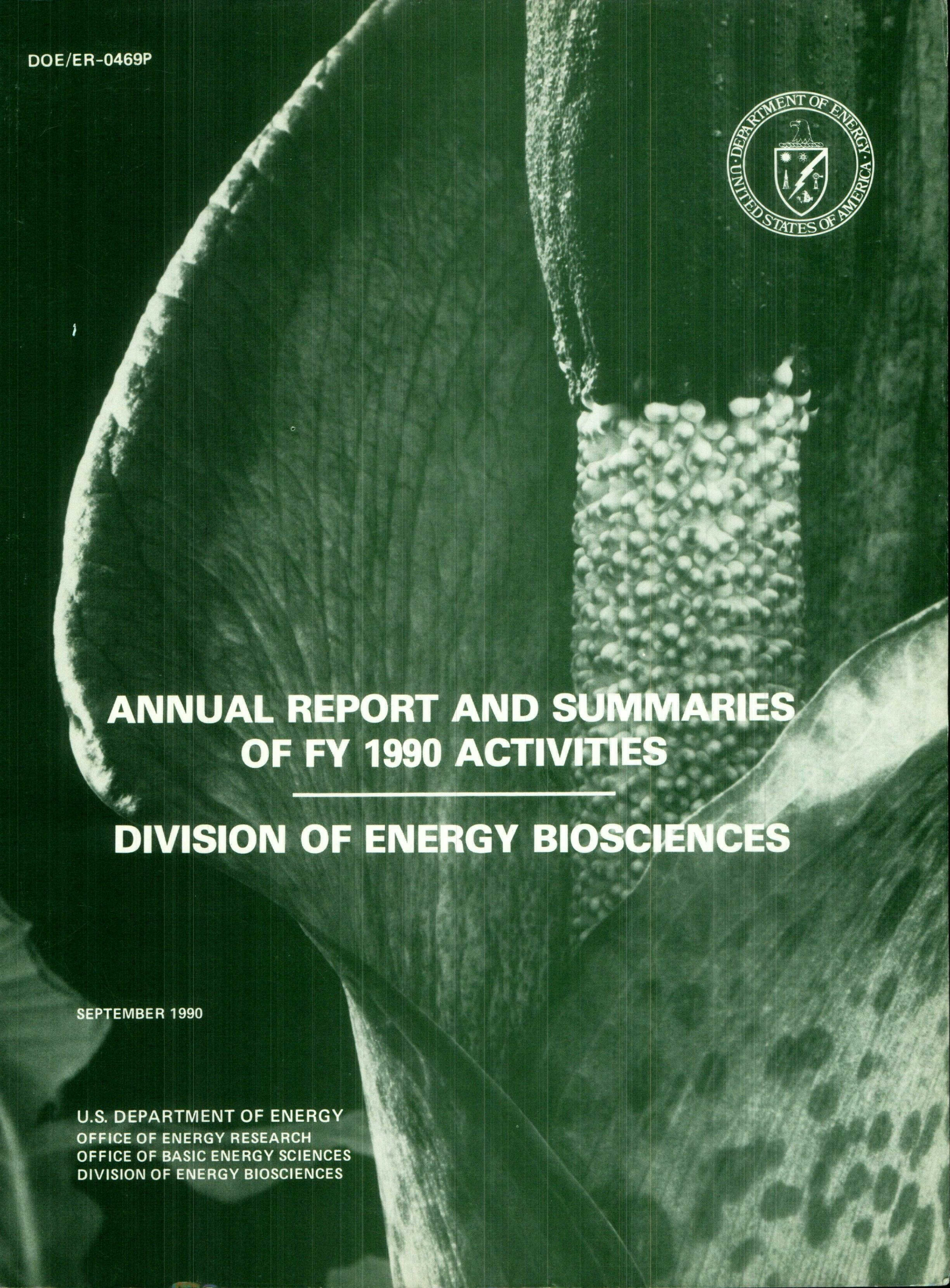

ANNUAL REPORT AND SUMMARIES OF FY 1990 ACTIVITIES

DIVISION OF ENERGY BIOSCIENCES

SEPTEMBER 1990

U.S. DEPARTMENT OF ENERGY
OFFICE OF ENERGY RESEARCH
OFFICE OF BASIC ENERGY SCIENCES
DIVISION OF ENERGY BIOSCIENCES

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Cover Picture: **(Photo courtesy of Kurt Stepnitz)**

The picture is a close-up view of the inflorescence of Amorphophallus, a plant of the Arum family. This group of plants has an unusually active alternative mitochondrial respiratory pathway which is not found in animal systems. Many plants contain this branched respiratory pathway in their mitochondria. The regulation of the partitioning of electron flow into the different pathways ultimately determines the energy balance of the plant.

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WASHINGTON, D.C. 20585

Program Overview of the Division of Energy Biosciences

Recent world events have strongly reaffirmed the necessity for constantly conserving energy and making available alternative energy sources. Biologically derived renewable resources have historically been extensively used throughout the world. However, if we are to take advantage of the powerful new biological technologies of genetic manipulation for improving the production and diversity of renewable energy resources as petroleum substitutes, a strong effort in fundamental research on plants and microorganisms is imperative.

The Energy Biosciences (EB) program encompasses research on the basic mechanisms of plants and microbes designed to provide the underpinning for future novel biotechnologies applicable to plant productivity, bioconversion processes and biology based energy conservation measures. The information base being garnered is also designed to gain understanding relevant to environmental problems. For example, an enhanced understanding of the capabilities of microorganisms to perform metabolic conversions may lead to new bioremediation procedures. Thus the body of data produced by EB program efforts has several critical impact areas. While the program is not involved with the near-term application of the information, the interactions and exchanges between EB basic researchers and users of the information is encouraged. Other units in the Department of Energy (DOE) and in industry are responsible for work on applications and process development.

The Energy Biosciences program, which is now a decade old, originated and grew amid probably the most exciting period of biological research. The new developments in genetic manipulation and the ways in which a wide array of new research technologies could be used has provided opportunities for rapidly obtaining remarkable insights into biological phenomena. The resolution of problems in genetic expression and regulation has been most impressive. In many instances the employment of new research technologies has allowed certain areas to surge ahead after years of frustrating difficulties. For example, major new findings about plant development are making investigators revise their thinking about development processes. Likewise, the cell cycle is now being defined in terms of molecular components and actions.

In the context of the Energy Biosciences scope there remains much that has been only lightly touched by molecular biological approaches. For example, the areas of *biological metabolic diversity and adaptability* are still wanting in the understanding of basic metabolic pathways, the enzymology involved and the respective regulatory mechanisms. The application of molecular biological approaches integrated with studies on the biochemistry and physiology in plants and microorganisms undoubtedly will facilitate the broad and deep understanding being sought. With adequate information available, the options for application to potential biotechnologies will become more obvious.

The Energy Biosciences program makes a deliberate effort to identify and emphasize important research topic areas that have been relatively neglected by the scientific community. Such areas sometimes are not populated heavily because of the time required for developing requisite approaches and techniques. This may entail slow progress with a certain degree of risk. The program is balanced with a modicum of projects of the type described above, plus numerous others that fall in better developed topic areas where approaches, methodologies and an active community make progress swift, and the competition oftentimes keen. Overall it is the intent to support innovative research that is conducted with contemporary tools and rigorous thinking.

There is continuing liaison and coordination with other Federal agencies, most notably, the National Science Foundation (NSF), the Department of Agriculture (USDA), and the National Institutes of Health (NIH) and others, as well as with other offices in DOE (Health and Environmental Research, Conservation and Renewable Energy, Fossil Energy). Cooperative activities such as the three agency (USDA, DOE, NSF) Plant Science Centers program and more recently the Arabidopsis genome studies program (NSF, USDA, NIH, DOE) represent levels of interaction based on the spirit of cooperation in achieving common objectives.

The scope of the Energy Biosciences program has been defined in broad terms as indicated below. The scope of topics presented below is representative of program interests:

A. The Plant Science areas:

1. **Bioenergetic Systems** in plants and microorganisms including photosynthesis, the major solar energy transformation process and other processes in which energy trapping or conservation is implicated.

2. **Plant Growth and Development Control.** This integrated array of processes is the key to how much of the solar energy trapped by the plant is converted ultimately into chemical forms. The mechanisms involved are perceived in biochemical and physiological terms as:

    a. **Genetic Regulation**: How the heritable material of plants controls the turning on and off of genes of metabolic pathways and other physiological processes.

    b. **Metabolic Capabilities and Regulation**: The elaboration of important metabolic pathways, the biochemical intermediates and the enzymes involved in pathways. The manner in which the metabolic pathways are regulated by environmental conditions, by developmental signals and by other inherent genetic and biochemical controls.

c. **Hormonal and Environmental Regulation**: The perception of external signals, e.g., light (duration, intensity and quality), chemical cues such as plant growth substances and other factors that affect the activities of cells and organs and ultimately the development of the whole plant.

3. **Stress Response Mechanisms**: The mechanisms by which plants respond and adapt to acute or long term exposure to natural suboptimal environmental conditions that influence solar energy conversion and the net yield of renewable resources.

4. **Genetic Transmission and Expression in Plants**: This knowledge is crucial for development of strategies for attaining new and useful plant genotypes that would be employed for enhancing renewable resource production both quantitatively and qualitatively.

5. **Plant-Microbial Interactions**: The mechanisms underlying pathogenesis and symbiosis may heavily influence plant productivity. Understanding the molecular basis of recognition in these systems is one specific objective.

6. **Plant Cell Wall Structure and Function**: An area that encompasses a comprehension of the chemical **structure** and **synthesis** of the most abundant resource of fixed carbon, including polysaccharides and lignin, as well as the emerging physiological roles of cell wall components in growth regulation, reactions to pathogens and other cell functions.

B. The **Microbiological** elements of the program include:

1. **Lignocellulosic Degradation**: Understanding the genetic and biochemical regulation of the complexes of polysaccharide and lignin degrading enzymes including coordinated synthesis and function of component enzymes.

2. **Fermentations**: The probing of the basic mechanisms of anaerobic bioconversion of renewables into acids, fuels and solvents. The metabolic pathways of conversion and their control are the subjects of this category.

3. **Genetics of Neglected Microorganisms**: This effort provides the background genetic information for future genetic improvement of microbes for which little or no information is available. In particular, emphasis is given to understanding and developing genetic transformation systems in fermentative organisms, ligno-cellulose degraders and those organisms involved in plant-microbe interactions.

4. **Energetics and Membrane Phenomena**: The adaptive changes to conditions of stress (e.g. high temperature, oxygen deprivation, acidity or alkalinity) in microorganisms in respect to cellular energy generation and membrane changes are the emphases.

5. **Metabolic Capabilities and Regulation**: In addition to anaerobic conversions in fermentations and methanogenesis, aerobic microbes have an enormous range of metabolic, synthetic and catabolic capabilities; key pathways require definition along with their regulatory mechanisms.

6. **Thermophily and Thermotolerance**: How microorganisms cope in molecular organization with elevated temperatures is the orientation in this area. This relates to the potential for using heat adapted organisms or their enzymes in future technologies.

7. **Microbial Ecology Associations**: Understanding the manner in which microbes act in concert during mixed culture fermentations and in other consortial activities is the objective of this research. The mechanistic basis of phenomenon such as interspecific hydrogen transfer and syntrophy are examples of what is being investigated.

8. **One and Two-Carbon Metabolism**: The mechanisms utilized when massive quantities of simple one and two-carbon molecules, such as carbon monoxide and carbon dioxide, acetate and others are microbiologically transformed in nature is the aim of this work. This includes methanogenesis and other significant transformations that yield potential fuels and other chemicals of interest.

Obviously the scope is unusually broad and reflects diversity of research needs and opportunities. A program with limited resources such as Energy Biosciences necessitates making difficult choices. The criteria upon which choices are based include: quality of science, innovativeness of ideas, needs within the research area, how much of the kind of work is already supported by this and other programs, relatedness to the energy mission as well as the potential for development within an area.

Some recent notable developments within the Energy Biosciences program include:

CarbBank

In recognition of the need for improved organization and access to structural information about carbohydrates, a new database has been developed at the University of Georgia Complex Carbohydrate Research Center. The database has been named CarbBank. A computer program that portrays structural details of carbohydrate molecules has been produced and with the aid of numerous curators in different countries, the data base is being built to include an up-to-date resource of structural information about carbohydrates. CarbBank is being designed to interface with other key structural databases including those covering nucleic acids and proteins. The effort has been a truly international one with a steering committee with representatives from laboratories working on carbohydrates from around the world. A recent version of CarbBank containing some 3000 structures was recently made available to the research community. Energy Biosciences has been the principal contributor to this effort with assistance from the National Institute of General Medical Sciences.

Degradation Mechanisms in Fungi

Phanerochaete, a white rot fungus now heavily studied for its ability to degrade lignin also has the capabilities of breaking down some important man-made contaminants, such as chlorophenols and polychlorinated biphenyls. The mechanism by which the fungus degrades the pollutant 2,4-dichlorophenol has been studied and partially elaborated at the Oregon Graduate Institute of Science and Technology. The breakdown of 2,4-dichlorophenol is initiated with an extracellular oxidation catalyzed by lignin peroxidase or manganese peroxidase. This is followed by the shuttling of the oxidized substrate into the cell where novel reduction and methylation reactions follow. The transformed substrate is then exported from the cell to be acted on again by extracellular peroxidases. Successive cycles of oxidation, reduction and methylation result in the removal of the chlorine atoms from the aromatic ring making the molecule increasingly susceptible to other subsequent degrading reactions which ultimately lead to complete breakdown to carbon dioxide.

Plant Cell Wall Proteins

The biochemical and physiological characteristics of plant cell walls is a major research topic in the Energy Biosciences program. The carbohydrates and lignins present in plant cell walls constitute the world's major renewable energy/chemical resource. The cell wall also contains an assortment of proteins with unusual composition. Proteins especially enriched with the amino acid, hydroxyproline, were described over twenty years ago. In recent years, a number of glycine rich proteins have been discovered in cell wall material in several laboratories. A current study undertaken by Dr. Derek Lamport of Michigan State University on a number of plant types has shown that cell wall proteins exist in all representatives of the plant kingdom examined. Two novel cell wall proteins, both rich in hydroxyproline with either threonine or histidine, indicate that there are variations on the compositional theme, but the theme is extant throughout the plant kingdom. The chemical characterization of these proteins when combined with a subcellular imaging technology developed by Dr. Joe Varner and colleagues of Washington University at St. Louis should provide a fuller understanding of the role these proteins play in the physiological processes of providing structural support, morphology and defense responses commonly associated with plant cell walls. As a result of these cumulative findings, other investigators in the research community have been stimulated to explore questions on the novel proteins of plant cell walls.

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

	Number of projects	FY 90 funding (in thousands)	Percent of total funds
University and Non-Profit Institutions	133	$ 12,131	59%
Michigan State University Plant Research Laboratory	15	2,619	13%
Plant Science Centers at Universities	2	1,264	6%
National Laboratories Brookhaven National Lab Lawrence Berkeley Lab Los Alamos National Lab	12	2,341	11%
Solar Energy Research Institute	1	123	1%
Oregon Graduate Institute of of Science and Technology	2	500	2%
Small Business Innovation Research (SBIR) contribution, and Miscellaneous		970	5%
Conferences, Educational Activities	6	599	2.5%
Databases	1	100	0.5%
	172	$ 20,647	

The total funding figure is a net figure after a Congressionally mandated general reduction and Gramm-Rudmann-Hollings deficit reduction action.

Several items in the budget including $0.5 million for the Oregon Graduate Institute of Science and Technology, Small Business Innovation Research (SBIR), and other items are mandated by Congress or by administrative direction (almost 10% of total), whereas the majority of the budget is discretionary. Over 80% of the budget is invested in university projects with the remainder of the funds going to national laboratories, non-academic labs, conference support and other miscellaneous items.

The discretionary budget for FY 1990 remained essentially the same as the previous year. As a result relatively few new proposals could be funded, but many highly meritorious proposals which had been received could not. Inasmuch as the number of proposals received for FY 90 consideration increased over thirty percent with the budget not changing significantly, this resulted in a declining success rate. To diminish the time, effort and trauma of proposal writing in FY 91, Energy Biosciences emphasized for proposal submissions a system using short preapplications or preproposals for preliminary consideration. Of the preproposals received only a fraction of the investigators were encouraged to submit new proposals. As an expedient, some major areas were excluded from consideration in this round of proposals because of anticipated budget restraints. It should be strongly emphasized that it is not the intent of the program management to be as restrictive in the future, on the assumption that pressures will ease.

In addition to its support of research programs, EB also provides a small fraction of its resources to meetings and educational activities. Included are conferences/workshops/studies for which the EB program provided support:

1. Summer Investigations into the Metabolic Diversity of the Microbial World, Marine Biological Laboratory, Woods Hole, MA, Summer 1990.

2. Eighth International Congress on Nitrogen Fixation, University of Tennessee, Knoxville, May 20-26, 1990.

3. Michigan State University DOE Plant Research Laboratory 25th Anniversary Symposium, May 25-26, 1990.

3. Examination of the Current Status of Plant Science Programs in the United States, National Academy of Sciences, 1990 (study co-funded with NSF and USDA).

4. Third International Congress of Plant Molecular Biology, University of Arizona, Tucson, AZ, October 6-11, 1991. (Planning and organizational activities)

The EB supported Life Sciences Research Foundation post-doctoral awardee for this year's three-year fellowship is Dr. Barbara Kunkel, who works in the laboratory of Dr. Brian Staskawicz at the University of California, Berkeley.

A Note of Appreciation

The Energy Biosciences program, like most others in the Federal research support system, is vitally dependent on individual scientists in the research community for the maintenance of quality and exciting science. At a time when extreme competitiveness for research funding is the rule, the dependence on the community for reviews has become even more important. Those of us in the position of managing research funds are most indebted to the scientists, both in this country and abroad, who have contributed their time, effort and scientific judgement in providing reviews of proposals and programs. One can only conjecture how our system would fare without such inputs. The assistance is very much appreciated.

Anyone wishing more information about the Energy Biosciences program is invited to write or call:

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1. Molecular Organization in the Native State of Woody Tissue: Studies of Tertiary Structure Using the Raman Microprobe, Solid State ^{13}C NMR and Biomimetic Tertiary Aggregates

R.H. Atalla, Forest Products Laboratory *$165,000 (FY90 funds/two years)*

Studies of wood cell wall structure using the Raman Microprobe have revealed variations in the composition and molecular orientation of cell wall constituents, both within individual cells and between adjacent cells. These observations have raised questions about the relationship between molecular and morphological levels of organization and about the intermolecular interactions which underlie such organization. Our objectives in this program are twofold: (1) to achieve further definition of the variation of molecular organization and its variation within and between tissue types; (2) to explore the classes of intermolecular interactions which determine the patterns of aggregation and organization of the constituents of the cell walls. The efforts to achieve greater definition of cell wall organization in the native state are based on extension of our prior work using the Raman Microprobe, complemented by more limited studies utilizing Solid State ^{13}C NMR. The exploration of intermolecular interactions which dominate the organization of the cell walls is currently centered on the development of biomimetic tertiary aggregates which simulate the states of aggregation which prevail in the cell walls. These are prepared by culturing cellulose producing bacteria in media which contain analogs of the other cell wall constituents. Preliminary studies have shown that analogs of both hemicelluloses and lignin can alter the aggregation of the cellulose in ways that make it more similar to the celluloses of the higher plants. These results have led us to pose the hypothesis that one of the functions of the hemicelluloses and lignin precursors in the cell wall is the regulation of the aggregation of cellulose. We also continue to explore the pathways available for charge transport within the structures we are investigating, both native and biomimetic.

Arizona State University

Tempe, AZ 85287-1604

2. Antenna Organization in Green Photosynthetic Bacteria

R.E. Blankenship, Department of Chemistry *$74,936*

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 and mechanism of excitation transfer in chlorosome antennas. The chlorosome pigments are organized *in vivo* into pigment oligomers in which direct pigment-pigment interactions are of dominant importance. Time-resolved fluorescence spectroscopy has been used to identify the pathway and kinetics of excitation flow from the peripheral region of the chlorosome, to the site where the chlorosome attaches to the membrane, into the membrane-bound antenna complexes and finally into the reaction center. Recent work has identified a redox-activated control of energy transfer efficiency in green sulfur bacteria. Under high redox potential conditions, up to 90% of the excitations are quenched within the chlorosome, while at low redox potentials essentially all excitations are transferred to the reaction center. The quenching effect may be a control mechanism that protects the cell during conditions where light and oxygen are present simultaneously. Current work is directed toward identification of the mechanism of this redox-activated control effect.

Arizona State University
Tempe, AZ 85287-1604

3. Center for the Study of Early Events in Photosynthesis

R.E. Blankenship, J.P. Allen, W.D. Frasch, J.D. Gust, S.H. Lin, A.L. Moore, T.A. Moore, G.R. Seely, W.F.J. Vermaas, A.N. Webber and N.W. Woodbury *$1,200,000 (FY88 funds/30 months)*

A USDA/DOE/NSF Plant Science Center for the Study of Early Events in Photosynthesis has been established at Arizona State University. This Center serves as an infrastructure supporting individual ASU scientists who study photosynthesis using a wide range of different methods and approaches, ranging from molecular biology and biochemistry to organic chemistry, ultrafast laser spectroscopy, X-ray crystallography and theoretical chemistry. The Center is structured to foster multidisciplinary cooperative research projects. In addition, the Center brings visiting scientists to ASU. Graduate and postdoctoral training programs are central components of the activities of the Center.

The ultimate objective of the research that is carried out at the center is to elucidate the basic principles that govern the biochemical and biophysical processes of photosynthetic energy storage. This goal is being sought via investigation of the early events of photosynthesis, including: light absorption and excitation transfer in photosynthetic antennas; the mechanism of primary photochemistry in plant and bacterial systems; secondary electron transfer processes; structure and assembly of photosynthetic antennas, reaction centers and electron transfer proteins; pigment-protein interactions; artificial and biomimetic photosynthetic systems; and mechanisms of biological electron transfer reactions.

*(A unit of the USDA-DOE-NSF Plant Science Center program.)

Arizona State University
Tempe, AZ 85287-1601

4. Specific Mutagenesis of a Chlorophyll-Binding Protein

W.F.J. Vermaas, Department of Botany *$70,992*

Chlorophyll-binding proteins in thylakoids in plants and cyanobacteria are essential to provide chlorophyll with the proper orientation, localization and environment needed for efficient light harvesting and energy transfer to fuel the photosynthesis process. However, the protein-pigment interactions are not properly understood. To identify domains of the chlorophyll-binding protein involved in pigment binding, and to investigate the role of the protein in modifying spectral properties of the bound chlorophyll, specific mutations are introduced into CP47, one of the major central chlorophyll-binding proteins in photosystem II. To this purpose, a *psbB* gene (encoding CP47) with specific mutation(s) in one or more codons is introduced into the genome of a strain of the cyanobacterium *Synechocystis* sp. PCC 6803 from which the wild-type *psbB* gene has been removed. Thus, the effect of specific amino acid residue replacements in CP47 on chlorophyll binding, protein assembly, and structure and function of photosystem II can be studied *in vivo*.

We also have generated mutants in which part of the cyanobacterial *psbB* gene has been replaced by the homologous part of the *psbB* gene from spinach. The question we wish to address by such experiments is whether pigments associated with CP47 serve as intermediates in the energy transfer pathway from peripheral antenna pigments, which are different in plants as compared to cyanobacteria, to the reaction center. In a chimaeric spinach/cyanobacterial mutant, energy transfer between phycobilisomes and photosystem II as monitored by *in vivo* fluorescence decay kinetics measurements was found to be altered. This suggests that CP47 is involved in energy transfer from phycobilisomes (the peripheral antenna) to the photosystem II reaction center.

University of Arizona
Tucson, AZ 85721

5. Controls of the Plant Endomembrane-Secretory Pathway
D.W. Galbraith, Department of Plant Sciences *$164,000 (FY89 funds/two years)*

An understanding of the molecular controls underlying the biosynthesis and targeting of components to the plant cell wall/plasma membrane interface is central to an understanding of plant growth and development. We are studying these controls through the analysis of various glycoproteins that are specifically localized to the *Nicotiana* plasma membrane; we are particularly interested in identifying those that are developmentally up-regulated during the induction of proliferative growth in plant cells. Firstly we have constructed monoclonal libraries directed against plant cell surface antigens, and have been characterizing the epitopes recognized by these antibodies. Secondly, we have prepared and screened bacteriophage expression libraries in order to identify genes whose expression is correlated with proliferative growth; one of the genes that we have characterized appears to encode a nuclearly-targeted transcription factor. Thirdly, we have developed a novel system, involving expression of the vesicular stomatitis virus G-protein, as a marker both *in vivo* and *in vitro* for the plant endomembrane/secretory pathway leading to the plasma membrane. Finally, we have continued the development of techniques of *in vivo* flow cytometric analysis and sorting of protoplasts for the analysis and isolation of rare variants that exhibit altered levels of cell surface expression of the various cell surface marker species. This research should lead to an improved understanding of the mechanisms that control plant cell growth and division.

University of Arizona
Tucson, AZ 85745

6. Phytoalexin Detoxifying Enzymes in the Plant Pathogenic Fungus Nectria Haematococca
H.D. VanEtten, Department of Plant Pathology *$143,500 (FY89 funds/two years)*

The production of antimicrobial compounds (phytoalexins) by plants is believed to function as an active mechanism for disease resistance. Our research has indicated one way that successful pathogens can overcome this resistance mechanism is by detoxifying their hosts' phytoalexins. Our primary model system has been the disease caused by the fungus *Nectria haematococca (Fusarium solani)* on pea (*Pisum sativum*). Our results have indicated that the pathogenicity of this fungus on pea requires pisatin demethylase (pda), a substrate-inducible cytochrome P-450 that detoxifies the pea phytoalexin pisatin. A gene encoding this cytochrome P-450 has been isolated and used, in conjunction with conventional genetic analyses, to demonstrate that there is a family of *Pda* genes in *N. haematococca.* Furthermore, a survey of other fungal pea pathogens has demonstrated the common occurrence of pda activity, suggesting that pisatin detoxification may be a common requirement for pathogenicity on pea. Studies during this past year have revealed that only one other fungal pathogen of pea, *Fusarium oxysporium*, contained DNA with significant homology to the *Pda* genes of *N. haematococca*. Biochemical tests of other pea pathogens having pda activity indicate that, as in *N. haematococca*, both the induction of pda and the substrate preference of the enzyme are specific for pisatin. If pisatin detoxification is a general requirement for pathogenicity on pea, then these results suggest that the *Pda* genes evolved independently in the different fungal pathogens.

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

7. Differential Regulation of Plastid mRNA Stability
D.B. Stern *$147,990 (FY90 funds/two years)*

The expression of photosynthetic proteins requires a cooperative interaction between the nuclear and plastid genomes, and the development of molecular tools to manipulate plants depends in part on understanding how these genes are regulated. Our research focuses on the control of mRNA stability in higher plant chloroplasts, since transcript stability is an important regulatory mechanism during plastid differentiation. Plastid mRNA lifetime is influenced by RNA secondary structure, RNA-binding proteins, and several types of ribonucleases. An *in vitro* system has been developed from spinach chloroplasts that allows study of each of these factors. Thus far, we have found that a stem/loop structure located at the mRNA 3' end is required for RNA stability *in vitro*, and that the sequence of the loop as well as the stem can significantly affect RNA half-life. We have characterized and partially purified several RNA-binding proteins from spinach chloroplasts. At least one of these proteins binds at the stem/loop in a base-specific manner, and may affect RNA structure and/or alter susceptibility to nuclease cleavages. At least two ribonuclease activities have been characterized: one is a non-specific exoribonuclease that may be a scavenger enzyme required for nucleotide turnover, and the other is an endonuclease that cleaves within the stem/loop and is potentially a rate-limiting enzyme for mRNA decay *in vivo*. We are continuing to purify these nucleases and RNA-binding proteins, and have initiated work at the organellar level to relate our *in vitro* results to the expression of genes in intact chloroplasts. One long-term goal is to test the results obtained *in vitro* using a chloroplast transformation system.

Brandeis University
Waltham, MA 02254

8. Carbon and Hydrogen Metabolism of Green Algae in Light and Dark
M. Gibbs, Department of Biology *$65,076*

After adaptation to a hydrogen metabolism, *Chlamydomonas reinhardtii* can photoanaerobically metabolize acetate with the evolution of H_2 and CO_2. An enzyme profile of the chloroplastic, cytoplasmic, and mitochondrial fractions were obtained with a cellular fractionation procedure that incorporated cell wall removal by autolysine, digestion of the plasmalemma with digitonin and fractionation by differential centrifugation on a Percoll step gradient. The sequence of events leading to the photoevolution of H_2 from acetate includes the conversion of acetate into succinate via the extraplastidic glyoxylate cycle, the oxidation of succinate to fumarate by chloroplastic succinic dehydrogenase and the oxidation of malate to oxaloacetate in the chloroplast by NAD dependent malate dehydrogenase. The level of potential activity of the enzymes was sufficient to accommodate the observed rate (22 umole per mg chlorophyll per hour) of gas evolution. Gas evolution by the intact, adapted cell was sensitive to rotenone, amytal, antimycin A, salicylhydroxamic acid (SHAM), 2,5-dibromo-3-methyl-0-isopropyl-p-benzoquinine, fluoroacetate but not by cyanide indicating involvement of the citric and glyoxylate cycles and the reactions of photosystem I. The isolated darkened chloroplast evolves aerobically CO_2 from [1-^{14}C] glucose indicating a chloroplastic respiratory pathway. Evolution of CO_2 is blocked by mitochondrial inhibitors.

Brookhaven National Laboratory
Upton, NY 11973

9. Plant Molecular Genetics
B. Burr and F.A. Burr, Biology Department *$290,000*

We have developed a means of rapidly mapping genes in maize using families of recombinant inbreds. The recombinant inbred families are also ideal populations in which to map quantitative trait loci - those genes having metric rather than qualitative effects upon the traits they control. Molecular markers mapped using the recombinant inbreds are useful in mapping new mutations in other populations. We have employed these tools in thestudy of the regulation of anthocyanin biosynthesis.

In addition to supplying energy for photosynthesis, light provides signals for many aspects of plant development. Light intensity, wavelength, and photoperiod are responsible for plant growth and flowering. Anthocyanin pigment synthesis is also photoregulated and provides a convenient assay for light reception and the transduction of its signal. The end result of this signal is the activation of structural genes encoding enzymes that catalyze steps in flavonoid biosynthesis. In maize most of these genes have been identified and cloned. In addition, genes that are likely to be directly involved in the coordinate regulation of structural gene transcription have been identified and cloned. *C1* acting in the seed and its plant homologue,*pl* have been cloned in this laboratory. We have characterized *b1*, a gene active largely in the plant with homology to *myc* oncogenes. We are interested in learning how *bl* and *pl* interact with themselves and other regulators to control light dependent anthocyanin biosynthesis.

Mutational analysis, coupled with subsequent gene isolation, is likely to supply information about earlier steps in the light signal transduction pathway. We have identified five mutations that drastically reduce plant pigmentation that do not involve any of the structural genes or the light dependent *b1* regulatory gene. Not surprisingly, some of these mutations have pleiotropic effects, reducing plant stature and/or chlorophyll content. We have also found two mutations that enhance plant pigmentation in a light dependent fashion. Three of these mutations have been mapped. A variant that lacks response to UV-B light, identified in another laboratory, has been shown to be a simple recessive. It is expected that isolation and characterization of the products of these genes will lead to an understanding of how the light signal is received by the plant and transduced into gene action.

Brookhaven National Laboratory
Upton, NY 11973

10. Molecular Bases and Photobiological Consequences of Light Intensity Adaptation In Dunaliella tertiolecta
P.G. Falkowski, Department of Applied Science *$63,856*

The long term goal of this research effort is directed towards elucidating the molecular bases for changes in abundance and composition of pigment proteins in response to variations in irradiance. The primary organism under investigation is a unicellular marine chlorophyte, *Dunaliella tertiolecta*, which has a similar photosynthetic apparatus to that found in higher plants. The project aims at understanding how pigment synthesis and protein synthesis are coordinated and regulated so that functional pigment protein complexes are formed or degraded in response to light intensity. Attention is focused on level of control of light harvesting complex. Four genes which encode for light harvesting chlorophyll a/b proteins of photosystem II have been isolated and one cDNA clone has been sequenced. Northern blots of total RNA probed with the *cab* gene revealed a large increase in *cab* mRNA levels following a shift from high to low irradiance levels. The possibility that a shift from high to low light intensity stimulates the transcription of the *cab* genes is being studied on isolated nuclei using *in vitro* run off transcription assays. Several genomic clones are being characterized to identify upstream control regions.

Brookhaven National Laboratory
Upton, NY 11973

11. Regulation of Energy Conversion in Photosynthesis
G. Hind, Biology Department *$404,000*

The project goal is to discover how energy transformation is regulated in photosynthetic membranes. Electron transport is studied in intact chloro-plasts of the C-3 plant, *Spinacia oleracea* and the C-4 plant, *Zea mays*. Relative electron fluxes through the cyclic and linear pathways are explored using flash and steady-state spectroscopy. Passage of electrons through the cytochrome complex is coupled to potential generation and vectorial H^+ transport; the stoichiometry of this coupling and its dependence on ambient redox poise are explored to elucidate the coupling mechanism.

Circular dichroism studies reveal that light-harvesting complexes in the thylakoid experience a partial loss of macrohelicity when the membrane is energized. Associated changes in protein secondary structure are evidentfrom ultraviolet CD spectroscopy. The influence of these conformational events on energy transfer, and their relation to known kinetic fluorescence phenomena is being investigated.

Slow structural adaptations known as state transitions also involve light-harvesting complexes and can be monitored by kinetic fluorescence spectroscopy. They fine tune the apportioning of excitation energy between the photosystems and are reversibly effected through activity of one or more membrane-bound kinases and phosphatases which are being isolated and characterized. The cDNA for these enzymes will be cloned and sequenced to yield protein sequence information. The mechanism through which kinase activity is controlled by ambient redox poise is unknown and will be studied by biochemical and genetic approaches.

These investigations will provide knowledge of mechanisms within the thylakoid that optimize overall photosynthetic productivity. Evidence is accumulating that state transitions also have a role in protecting the thylakoid against photoinhibition and photodestruction.

Brookhaven National Laboratory
Upton, NY 11973

12. The Physiology and Biochemistry of Cyanobacteria
H.W. Siegelman, Biology Department *$220,000*

The energy collection system of the cyanobacteria consists of two domains of phycobiliproteins: rods (80% of the mass) and a triangular core of allophycocyanin (20% of the mass). The trimer-monomer aggregation states of allophycocyanin are a function of pH and protein concentration. The fluorescence lifetime of the trimer is much longer than the monomer. The physiology of infection of cyanobacteria by the cyanophage LPP-1 is being examined. Phage increases of the order of 10^4 were obtained. The phage was resolved by sequential CsCl gradient and equilibrium centrifugation into a major band and 5 minor bands of lower density. The purified major band, examined by STEM, consisted of an icosohedral head with a very short tail. The mass of the phage was $50 \times 10^6 \pm 1.5 \times 10^6$ Da determined by electron scatter in the STEM. The phage DNA consists of about 40,000 base pairs or about 50% of the mass of the phage. Comparisons of the size, shape, general morphology, and DNA and protein content of LPP-1 and T7 were made. Polyacryamide gel electrophoresis of LPP-1 and T7 proteins showed that the size of the principal and minor head proteins were not identical, but of equivalent complexity. Agarose gel electrophoresis of several restriction enzyme digests of the LPP-1 and T7 DNAs showed that the size of the restriction fragements were not identical, although similar in complexity. A primary objective is to determine the entire genomic DNA sequence of the LPP-1 phage for comparison with the T7 coliphage sequence.

Brown University

Providence, RI 02912

13. δ-Aminolevulinate Biosynthesis in Oxygenic Prokaryotes

S. Beale, Division of Biology and Medicine *$82,824*

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. We are characterizing the reaction components for ALA biosynthesis derived from oxygenic prokaryotes, comparing them to their counterparts in plants, and studying the regulation of their activity in response to light and nutritional status. The potential of the prokaryotes for molecular genetic studies is being exploited by generating ALA auxotrophs, and identifying the enzymatic lesions by *in vitro* reaction complementation with purified, identified reaction components obtained from wild-type cells. Genetic complementation of the auxotrophic cells will be carried out by plasmid transformation with genomic libraries obtained from wild-type cells and carried in *E. coli*. The genes coding for the macromolecular reaction components will be isolated, identified, and made available for use as probes for studying 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 analogous genes in eukaryotic algae and higher plants.

California Institute of Technology

Pasadena, CA 91125

14. Genetics in Methylotrophic Bacteria

M.E. Lidstrom, Environmental Engineering Sciences *$179,999 (FY90 funds/two years)*

The purpose of this project is to use genetic techniques to study the transcriptional regulation of C-1 specific functions in methylotrophic bacteria. We have focused on methanol oxidation genes, and have cloned the *mox*F, A, K and L genes from both methanol and methane utilizing bacteria. We have cloned promoter regions from these genes into broad host range promoter cloning vehicles using *lacz* as the reporter gene and have mapped transcriptional start sites. Sequence analysis has revealed a highly conserved sequence at the -10 and -35 positions that may represent the promoter for C-1 genes. Current studies are aimed at defining the key elements of these promoter sequences.

California Institute of Technology
Pasadena, CA 91125

15. Molecular Analysis of Ethylene-Insensitive Mutants in Arabidopsis
E. Meyerowitz, Division of Biology *$81,344*

Ethylene is a plant hormone known to be necessary for, or involved in, a large range of developmental processes and environmental responses of higher plants. While response to the hormone is critical in many aspects of plant biology, the mechanism by which the hormone acts is unknown. To elucidate this mechanism, we are applying a combination of genetic, molecular and physiological techniques. We are concentrating on a single plant, *Arabidopsis thaliana*, and on a single gene in this plant, called ETR1. One studied mutation in this gene confers dominant resistance to ethylene, so that all tested ethylene responses are absent in the mutants.

Most of our work is devoted to cloning the mutant ETR1 gene. To this end we have genetically mapped the mutation relative to the cloned DNA fragments that are the markers on the *Arabidopsis* restriction fragment length polymorphism genetic map. One marker maps very close to ETR1, and has served as a starting point for a chromosome walk. This walk has proceeded by isolation of a series of overlapping cosmid and yeast artificial chromosome clones, such that a region of more than 150 kilobase pairs of contiguous DNA in the region of the starting point has been obtained. Genetic mapping shows that, in at least one direction, the region of the genome that cosegregates with ETR1 has been exceeded. This may be true in the other direction as well, so that we may now have a series of cloned fragments, one or more of which contain the gene. Each of the cosmid clones from the walk is being tested by a transformation assay to see which contain the gene.

University of California
Los Angeles, CA 90024

16. Energy Capture and Use in Plants and Bacteria
P.D. Boyer, Molecular Biology Institute *$98,948*

The main emphasis continues to be to gain understanding of the mechanism of the ATP synthase in plants and bacteria. A central goal is to critically evaluate the proposed binding change mechanism. A new aspect of the hypothesis to be tested is that one site on a catalytic subunit interchanges bound substrates readily with medium substrates, another site has tightly bound and interconverting ADP, P^i, and ATP present at an equilibrium near unity, and the third catalytic site has tightly bound ATP present. For ATP synthesis an energy-linked binding change is proposed to interconvert the three sites with release of the tightly bound ATP. One approach will be to attempt to measure the amount of bound ADP and ATP on catalytic sites during steady state ATP formation by the intact synthase or ATP hydrolysis by the isolated CF_1 ATPase as a function of substrate concentration. For example, adequate measurements of hexokinase inaccessible ATP and, in rapid mixing experiments, of bound ADP committed to form ATP will reflect catalytic site occupancy. Total functional catalytic sites can be assessed by the amount of readily replaced tightly bound ADP. Other experiments will concern how the nucleotides bound at the three noncatalytic sites modulate catalytic events, and the effects of covalent inhibitors and of the antibiotic aurovertin on catalytic pathways by use of ^{18}O probes as developed in our laboratory.

University of California
Davis, CA 95616

17. Restriction of Virus Infections by Plants
G. Bruening, Department of Plant Pathology *$90,712*

The productivity of a given plant line, in terms of biomass or food or fiber production, often is limited by the action of plant pathogens. An obvious and direct, and usually ecologically sound, approach to limiting the effects of a pathogen is to develop a plant line that is resistant to the pathogen. In the first of two approaches we investigate "genotypic resistance," in which one or a few lines of an otherwise susceptible plant species exhibit resistance against a specific virus. The basis of genotypic resistance of the Arlington line of cowpea against cowpea mosaic virus (CPMV) has been associated, in the course of the previous research, with an inhibitor of the processing of CPMV polyproteins. We are attempting to purify protein(s) that exhibit this activity and which have the expected virus specificity and correlations in their inheritance in cowpea crosses. Experiments designed to identify and isolate a gene(s) for the inhibitor also are in progress. We are investigating polyprotein processing and other aspects of cowpea severe mosaic virus, which is similar to CPMV but not affected by the inhibitor of polyprotein processing. The second aspect of this research is concerned with understanding how a small satellite RNA associated with tobacco ringpot virus can interfere with the replication of cherry leafroll virus (CLRV).

University of California
La Jolla, CA 92093

18. Structure, Biosynthesis and Role of Complex Protein-Bound Glycans
M.J. Chrispeels, Department of Biology *$85,782*

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. These modifications result in a variety of complex glycans. We are studying the enzymes which are involved in these modifications and the sequence in which these various reactions occur. The purpose of this work is to understand how these particular complex carbohydrates are formed in plants. In addition, we are investigating the role of these glycans and the relationship between glycan modification and protein targeting. Our most recent work concerns the identification of a bean seed protein that inhibits the activities of insect and mammalian α-amylases. This plant defense protein is the product of a lectin gene, and is therefore the first lectin-type protein that has a known biological function. It is also a glycoprotein with complex and high-mannose glycans. We are investigating the role of these glycans in its activity.

University of California
Davis, California 95616

19. Modifying K^+/Na^+ Discrimination in Salt-Stressed Wheat Containing Chromosomes of a Salt-Tolerant Lophopyrum

E. Epstein and J. Dvorak, Departments of Land, Air and Water Resources, and Agronomy and Range Science — *$162,000 (FY89 funds/two years)*

Salinity of soils and water is a major impediment to the capture of solar energy by green plants in those large areas of the world that are arid or semi-arid. This project is devoted to generate knowledge applicable to the development of salt tolerant crops. Specifically, the project is meant to advance our genetic and physiological understanding of how K^+/Na^+ discrimination bears on salt tolerance. The experimental materials are lines of hexaploid (A, B and D genomes) wheat, *Triticum aestivum* cv 'Chinese Spring' into which has been incorporated each of the chromosomes of the E genome from the salt-tolerant diploid wheatgrass, *Lophopyrum elongatum*. During the 1988-1989 season, a field trial was conducted to evaluate correlations between the salt tolerance of the *L. elongatum* chromosome substitution lines and K^+/Na^+ discrimination at two salinity levels and under control conditions. Ion analyses of plant material and comparisons with yield showed a positive correlation between the K^+/Na^+ ratio and salt tolerance. The trial is being replicated in the 1989-1990 season. The physiological aspects of enhanced K^+/Na^+ discrimination are being examined in experiments on the absorption and translocation to the shoot of K^+ labeled with ^{42}K (or ^{86}Rb) and Na^+ labeled with ^{22}Na. Amphiploid seedlings absorb K^+ at a higher rate than do 'Chinese Spring' seedlings. The locus or loci conditioning this response are being determined.

University of California
Berkeley, CA 94720

20. Transcription Control Elements and Manipulation of Chloroplast Genes

W. Gruissem, Department of Plant Biology — *$97,614*

A prerequisite for the manipulation of crop plants by introducing desirable genes into plant cells, or by transformation of chloroplasts, is our understanding of regulatory processes that are involved in the expression of genes. Our research project focuses on the molecular mechanisms of chloroplast gene expression in higher plants. Most monocistronic and polycistronic chloroplast transcription units contain proximal to their transcription start site promoter elements that dictate the relative transcriptional activity of these genes by their respective promoter strength. We have found that the relative transcriptional activities of most chloroplast genes are maintained during different stages of plant development. The transcription activity of certain genes (e.g., genes for photosystem I proteins, the gene for the intron-containing $tRNA^{Lys}$), however, is regulated in response to the plant development program or light quality changes. The experimental analysis of their promoter regions, together with the quantitation of RNA polymerase levels and the analysis of proteins other than RNA polymerase binding to these regions, will allow us to devise a model for this type of transcriptional regulation. As part of this experimental work we are isolating a chloroplast RNA polymerase transcription initiation complex using the *psbA* promoter as a model. The proteins that constitute this complex will be characterized by immunological and protein sequencing techniques. To support the *in vitro* analysis of regulatory elements, we continue our work to develop a high efficiency chloroplast transformation system and to construct stable chloroplast transformants.

University of California
Los Angeles, CA 90024-1489

21. Molecular Biology and Genetics of the Acetate-Utilizing Methanogenic Bacteria
R.P. Gunsalus, Department of Microbiology *$80,841*

Acetate conversion to methane and CO_2 by the methanogenic archaebacteria is a rate limiting step in anaerobic degradative processes. Current evidence indicates that acetate is catabolized by carbon monoxide (CO) dehydrogenase which is regulated at the gene level in response to the presence of substrate. Until recently it has been technically difficult to study the molecular biology and genetics of most acetate-utilizing *Methanosarcina* spp. because cellular aggregation prevented the growth and plating of individual cells which is necessary for mutant selection and isolation. *Methanosarcina thermophila* can be conditioned to grow as single cells and methods have been developed for high plating efficiency on agar medium. Methods are being refined for the production of auxotrophic and catabolic mutants by irradiation and chemical mutagenesis. The feasibility of a hybrid shuttle vector is being examined by identifying the origin of replication and the location of open reading frames in a plasmid isolated from *Methanosarcina acetivorans*. This information may be of later use for inserting selective marker genes which can be expressed from the plasmid encoded promoter. Chromosomal libraries from *M. thermophila* are being screened for CO dehydrogenase genes and positive DNA fragments are being sequenced. Evaluation of the CO dehydrogenase gene sequences in conjunction with genetic studies will provide insight into the molecular mechanisms that are responsible regulatory control of CO dehydrogenase which will serve as a model system for gene regulation in the methanogenic archaebacteria.

University of California
Irvine, CA 92717

22. Membrane Bioenergetics of Salt Tolerant Organisms
J.K. Lanyi, Department of Physiology and Biophysics *$168,000 (FY90 funds/two years)*

The mechanism of energy transduction in extremely halophilic archaebacteria is explored in two kinds of membrane-bound systems: the proton and chloride transporting bacterial rhodopsins, and the proton transporting ATPase. Studies of protonation changes during the photoreactions of bacteriorhodopsin and halorhodopsin are expected to reveal steps by which protons are transferred from one side of the membrane to the other in the former pigment (proton pump), and stratagems which prevent these processes to occur in the latter pigment (chloride pump). Structural and active site studies of the halobacterial ATPase will establish features shared with, and different from, eubacterial and other archaebacterial systems. Cloning and sequencing of the structural genes which code for the subunits of this enzyme support these studies, and provide the basis for further approaches to the molecular biology of this system.

University of California
Berkeley, CA 94720

23. Determination of Genes and Phenotypes of Bacteria Necessary for Epiphytic Colonization and Survival on Plants

S.E. Lindow, Department of Plant Pathology *$68,035*

Bacteria that live as epiphytes on the surface of healthy plants are important as plant pathogens, in causing plant frost injury by catalyzing ice formation, and in other processes. The objectives of this study are to determine the traits of these epiphytic bacteria which allow them to grow and/or survive in the hostile leaf surface environment. The genes and phenotypes of a strain of *Pseudomonas syringae* that are necessary for epiphytic fitness on bean leaves are being determined by an evaluation of the fitness and phenotypes of 5,300 individual Tn*5*-induced insertion mutants. Attention is being placed on several mutants which exhibit no known phenotypic alteration, but which while growing normally on moist leaf surfaces succumb to the stresses associated with dry leaf surfaces, unlike the parental strain. The randomness of Tn*5* insertions in *P. syringae* was further demonstrated by showing a normal distribution of *Xbal* restriction fragment sizes, separated by pulsed field electrophoresis, that contained Tn*5*. Tn*5* was located in relatively few *Xbal* restriction fragments in epiphytic mutants however. The genes from stress-sensitive epiphytic fitness mutants are being identified in a cosmid library of the parental *P. syringae* strain by sequence homology to Tn*5*-containing fragments cloned from mutant strains. Fusions of these regions with a promoterless *lnaZ* genes are being made to determine the regulation of transcription of these regions *in situ* by measuring the ice nuclei produced in merodiploid strains containing these fusion genes.

University of California
Davis, CA 95616

24. Transposon Tagging of Disease Resistance Genes

R.W. Michelmore, Department of Vegetable Crops *$67,048*

We are developing a transposon mutagenesis system for lettuce to clone genes for resistance to the fungal pathogen, *Bremia lactucae*. Activity of heterologous transposons is being studied in transgenic plants. Southern analysis of T_1 and T_2 plants containing *Tam3* from Antirrhinum provided ambiguous results. Multiple endonuclease digests indicated that transposition had occurred; however, in no plant were all endonuclease digests consistent with a simple excision event. Southern or PCR analysis of over 50 plants containing *Ac* from maize have also failed to reveal clear evidence of transposition; this is contrast to experiments by others with the same constructs who have observed high rates of *Ac* excision in other plant species. Nearly all of 65 T_2 families containing *Ac* interrupting a chimeric streptomycin resistance gene (Courtesy J. Jones, Sainsbury Lab., UK) clearly segregated for streptomycin resistance. Southern analyses, however, showed no evidence of transposition, indicating restoration of a functional message by other mechanisms, possibly mRNA processing. Transgenic plants have also been generated containing CaMV 35S or *hsp70* promoters fused to transposase coding sequences or a *Ds* element interrupting a chimeric GUS gene (Courtesy M. Lassner, UC Davis). F_1 plants containing both constructs were analyzed for transposition. Only two plants containing both constructs were obtained from 48 progeny, far fewer than expected, and neither showed evidence of transposition in Southerns and GUS assays. We are currently constructing further chimeric transposase fusions. To test for the stability of the targeted disease resistance genes, 50,000 F_1 plants heterozygous for three resistance genes were generated; no mutants have been identified in the 5,000 so far screened.

University of California
Berkeley, CA 94720

25. Characterization of a Defective Interfering RNA That Contains a Mosaic of a Plant Viral Genome

T.J. Morris and A.O. Jackson, Department of Plant Pathology *$78,387*

Viruses are important pathogens responsible for significant yield loss in crop plants. This research addresses the identification of viral sequences important in virus replication, encapsidation and other events that effect viral pathogenicity through the molecular characterization of a unique class of symptom modulating RNAs called defective interfering RNAs (DI RNAs). DI RNAs are linear deletion mutants of viral genomes that compete with and interfere with the helper virus thus reducing the severity of the disease. Although DIs are common components of animal virus infections, they are uncommon in plant infections. We first discovered DIs in association with tomato bushy stunt virus (TBSV) and have since identified similar DIs in association with turnip crinkle virus (TCV). To evaluate the essential sequences of the DI RNAs, we have completely cloned and sequenced the helper virus genomes and have begun to investigate the origin and evolution of DI RNAs which arise *de novo* when host plants are inoculated with RNA transcripts derived from complete clones of the viral genomes. We have also developed a procedure to permit rapid cloning, sequencing and the evaluation of the biological activity of DI RNAs using PCR amplification methods. To address the mechanism of virus-host interactions leading to symptom attenuations, we have used molecular probes to study the replication and interference effects of DIs in plants and protoplasts. In addition, we are in the process of constructing plasmids to permit the expression of DIs in transgenic tomatoes with the longer term goal of constructing plants with engineered resistance to plant viruses.

University of California
Los Angeles, CA 90024

26. The Gibberellin A_{20} 3β-hydroxylase: Isolation of the Enzyme and its Molecular Biology

B.O. Phinney and J. MacMillan, Department of Biology *$137,000 (FY90 funds/two years)*

The early-13-hydroxylation pathway of gibberellin (GA) biosynthesis in maize shoots has been documented using the single gene dwarf mutants, *d1, d2, d3, d5*, and *an1*. The results have provided an understanding of the control of stem elongation by the GAs in maize shoots and have led to the hypothesis that GA_1 is the primary GA responsible for shoot elongation in maize. The goal of the project is to extract and purify the 3β-hydroxylase catalyzing the conversion of bio-inactive GA_{20} to active GA_1. In preliminary experiments, the PIs have found that the cortical tissue from maize stem is an excellent source for the enzyme. The enzyme will be extracted from this tissue and purified by column chromatography followed by immunoaffinity chromatography using either a monoclonal antibody that the PIs have prepared to the corresponding enzyme from bean seed, or a monoclonal antibody prepared to a partially purified enzyme from maize. (Column chromatography has already been used by the PIs to partially purify the 3β-hydroxylase from bean seed.) The purified enzyme from maize cortex will be partially sequenced; synthetic oligonucleotides will be constructed and used to probe a cDNA library prepared from maize cortex mRNA. The isolated clones will be probed with oligonucleotides corresponding to different regions of the protein sequence to identify the clone for the 3β-hydroxylase. The cDNA for the 3β-hydroxylase will be inserted into a high expression vector to produce large quantities of the enzyme. The structure of the enzyme will be investigated by X-ray crystallography. Transcriptional and translational control of the protein will be examined. We will also isolate the enzymes from two *dwarf-1* alleles ("leaky" mutants which block the step GA_{20} to GA_1); comparisons of the structure and properties of the gene and its product(s) will provide information on the enzymatic basis for the *d1* control of this step.

University of California
Berkeley, CA 94720

27. Phytochrome from Green Plants: Assay, Purification and Characterization
P.H. Quail, Department of Plant Biology *$87,754*

There is strong evidence that the molecular species of phytochrome that predominates in fully-green tissue (Type 2 phytochrome) is distinct from the well-characterized species that predominates in etiolated tissue (Type 1 phytochrome). Our goal is to define the molecular properties, biogenesis, and mechanism of action of Type 2 phytochrome. *Arabidopsis thaliana* has provided the key to this problem. DNA blot analysis indicates that the *Arabidopsis* genome contains four to five phytochrome-related gene sequences. We have isolated and sequenced cDNA clones corresponding to three of these genes. The deduced amino acid sequence of one of the encoded proteins (*phy A*) shows 65-80% sequence identity with the major Type 1 phytochrome apoproteins described previously in other plant species. The other two polypeptides (*phy B* and *phy C*) are unique in that they have low sequence identity (~50%) with each other, with *phy A*, and with all previously described phytochromes. The *phy A, phy B* and *phy C* proteins are of similar molecular mass, have related hydropathic profiles, and contain a conserved chromophore attachment region. However, the sequence comparison data indicate that the three phy genes diverged early in plant evolution well before the divergence of the two major groups of angiosperms, the monocots and dicots. The steady-state level of the *phy A* transcript is high in dark-grown seedlings and is down-regulated by light. In contrast, the *phy B* and *phy C* transcripts are present at lower levels and are not strongly light-regulated. This constitutive expression of the *phy B* and *phy C* transcripts is similar to that observed for the Type 2 phytochrome protein in oats.

University of California
Berkeley, CA 94720

28. Genetic and Biochemical Basis of Race-Specific Incompatibility in Pseudomonas syringae pv. glycinea-Soybean Interactions
B.J. Staskawicz, Department of Plant Pathology *$84,796*

The expression of disease resistance in soybean towards the bacterial pathogen *Pseudomonas syringae* pv. *glycinea* is genetically determined by the interaction of specific avirulence genes in the pathogen that correspond to specific resistance genes in the host. Our work has focused on studying the interaction between the avirulence gene *avr*B and the soybean resistance gene Rpg1. Our recent work has concentrated on studying the promotor region of *avr*B. We have characterized the promotor by determining the initiation of transcription by primer extension and have shown that the *avr*B gene is transcribed from two initiation sites depending on whether the cells are grown in a fructose induction medium or whether they are grown on a citrate suppression medium. (Huynh et. al. 1989, *Science* 245: 1374-1377).

In addition, we have cloned and identified the Hrp gene cluster from *Pseudomonas syringae* pv. *tomato* strain DC3000. We have characterized the physical organization of this gene cluster by restriction enzyme mapping and functional analysis has been performed by marker exchange mutagenesis with a ß-glucuronidase reporter transposon. Work is in progress to identify transcriptional units and to determine if this region is similar to the Hrp cluster of *Pseudomonas syringae* pv. *glycinea*. Interestingly, several of these Hrp cosmid clones from *Pseudomonas syringae* pv. *tomato* also suppress both pathogenicity and the ability to elicit the hypersensitive reaction when introduced into *Pseudomonas syringae* pv. *glycinea*. We are currently in the process of localizing this region and we plan to characterize this region in hopes that it may elucidate the biochemical basis of suppression and ultimately to help us to determine how the Hrp gene cluster and avirulence genes work in concert to elicit a race-specific resistance reaction.

University of California
Santa Cruz, CA 95064

29. Tonoplast Transport and Salt Tolerance in Plants
L. Taiz, Biology Department *$136,170 (FY89 funds/22 months)*

The tonoplast VoV1 ATPase provides the primary energy source for solute uptake into plant vacuoles. The enzyme is a large hetero-oligomer, ~500 kDa, composed of a water soluble catalytic complex (V1) and a membrane channel complex (Vo). V1 consists of three copies each of two major subunits, A (70 kDa) and B (60 kDa), and one copy each of about three minor subunits. We investigated the 5'-upstream region of the gene encoding the catalytic A subunit of *Daucus carota*. A genomic sublibrary was screened with a cDNA probe and a 4kb genomic clone was obtained covering the first two exons and about 3 kb of 5'-upstream sequence. Within 240 bp upstream of the initiation codon three putative TATA-boxes were found. Ribonuclease protection analysis indicated that the three TATA-boxes corresponded to two major and one minor transcription start site. Sequence motifs with putative regulatory function are two CCAAT-boxes, an Sp1-binding consensus sequence, and long (TATA)n stretches within 800 bp of the 5'-upstream sequence. Fusions to the beta-glucuronidase (GUS) reporter gene were made for two different promoter constructs and the resulting plasmids were mobilized into *Agrobacterium tumefaciens*. The analysis of GUS activities in the transformed carrot calli showed that 240 bp of upstream sequence, including all three TATA-boxes, led to low but detectable GUS-expression; however, the larger construct, which included the putative Sp1-binding sequence and the (TATA)n stretches, led to an approximately 6-fold higher GUS-expression. Studies on the promoter for the B subunit gene are in progress.

University of California
Berkeley, CA 94720

30. Analysis of the Proteins Essential for Agrobacterium Mediated DNA Transfer to Plant Cells
P. Zambryski, Department of Plant Biology *$92,684*

The major goal of the laboratory is to understand how *Agrobacterium* can genetically transform plant cells. The proposed research focuses on the proteins, encoded by the Ti plasmid virulence (*vir*) region that mediate the DNA transfer event. There are two general classes of *vir* proteins, DNA binding proteins and membrane associated proteins. During the last year, we have characterized the *virB* operon, the largest of the *vir* loci, spanning over 9 kbp. Genetic studies have shown that *virB* products are essential for T-DNA transfer, but their functions remain unknown. To provide information relevant to *virB* function, the nucleotide sequence of the *virB* operon of the nopaline plasmid, pTiC58, was determined. Eleven open reading frames (ORFs) were predicted from the sequence. The predicted sizes of 10 of the 11 *virB* polypeptides were verified by specific expression in *E. coli*. Only the smallest ORF, potentially encoding a 5.8 kDa polypeptide, was not detected. Interestingly, the initiation of translation of 5 different *virB* ORF's occurs at codons that overlap the termination codons of the immediately upstream ORF; thus, translational coupling may be an important mechanism for efficient translation of the large *virB* polycistronic mRNA, i.e., by keeping ribosomes bound thereby minimizing nucleolytic attack of intercistronic regions. Based on hydropathy plot analysis, 9 of the *virB* ORFs encode proteins that may interact with membranes; these data support the hypothesis that *virB* gene products may form a membrane pore or channel to mediate exit of T-strands from *Agrobacterium* to the plant cell. A comparison of the two published octopine Ti plasmid *virB* regions with the nopaline sequence indicate several errors in the earlier reports.

University of California
Los Angeles, CA 90024

31. CO_2 and the Stomatal Control of Water Balance and Photosynthesis in Higher Plants
E. Zeiger, Department of Biology *$150,000 (FY90 funds/two years)*

Guard cells perceive many variables in the leaf environment - light, intercellular CO_2 concentrations, water vapor pressure differences, temperature - and transduce these signals into metabolic reactions that modulate stomatal apertures. The control of stomatal apertures is critical for the adaptation and acclimation of the plant to its environment. Intercellular CO_2 concentrations are central to the interaction between the stomata and the photosynthesizing mesophyll. Our working hypothesis on the mechanism transducing the CO_2 signal into modulated stomatal apertures postulates that the proton pump at the guard cell plasma membrane and photosynthetic carbon metabolism in the guard cell chloroplast compete for ATP and a second, yet to be identified, photosynthetic product. In current work, we have developed a method to isolate intact chloroplasts from guard cell protoplasts of *Vicia faba*. Key metabolic intermediates in the chloroplasts and their suspension medium are analyzed by HPLC. Using light quality as modulator of chloroplast activity, we are characterizing changes in photosynthetic activity and the export of photosynthetic products to the suspension medium. The goal of this study is to identify mechanisms regulating carbon fixation in the guard cell chloroplast, and the nature of the photosynthetic products exported to the cytoplasm.

Case Western Reserve University
Cleveland, OH 44106

32. Cloning and Analysis of Genes Regulating Plant Growth
C.D. Town, Biology Department *$83,810*

The long term aims of this work are to isolate and characterize genes involved in the control of proliferation of plant cells. We have induced tumors on the model plant *Arabidopsis thaliana* using ^{60}Co gamma rays and shown that, in contrast to normal tissues, they grow in the absence of hormones when transferred to culture. Individual tumors show interesting differences in gross morphology, degree of cytodifferentiation and growth rate. We propose that these tumors arise by radiation-induced genetic changes which activate the expression of either the growth hormone genes themselves, or of other genes involved in the control of cell proliferation, in direct analogy to the activation of oncogenes in animal cells by radiation, chemicals or other genotoxic agents. We have investigated the hormone sensitivity and hormone content of the tumors. In some tumors one or both of these parameters were changed, while in others they were not, indicating that a tumor can arise without a change in either the hormone content or hormone sensitivity of the progenitor tissue. To identify possible plant "oncogenes" we screened northern blots of $polyA^+$ RNA at low stringency with a variety of animal oncogene probes (20 in all). One probe (*int-2*) hybridized strongly to a 900 base transcript which was expressed in all the tumors, but absent from callus formed from normal tissue grown on 2,4-D. Sequencing of cross-hybridizing cDNAs isolated from a tumor library suggests that the transcript encodes a glycine-rich protein. Further northern analysis showed that the transcript was present in roots and stems of plants and also in callus grown on NAA rather than 2,4-D as auxin. Thus this transcript is not entirely tumor-specific, but does show interesting hormonal regulation. As a more general approach, we have begun the construction of subtraction libraries, whereby sequences common to both tumor and normal callus cDNA libraries are removed by cross-hybridization so that the residual library is highly enriched for tumor-specific sequences. We also plan to apply the same subtraction technique to identify sequences which are newly expressed when a slow-growing tumor spontaneously progresses to a fast-growing variant, as has happened on three separate occasions over the last two years in culture. Identification of genes involved in tumor formation and growth rate will further our understanding of growth control in plants and could contribute to the development of new varieties of crop plants with improved yields, nutritional value and environmental tolerance.

University of Chicago
Chicago, IL 60637

33. Organization and Regulation of the Genes for Nitrogen Fixation in Rhodobacter capsulatus
R. Haselkorn, Department of Molecular Genetics and Cell Biology

$172,000 (FY89 funds/two years)

We are attempting to elucidate the control circuit that regulates expression of the genes for nitrogen fixation in *Rhodobacter capsulatus*. In common with other diazotrophs such as *Klebsiella*, the *Rhodobacter nif* genes are normally expressed only under anaerobic, nitrogen starvation conditions. Based on our genetic studies and DNA sequencing, we know that transcription of *nif*H requires the products of the *nif*R4 (*ntr*A), *nif*R1 (*ntr*C) and *nif*R2 (*ntr*B) genes. W. Klipp showed that *R. capsulatus* has two functional copies of the *nif*A gene, whose product is required to activate *nif*H transcription in *Klebsiella*. During the past year we completed construction of *nif*A::*lac*Z fusions and subcloned probe fragments for determining the start site for transcription of the *nif*A, *nif*R2 and *nif*R4 genes. Thus far, these studies have shown that expression of *nif*A and *nif*R4 are both higher in glutamate-grown cells than in ammonia-grown cells. The *nif*R4 gene has a single transcription start site while *nif*A1 and *nif*A2 each have a pair of closely spaced start sites. Possible alternate start sites, closer to the translation starts, are revealed in RNA prepared from a derepressed *nif*R1 mutant. We also constructed a plasmid capable of replication in *Rhodobacter* and small enough to serve as a good monitor of negative supercoiling. Our purpose was to transfer to this plasmid various *nif::lac* fusions in order to study the relationship between O_2 level, supercoiling, and expression. However, to date we have been unable to detect any difference in extent of supercoiling in plasmid DNA prepared from cells grown aerobically vs. anaerobically. The purification of *R. capsulatus* RNA polymerase was also pursued with the view of establishing an *in vitro* system for studying transcription of the *nif* regulatory genes. The nucleotide sequence of the 5-kb *R. capsulatus rpoB* gene, encoding the ß subunit of RNA polymerase, was completed except for two small gaps.

Finally, we began a program to search for genes whose expression depends on NifR4. A library of *R. capsulatus* DNA fragments was screened by subtractive hybridization, first saturating with unlabeled RNA from the NifR4$^-$ mutant LJ1 and then hybridizing with labelled RNA from derepressed wild type cells. Six clones were isolated and fragments of these are being sub-cloned for further study. Several of these fragments contain genes whose transcription depends on NifR1 as well as being repressed by ammonia.

Clemson University
Clemson, SC 29634-1903

34. The Magnesium Chelation Step in Chlorophyll Biosynthesis
J.D. Weinstein, Department of Biological Sciences *$61,511*

In photosynthetic organisms, the biogenesis of energy transducing membranes requires the coordinate synthesis of prosthetic groups, proteins, and various lipids. Two of the major prosthetic groups, chlorophyll and heme, share a common biosynthetic pathway that diverges at the point of metal insertion into protoporphyrin IX. Insertion of iron leads to the formation of hemes, while insertion of magnesium is the first step unique to chlorophyll formation. This project is directed toward elucidating the mechanism and regulatory features of the enzyme(s) responsible for magnesium chelation in isolated chloroplasts. An assay of this activity has been developed wherein it is possible to measure initial rates of reaction, and we have confirmed the reported requirements for Mg^{2+}, protoporphyrin, and ATP. The chloroplast system has an absolute requirement for membrane intactness, and the activity has been tentatively localized to the plastid envelope on the basis of its sensitivity to a membrane impermeable mercurial reagent. Possible relationships between the ATP and membrane intactness requirements will be probed by the use of uncouplers, ionophores, and inhibitors of ATPase. Possible localization of the activity to the plastid envelope will be assessed by inhibition and/or labeling with a variety of protein modifying reagents, and by limited proteolysis. Chloroplast components will be fractionated to identify and characterize the Mg-chelatase enzyme.

Cornell University
Ithaca, NY 14853

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

J. Gibson, Section of Biochemistry, Molecular and Cell Biology, Division of Biological Sciences *$64,090*

Very large quantities of aromatic compounds, many of them recalcitrant and potentially carcinogenic, are released into the biosphere as industrial byproducts. Lignins derived from plant sources are an even larger source of benzenoid rings in Nature. Many of these compounds find their way into anaerobic environments, where they can be degraded, if slowly, by anaerobic bacteria. Little is known concerning the metabolic pathways, let alone the detailed enzymology or regulation, employed in these processes. The major objectives of this project are to elucidate the biochemistry of the degradation of benzoate and of 4-hydroxybenzoate by the phototrophic bacterium *Rhodopseudomonas palustris*, one of the relatively small number of microorganisms known to grow well with these and related compounds as sole carbon source. We have shown that these two compounds, or close derivatives, appear to be key intermediates in the degradation of a range of more complex aromatic acids utilized for growth. The first step in the anaerobic attack on these model compounds involves the formation of Coenzyme A thioesters by apparently specific enzymes. The main thrust of work in the next year will be to complete the purification and characterization of 4-hydroxybenzoate:CoA ligase, and to establish the nature and number of enzymatic steps and cofactors involved in the next, reductive, stages of aromatic acid thioester metabolism. A number of mutants with restricted substrate utilization have been isolated, and will be used for determining sequence and regulation of these reactions. Our work will be closely correlated with that of Dr. Harwood at the University of Iowa. The overall biochemistry is likely to be similar in other types of microorganisms that degrade aromatics anaerobically, so that these studies will contribute ultimately to possibilities for engineering more rapid or diversified biodegradations in such environments.

Cornell University
Ithaca, NY 14853

36. Molecular Analysis of Cytoplasmic Male Sterility

M.R. Hanson, Section of Genetics and Development *$70,006*

The ultimate aims of the project are to understand the molecular mechanism of the disruption in pollen development which occurs in cytoplasmic male sterile plants and to understand the control of respiratory energy flow in the higher plant cell. A mitochondrial locus termed S-*pcf* segregates with sterility and with an alteration in respiration in *Petunia*. This cloned locus contains three genes, an abnormal fused gene termed *pcf*, a gene for a subunit of an NADH dehydrogenase complex, and a small ribosomal subunit protein. The *pcf* gene is comprised of partial sequences of ATPase subunit 9, cytochrome oxidase subunit II, and an unidentified reading frame. Components of the S-*pcf* locus will be introduced into the nucleus of a fertile genotype under the control of appropriate regulatory signals, and polypeptide products of introduced genes will be directed to the mitochondrion with a transit peptide. By examining transgenic plants, we can determine what elements of the locus are critical for altered respiration or sterility. Such knowledge could explain how mitochondrial DNA affects pollen development in the large number of plant species which exhibit the agronomically important trait of male sterility.

Cornell University
Ithaca, NY 14853

37. Structure and Function of the Self-Incompatibility Proteins of Brassica oleracea
M. Nasrallah, Division of Biological Sciences *$74,936*

The specificity of the pollen-stigma interaction in self-incompatible species belonging to the mustard family, the Brassicaceae, is controlled by more than fifty alleles at the *S* locus. In *Brassica*, the genotype of the pollen parent determines the pollen reaction and characterizes the incompatibility system as a sporophytic type. Pollen fails to produce a pollen tube on a stigma expressing the same *S* allele as the pollen parent. We have shown by a number of genetic and molecular analyses that self-incompatibility in this species is mediated by a glycoprotein encoded by the *S* locus and expressed at high levels in the stigma, and to a lesser extent in the anther prior to microspore matura.

We have demonstrated by ultrastructural immunocytochemistry that, in the stigma, the *S*-locus specific glycoproteins accumulate in the walls of the surface papillar cells. We also found that these molecules exist as phosphorylated glycoforms. The significance to the recognition of self, of the two post-translational modifications, phosphorylation and addition of complex glycan side-chains, is being addressed under this project. In addition, we have identified other proteins that are encoded by independent genes belonging to the *S*-multigene family. The characterization of the *S* proteins and their homologs in pollen should clarify their involvement in the cellular interaction between stigma and pollen.

Cornell University
Ithaca, NY 14853

38. Mechanisms of Inhibition of Viral Replication in Plants
P. Palukaitis, Department of Plant Pathology *$75,921*

Viruses are a major class of plant pathogens that are responsible for crop losses and reductions in plant biomass. In some cases natural resistance genes are known which can lessen the effects of such pathogens. However, none of these resistance genes has been isolated, and their mechanism of action is not understood. This project is concerned with analyzing the molecular mechanisms of inhibition of viral replication and movement in plants, and how viruses mutate to overcome such natural resistance mechanisms. Several approaches are being used to examine the above mechanisms: (1) Protoplasts from plants resistant to infection by either cucumber mosaic virus (CMV) or tobacco mosaic virus (TMV) are being used to analyze various hypothesized mechanisms of resistance, as well as to delimit viral sequences involved in resistance breakage. (2) Transgenic plants expressing CMV and/or TMV movement genes will be used to determine the nature of the interactions between viral movement gene products and host genes for resistance to viral movement. (3) RNase protection/heterogeneity assays and sequence analysis are being used to delimit sequences alterations involved in resistance breakage. These techniques are being used to differentiate sequence alterations in the TMV movement gene involved in breaking resistance of the tomato Tm-2^a gene vs. the Tm-2 gene. And (4), using biologically-active cDNA clones of different strains of CMV to map sequence alterations that effect resistance to this virus, we have delimited a cultivar-specific resistance in zucchini squash to CMV infection, to specific regions of RNA 3.

Cornell University
Ithaca, NY 14853

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

P.L. Steponkus, Department of Agronomy *$87,162*

The goal of our program is to provide a mechanistic understanding of the cellular and molecular aspects of freezing injury and cold acclimation from a perspective of the structural and functional integrity of the plasma membrane. The current focus is on dehydration-induced alterations in the ultrastructure of the plasma membrane that result in the loss of osmotic responsiveness (LOR). These alterations include the formation of aparticulate domains in the plasma membrane, aparticulate lamellae subtending the plasma membrane, and lamellar-to-hexagonal$_{II}$ phase transitions in the plasma membrane and subtending lamellae. Current studies include i) characterization of the incidence of LOR and lamellar-to-hexagonal$_{II}$ phase transitions as a function of the stage of cold acclimation and artificial enrichment of the plasma membrane lipid composition; ii) determination of the hydration characteristics and force-distance relationships of plasma membrane lipids and model lipid mixtures; and iii) studies of dehydration-induced phase transitions and demixing in model systems of plasma membrane lipids. These studies indicate that i) the ultrastructural alterations are a consequence of the close approach of bilayers that occurs during freeze-induced dehydration and which results in the demixing of membrane components; ii) the decreased propensity for dehydration-induced lamellar-to-hexagonal$_{II}$ phase transitions occurs during the first week of the cold acclimation period and is associated with increased hydration of the plasma membrane lipids; and iii) this change is effected by an increase in the proportion of unsaturated species of phosphatidylcholine.

Cornell University
Ithaca, NY 14853

40. Studies of the Genetic Regulation of the Thermomonospora Cellulase Complex

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

$142,000 (FY90 funds/two years)

The goals of this project are to determine the molecular mechanisms regulating cellulase synthesis in the soil bacterium *Thermomonospora fusca* and to determine the molecular mechanism by which *T. fusca* cellulases degrade crystalline cellulose. The genes for *T. fusca* cellulases E_1,E_2,E_4 and E_5 have been sequenced and show no significant homology with each other beyond the presence of a common 14 bp control sequence just upstream of their initiation codons. The amino acid sequence of E_5 shows 37% identity to a *Bacillus* cellulase. The amino acid sequence of E_2 shows 50% identity to a *Microbispora* cellulase and 37% identity to a *Cellulomonas fimi* endogluconase, while the E_4 amino acid sequence shows 35% identity to an avocado cellulase. Proteolytic derivatives of both E_2 and E_5 have been isolated that are missing about 25% of the parent protein from the C-terminus and the N-terminus respectively. These proteins have full catalytic activity on CMC but bind much less tightly to cellulose than the native proteins. Both sequences that are removed contain regions resembling the cellulose binding sites of other cellulases. The E_2 proteolytic product has been purified and is being crystallized and if suitable crystals are obtained, its 3 dimensional structure will be determined. We are trying to determine the active sites of these enzymes by sequence comparisons with homologous cellulases, *in vitro* mutagenesis and the synthesis and use of affinity labeling reagents. Once potential active site residues are identified, they will be modified by site-directed mutagenesis and the properties of the mutant enzymes will be determined.

Cornell University
Ithaca, NY 14853-7201

41. Conversion of Acetic Acid to Methane by Thermophiles
S.H. Zinder, Department of Microbiology *$192,000 (FY90 funds/two years)*

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 isolation of the thermophilic *Methanothrix* sp. strain CALS-1 which grows much more rapidly (Td = 24 h) than do mesophilic cultures; 2) the demonstration that acetate utilization by the thermophilic *Methanothrix* proceeds at maximal rates at acetate concentrations as low as 100 μM, lower than that described for any other aceticlastic methanogen; 3) the demonstration of thresholds for acetate utilization of 15-20 μM and 1-2.5 mM for a thermophilic *Methanothrix* and *Methanosarcina*, respectively; 4) the demonstration that *Methanothrix* sp. strain CALS-1 has high levels of a very thermostable carbon monoxide dehydrogenase which associates with other proteins; 5) the isolation in axenic culture of the acetate-oxidizing rod-shaped (AOR) member of a thermophilic two-membered coculture which converts acetate to methane using interspecies hydrogen transfer; 6) the demonstration that axenic cultures of the AOR can also growth on H_2-CO_2 and produce acetate, the reverse of the reaction it carries out in coculture and that the AOR has high levels of CO dehydrogenase, while levels of formyltetrahydrofolate synthetase and of folates were extremely low; 7) the finding that entropy effects needed to be accounted for to explain the high partial pressure of hydrogen found in the syntrophic acetate oxidizing coculture when growing at 60°C; 8) the isolation and characterization of *Desulfotomaculum thermoacetooxidans*, a thermophilic sulfate-reducing bacterium capable of using acetate. Current research centers on further characterization of the thermophilic *Methanothrix* and factors which allow it to compete with *Methanosarcina*.

University of Delaware
Lewes, DE 19958

42. Metabolic Mechanisms of Plant Growth at Low Water Potentials
J.S. Boyer, College of Marine Studies *$75,842*

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 in germinating seedlings, turgor in most of the cells is completely maintained when water potentials are low enough to inhibit growth. However, gradients in water potential decrease between the vascular tissue and the enlarging cells, which inhibits water entry and therefore growth. A few hours later, the extensibility of the cell walls decreases and a 28kD protein accumulates in the walls. The protein does not accumulate in the mature tissue of soybean stems nor in the roots, where growth continues unabated. Antibodies to this protein show an immunologically related protein in the cytoplasm. The gene deduced from cDNA clones for the wall protein is identical to the gene for a protein that also accumulates in vacuoles of depodded soybean plants. The relationship between the wall protein and vacuolar protein is being investigated by exploring changes in cell content of mRNA for the proteins. The correlation between the appearance of the wall protein and the growth response to low water potentials suggests that the protein could play a role in growth.

Duke University
Durham, NC 27706

43. Stable Isotope Fractionation in Photosynthesis
C.B. Osmond, Department of Botany *$174,000 (FY89 funds/two years)*

Laboratory systems for stable isotope analyses of carbon, hydrogen and oxygen have been installed, calibrated and applied to a test system, the hypsophylls (shucks) of the C_4 plant *Z. mays* which have $\delta^{13}C$ values 3 - 4$^{o}/_{oo}$ more negative than other parts of the plant. The proportions of carbon derived from heterotrophic sources (sucrose from C_4 photosynthesis in other leaves), and from concurrent C_4 photosynthesis and C_3 photosynthesis in the poorly differentiated mesophyll and bundle sheath complex of the outer hypsophylls, were determined from analysis of $\delta^{13}C$ and $\delta^{2}H$ values of shuck cellulose. These methods have been applied to algal colonies on agar plates. In preliminary experiments, wild-type *Chlamydomonas reinhardtii* provided with CO_2 and acetate is mixotrophic, acquiring only 29% of total carbon from CO_2. The *psbA* deletion mutant, transformed with a herbicide resistant gene, *psbA* remains 100% heterotrophic in the presence of CO_2 and acetate, even though it is feebly autotrophic on CO_2 alone. Improved control of copy number during transformation has been obtained, and a program of site directed mutation of the *psbA* and *psbD* genes has begun. Protocols for the biochemical evaluation of these transformants, using fluorescence analysis and photosynthetic O_2 evolution, have been developed. Physiological evaluation of the performance of these transformants will be related to biochemical characteristics and specific structural modifications in the D1 polypeptide of PSII using stable isotopes.

Duke University
Durham, NC 27706

44. Molecular Studies of Functional Aspects of Higher Plant Mitochondria
J.N. Siedow, Department of Botany *$148,000 (FY90 funds/two years)*

Mitochondria isolated from *cms*-T lines of maize are sensitive to a toxin (BmT-toxin) derived from the fungus *Bipolaris maydis*, race T. Toxin sensitivity is associated with a mitochondrial-encoded 13 kDa protein, URF13, that interacts with BmT-toxin to produce channels within the inner mitochondrial membrane. The goal of this research (carried out in collaboration with C.S. Levings, N.C. State University) is to characterize the mechanism by which URF13 and BmT-toxin interact to permeabilize biological membranes. The expression of URF13 in *E. coli* confers BmT-toxin sensitivity on the resulting cells, and binding studies with radiolabeled toxin have established that toxin binds specifically to *E. coli* expressing URF13. Site-directed mutagenesis of URF13 in *E. coli* has been used to generate cells that are no longer sensitive to toxin. Mutants that confer toxin resistance can be separated into those that do not bind toxin and those that bind toxin but no longer form a membrane channel. This allows characterization of the URF13 protein domains responsible for toxin binding versus channel formation. Labeling studies are being used to determine the topological orientation of URF13 within the membrane while crosslinking studies have established the oligomeric nature of URF13 within the membrane. Both approaches are being coupled with site-directed mutagenesis to determine the structural basis of the URF13/BmT-toxin interaction and channel formation. Finally, attempts are being made to purify URF13 with the goal of reconstituting URF13 into liposomes to develop a more well-defined system for looking at URF13/toxin interactions.

Florida State University
Tallahassee, FL 32306

45. Guard Cell Biochemistry - Response to Environmental Stimuli Causing Changes in Gas Exchange

W.H. Outlaw, Jr., Department of Biological Sciences *$100,572*

Stomatal aperture size is regulated to effect a compromise between the opposing priorities of avoiding water loss and of admitting CO_2. Pore enlargement is brought about by swelling of the subtending guard cell pair, a result of accumulation of solutes from the apoplast and synthesis of low MW substances. The specialized metabolism that these cells have evolved to fulfill their crucial role in the plant's physiology extends to more subtle aspects. Using methods developed with DOE support, we have followed the kinetics of ABA-concentration changes in various leaf cells *in planta*. The ABA concentration in guard cells increases before it does in other cells after water stress is imposed. Guard cells do not immediately have lowered ABA concentration after stress is relieved, as do other leaf cells. We have determined the effect of pH, malate and glucose-6-P on guard-cell PEPC, using real-time methods with sufficient sensitivity for single-cell assays. These results indicate that cellular glucose-6-P (which we have measured) is a potent regulator and more important than previously considered. Several aspects of the PEPC assay have been studied, including the appraisal of the effects of PEP.Mg dissociation. We are pursuing to the end cytoplasmic malate determinations. (These values are required to interpret data from many areas of plant physiology.) We have turned to root hairs, which at some stages are virtually devoid of vacuoles (where the bulk of cellular malate is).

University of Florida
Gainesville, FL 32611

46. Ethanologenic Enzymes of Zymomonas mobilis

L.O. Ingram, Department of Microbiology and Cell Science *$77,894*

The glycolytic and ethanologenic enzymes in the bacterium, *Zymomonas mobilis*, constitute 30% to 50% of the soluble cellular protein. These high levels of enzymes are essential for ATP production via the Entner-Doudoroff pathway in this obligately fermentative organism. This differential expression of central pathway and biosynthetic enzymes represents one of the most fundamental forms of regulation, balancing enzymatic requirements for the production of carbon skeletons and energy against the need to divert portions of these skeletons for the biosynthesis of macromolecular precursors.

Over 95% of the glucose metabolized by this organism is converted to ethanol and carbon dioxide, both easily measured fermentation products. This coupled with the lack of allosteric control in the glycolytic enzymes make *Z. mobilis* an attractive organism for investigations of glycolytic flux and the extent to which control resides in individual enzymes.

Five of the glycolytic genes from *Z. mobilis* have been cloned, sequenced, and their features compared to determine possible mechanisms for high level expression. These genes are present as single copies on the chromosome of *Z. mobilis* but are expressed at very high levels. Four of the five contained a highly biased usage of codons and canonical Shine-Dalgarno sequences appropriately spaced for ribosomal binding. All contained tandem promoters with extended untranslated leader sequences which may form extensive stem loop structures. Partial conservation of promoter structure was evident in the "-10" and "-35" regions.

Antibodies have been prepared to most of these enzymes and a 2-dimensional IEF/PAGE map of these proteins has been made. Two isoenzymes of alcohol dehydrogenase are present which are genetically unrelated; both function during normal growth. ADHI is a typical zinc enzyme related to the eukaryotic enzymes. The iron-activated ADHII defined a new evolutionary group which now includes butanol dehydrogenase from *Clostridium acetobutylicum* and propanediol oxidoreductase from *Escherichia coli*.

Although the genes encoding glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase form an operon, GAP protein is approximately 5-fold more abundant than PGK. This differential expression does not involve attenuation but results from endolytic processing of the longer message to inactivate PGK. Messages for the glycolytic enzymes appear to be very abundant and unusually stable with half-lives of around 15 min. The insertion of multiple copies of these genes into *Z. mobilis* results in much less than the expected level of expression of mRNA, protein, and enzymatic activity, suggesting the possible existence of soluble factors which place an upper limit on their transcription.

University of Florida
Gainesville, FL 32611

47. Gene-Enzyme Relationships in Somatic Cells and Their Organismal Derivatives in Higher Plants
R.A. Jensen, Department of Microbiology and Cell Science *$99,586*

The biochemical genetics of aromatic amino acid biosynthesis in higher plants is under study. We have proposed a "dual-pathway" hypothesis of compartmentation for enzymes functioning in either the chloroplast (or other plastids) or in the cytosol. Comparative work indicates that enzymological features of pathway organization and regulation are generally similar in higher plants. We are developing a rigorous system of tissue culture in *Nicotiana silvestris*, an initial emphasis being the characterization of exact growth rates in the presence of amino acids, amino-acid analogues, and herbicides such as glyphosate. Environmental treatments such as mechanical wounding have been shown to induce elevated enzyme levels of the entire primary pathway of aromatic biosynthesis, and the molecular basis for this is under study. The long-term goal is to isolate a complete set of structural-gene and regulatory-gene mutants in totipotent cells that will regenerate to the organismal stage. This will facilitate an understanding of the basic nature of developmental processes by exploitation of defined genetic alterations. Analysis of this pathway should contribute heavily to an understanding of the biology of the plant because the pathway generates protein precursors, vitamins, growth regulators, a vast array of secondary metabolites, and medically significant pharmacological agents.

University of Georgia
Athens, GA 30602

48. The Metabolism of Hydrogen by Extremely Thermophilic Bacteria
M.W.W. Adams *$74,902*

Extremely thermophilic bacteria are a remarkable and unique group of microorganisms that grow optimally up to 105°C. They are a very recent discovery and have been isolated only from volcanic areas which include deep sea hydrothermal vents. We are studying the metabolism of hydrogen (H_2) by five extreme thermophiles grown *in vitro* and are examining their hydrogenases, the enzyme responsible for catalyzing H_2 production and H_2 oxidation, and related enzymes. For example, the archaebacterium, *Pyrococcus furiosus*, grows optimally at 100°C and produces H_2 via a fermentative-type metabolism. We have purified the hydrogenase and its physiological electron carrier, a novel ferredoxin, together with a rubredoxin, pyruvate ferredoxin oxidoreductase and a novel tungsten-containing enzyme which oxidizes aldehydes to acids. The latter two enzymes couple substrate oxidation to H_2 production via the ferredoxin and hydrogenase. The hydrogenase is a nickel-iron-sulfur protein and the optimum temperature for catalyzing both H_2 evolution and H_2 oxidation is above 95°C. It preferentially catalyzes H_2 production at all temperatures and appears to represent a new type of "evolution" hydrogenase. In contrast, the hydrogenase of *Thermotoga maritima*, the most thermophilic eubacterium currently known (T_{max}=90°C), contains iron-sulfur clusters but not nickel and preferentially catalyzes H_2 activation. It lacks the H_2-activating Fe-S cluster of mesophilic hydrogenases and appears to be a new type of "uptake" Fe-hydrogenase. Since molecular H_2 plays a central role in the commercial production of fuels and numerous chemicals, our long term objective is to assess the potential utility of extremely thermophilic hydrogenases in industrial energy conversions.

University of Georgia
Athens, GA 30602

49. The Structures and Functions of Oligosaccharins
P. Albersheim, Complex Carbohydrate Research Center *$162,713*

This project is concerned with the isolation and characterization of oligosaccharins, which are naturally occurring complex carbohydrates that possess biological regulatory activities. We have evidence that oligosaccharins, when released from the complex carbohydrates of cell walls, regulate various biological functions within plants. We are studying the following oligosaccharins. [1] Oligosaccharins isolated from plant cell walls that elicit phytoalexin (antibiotic) accumulation in plant tissues. Research in this area is emphasizing the involvement of fungal *endo*polygalacturonases (EPGs) and a plant-derived inhibitor (PGIP) of the fungal EPGs in the release of elicitor-active oligogalacturonides (DP=10-14) from plant cell walls. We are particularly interested in elucidating the mechanism by which EPGs cause necrosis in some plants. [2] An oligosaccharin that may trigger the hypersensitive resistance response in plants. We have purified to homogeneity an *endo*xylanase and an arabinosidase secreted by *Magnaporthe grisea* that act in concert to release the oligosaccharin from isolated plant cell walls. We are in the process of identifying the bioactive oligosaccharide(s). [3] Fungal glucans when sprayed on *Nicotianae* protect the *Nicotianae* from virus infection. The resistance does not involve any of the well-characterized mechanisms of resistance such as phytoalexin, lignin, callose, or hydroxylproline-rich glycoprotein synthesis, or the accumulation of pathogenesis-related proteins. We have evidence that the glucan selectively activates synthesis of the mRNA of a glycine-rich cell wall protein. We are trying to ascertain whether accumulation of these glycine-rich proteins is involved in defense against virus infection. We are also trying to identify the glucan oligosaccharide (it is not the phytoalexin elicitor) that activates viral defense. [4] Oligosaccharins that are able to induce flowers and vegetative shoots and inhibit roots in tobacco epidermal explants. Our studies with tobacco explants are emphasizing an oligosaccharin, released from plant cell walls by *endo*-α-1,4-polygalacturonase, that stimulates floral development and inhibits root development.

University of Georgia
Athens, GA 30602

50. CarbBank - A Structural and Bibliographic Database for Complex Carbohydrates
P. Albersheim, Complex Carbohydrate Research Center *$100,000*

CarbBank is a computer program that, for the first time, enables scientists to systematically and rapidly search for published complex carbohydrate structures. Carbohydrate structures linked to literature citations in a computerized database (i) allows comparison of known with newly discovered carbohydrate structures, (ii) brings to scientists an awareness of structural heterogeneity, for example, the attachment of several different oligosaccharides to the same amino acid of a protein, (iii) leads to comparisons of carbohydrate structures between species, and (iv) assists in predicting carbohydrate structures based on limited structural information or properties of known molecules. CarbBank is enhancing the research efficiency and capabilities of many scientists besides those specializing in carbohydrates and is, for the first time, making carbohydrate structures accessible to a wide spectrum of scientists.

The CarbBank program was written with extensive input from specialists in the various disciplines of carbohydrate chemistry in order to create an environment that meets the needs of as wide a range of investigators as possible. CarbBank is menu-driven and has a rich selection of context-sensitive help screens. CarbBank has an *editor* to create and/or edit complex carbohydrate structures and associated *text information*, a *filer* module that helps create, merge, and maintain database files, and a *search* module to find and retrieve structures based on search criteria that the user supplies. CarbBank can export entire databases (or partial databases depending on search results) to text files and can import text file information and transform it into a CarbBank database file. CarbBank uses a syntax checker during data entry (i.e., during import or editing) to warn the user of entry errors or violations of carbohydrate chemistry

conventions. CarbBank uses structure nomenclature that is similar to the nomenclature used by Carbohydrate Research, and the list of available building blocks for structures (glycosyl residue and non-carbohydrate) is expandable and under review by an international nomenclature committee. CarbBank uses a sophisticated system of data coding and indexing so that searches are very rapid.

The international CarbBank Board of Overseers, with the special assistance of its Executive Committee, has selected and approved some 40 curators in about 20 countries. Curators are identifying and providing the database with the structures and bibliographic information of over 100 complex carbohydrates per year that are entered into the Complex Carbohydrate Structure Database. CarbBank was initially distributed in October 1989 and at that time contained the structures and bibliographic information for about one-third (~2,000) of all published carbohydrate structures larger than a disaccharide (~6,000). It is hoped that all existing carbohydrate structures will be entered into CarbBank within three years. The database is distributed semi-annually on magnetic media (5.25-inch floppy diskette and 3.5-inch hardshell disk). For information about CarbBank contact: Dana Smith, CarbBank Data Manager, Complex Carbohydrate Research Center, The University of Georgia, 220 Riverbend Road, Athens, GA 30602, Telephone: 404-542-4484, Fax: 404-542-4412.

University of Georgia

Athens, GA 30602

51. The University of Georgia Complex Carbohydrate Research Center (CCRC) - A Department of Energy Unit of the USDA/DOE/NSF Plant Science Centers Program

P. Albersheim and A. Darvill, Complex Carbohydrate Research Center

$1,264,000 (FY90 funds/15 1/2 months)

The CCRC, with its multidisciplinary faculty and staff, was formed to serve as a national resource for basic research in complex carbohydrates. The CCRC actively assists in defining the structures and studying the biological functions of plant and microbial carbohydrates. Research, training, and service activities are components of this program. Research focuses on various aspects of carbohydrate science, including methods development, structural characterization, and function elucidation. Educational activities involve the training of graduate students, postdoctoral associates, and visiting scientists in the analytical methods used for studying carbohydrate structures. Two week-long laboratory training courses and a symposium were held in May 1990. Services offered involve conducting routine analyses of carbohydrate samples provided by scientists from other institutions. These analyses include determination of glycosyl-residue and glycosyl-linkage compositions, and acquisition and interpretation of one-dimensional NMR and FAB-MS spectra. The CCRC also forms collaborations with scientists on more extensive research projects. These services and collaborative investigations are limited to non-proprietary research. Those interested in assistance, collaboration or training should write to: Dr. Russell Carlson, Technical Director, Complex Carbohydrate Research Center, The University of Georgia, 220 Riverbend Road, Athens, GA 30602.

*(A unit of the USDA-DOE-NSF Plant Science Center Program.)

University of Georgia
Athens, GA 30602

52. Structural Studies of Complex Carbohydrates of Plant Cell Walls
A. Darvill, Complex Carbohydrate Research Center *$367,048*

The cell walls of a plant determine the plant's structure and morphology and act as a barrier to pests. Cell walls are also a source of complex carbohydrates with biological regulatory properties (oligosaccharins). This project involves the isolation and structural characterization of the complex carbohydrates that constitute approximately 90% of the walls of growing plant cells. These structural studies emphasize detailed analyses of two pectic polysaccharides, rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II), and the hemicellulosic polysaccharide xyloglucan. We are chemically and enzymatically fragmenting the backbone of RG-I to produce RG-I side chains, which will be purified and structurally characterized. We propose to use affinity chromatography to determine whether RG-I is a family of polysaccharides with different numbers, populations, and orders of side chains. We are continuing the characterization of the cell wall pectic polysaccharide RG-II by using a combination of chemical and enzymatic methods to determine the order and positions of attachment of the side chains to the backbone with the aim to elucidate the entire glycosyl sequence of this polysaccharide. We are continuing the structural characterization of cell wall xyloglucan by characterizing endoglucanase-resistant xyloglucan oligosaccharides (D.P. ≈15-20) and a novel undecasaccharide subunit. We are generating and characterizing monoclonal antibodies to specific cell wall epitopes for use in localizing specific polysaccharides and polysaccharide structures within the plant cell wall. The research supported in this project will increase our knowledge of the primary structures of cell wall polysaccharides and of their locations in the cell walls and, we hope, enable us to build a more complete model of the primary cell wall of plants.

University of Georgia
Athens, GA 30602

53. Molecular Biology of Lea Genes of Higher Plants
L. Dure III, Department of Biochemistry *$142,992 (FY90 funds/two years)*

The *Lea* genes of cottonseed produce proteins that are *l*ate embryogenesis *a*bundant. Some of these genes appear to be induced to high levels of expression by ABA and/or by water stress in mature tissues of the plant. Others appear to be expressed only in seeds. Presently, we are examining tissue/cell type specificity of 5 of these genes by *in situ* hybridization of their mRNAs. Several of these genes are being expressed as protein in *E. coli* to provide protein for antibody preparation which in turn will be used for *in situ* localization of *LEA* proteins within cells and for monitoring large scale protein purification.

The *in vivo* 3D structures of several of the *LEA* proteins are being studied by biophysical techniques and computer modelling. Two *LEA* proteins appear to exist as polymers of helical bundles whereas two others appear to have no set structure. The latter property, resulting from extreme hydrophilicity suggests a "chaperone" function during seed desiccation.

University of Georgia
Tifton, GA 31793

54. Development of Innovative Techniques That May Be Used as Models to Improve Plant Performance
W.W. Hanna, G.W. Burton, Department of Agronomy *$37,057*

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. A new stable cytoplasmic-nuclear male sterile cytoplasm, A_4, transferred from a wild grassy subspecies to cultivated pearl millet has been described. Five true-breeding (apomictic) interspecific hybrids produced forage yields equal to or exceeded up to 61% that of the best commercial pearl millet forage hybrids. Pearl millet grain in a poultry diet replaced corn without adversely affecting gain or feed efficiency of chicks. The male parent of the grain hybrid was derived from a wild *P. purpureum* in the secondary gene pool. Eight wild *Pennisetum* species with various chromosome numbers and ploidy levels were crossed with diploid and tetraploid pearl millet. Three species, *P. orientale, P. setaceum,* and *P. squamulatum* were successfully crossed with pearl millet. Average seed fertility of two tetraploid pearl millet inbreds was increased eight fold by crossing the inbreds and selecting for higher fertility. The overall impact is increased, more efficient, and more reliable production of food and forage.

University of Georgia
Athens, GA 30602

55. Environmental Stress-Mediated Changes in Transcriptional and Translational Regulation of Protein Synthesis in Crop Plants
J.L. Key, Department of Botany *$152,000 (FY89 funds/two years)*

The influence of high temperature stress (heat shock or HS) and other environmental stress agents on gene expression is under investigation. Areas of research during the current year included (1) identification of hsps synthesized on ER-bound polysomes (an indication of membrane localization); (2) analysis of the effects of HS on proton pumping by microsomal membranes; (3) molecular characterization of cDNAs/gene for a *hsp70* and a *hsp84* of soybean and *Arabidopsis*, respectively; (4) analysis of self-regulation of the HS response using amino acid analogs and cycloheximide (CH); and (5) use of the *Gmhsp17.5E* promoter cassette for high-level expression of various chimeric genes.

Of the many HS gene families, only those represented by cDNA clones pFS2033, pEV6, and pEV2 (possibly members of the same family) hybridized on Northern blots to mRNAs isolated from ER-bound polysomes. These and other data provide strong evidence that pFS2033-related hsps associate with some membrane system in a protease insensitive manner. mRNAs of other HS gene families are present only on free polysomes.

The amino acid analog, azetidine-2-carboxylic acid, induced the accumulation at 28°C of all HS mRNAs except for pFS2033-related and pEV3 (chloroplast-localized hsp) mRNAs; however, only limited hsp synthesis (primarily the high molecular weight families) occurred at 28°C. A full complement of HS mRNAs and hsps was synthesized at 40°C in the presence of analog. HS mRNAs in the absence of analog reach the maximum level by 1 to 2 hr and then slowly decay at HS temperatures; HS mRNAs continued to accumulate for several hr in analog-treated tissue. The addition of CH at a concentration

which inhibited protein synthesis by 60 to 70% also extended by a few hr the accumulation of HS mRNAs.These data, taken together, are consistent with the involvement of some protein(s) synthesized during HS in self-regulation of the HS response.

The HS cassette with its very strong promoter has been used in a number of chimeric gene constructs for over- and under-expression of a particular gene product (e.g., indoleacetamide hydrolase and the isopentenyl transferase) in a number of physiological and developmental studies. Transformed *Arabidopsis* plants (second generation) are being evaluated for phenotypic effects of over-and under-expression of the *hsc70* gene (under-expression of closely related hsc and *hsp70* genes might also be expected with these antisense constructs).

University of Georgia
Athens, GA 30602

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

R.B. Meagher, Department of Genetics *$81,838*

The goal of this research is to elucidate the mechanisms and determinants controlling RNA turnover and general chemical instability of RNA in higher plants. We are using several techniques to understand the general factors contributing to turnover of all RNAs and specifically to determine how the RNAs encoding soybean ribulose bisphosphate carboxylase small subunit (SSU) are degraded. These approaches include analysis of *in vivo* RNA structures by chemical modification; analysis of SSU transcription rates and RNA levels under a variety of light regimes to demonstrate differential RNA stability in light and darkness; analysis of SSU RNAs and control RNAs on polysomes to demonstrate that SSU RNA is preferentially loaded onto polysomes; and the analysis of soybean SSU RNA expression in transgenic plants. A project has just been initiated in which plant cDNAs encoding various RNases will be isolated by complementing conditional mutants in *E. coli* that have altered patterns of RNA processing. In the next year of this project we hope to finish mapping the major degradation products for one soybean and one petunia SSU transcript and design a general model for SSU RNA degradation in plants.

University of Georgia
Athens, GA 30602

57. Microbiology and Physiology of Anaerobic Fermentations of Cellulose

H.D. Peck, Jr., L.G. Ljungdahl, L.E. Mortenson, J.K.W. Wiegel, Departments of Biochemistry and Microbiology *$617,670 (FY89 funds/two years)*

This project involves the biochemistry and physiology of four major groups (primary, secondary, ancillary and methane bacteria) of anaerobic bacteria, that are involved in the conversion of cellulose to methane or chemical feedstocks. The primary bacterium, *Clostridium thermocellum*, has a cellulolytic enzyme system capable of hydrolyzing crystalline cellulose and consists of polypeptide complexes ranging in M_r from 5 to 100 million. The complexes attached to the substrate cellulose with the aid of a low molecular (about 1000 daltons) yellow affinity substance (YAS) produced by the bacterium in the presence of cellulose. Properties of the complexes and YAS are studied. Research on the secondary and ancillary bacteria includes acetogens, clostridia, methanogens and sulfate reducing bacteria (SRB). Aspects of metabolism are being studied which appear to be relevant for the interactions on consortia and their bioenergetics, particularly related to hydrogen, formate, CO, and CO_2. The molecular basis of interspecies H_2-transfer and H_2-cycling, electron-transfer proteins, ATPase system and enzymes of one-carbon metabolism will receive special focus. Most bacteria appear to produce different proteins with hydrogenase activity which are presumed to be regulated by conditions of growth. Five different hydrogenases have been characterized in detail from the bacteria of interest: The O_2-labile 12Fe

bidirectional hydrogenases from *C. pasteurianium* and *A. woodii*; the O_2-labile 8Fe uptake hydrogenase from *C. pasteurianium*; and O_2-stable 12Fe hydrogenase from *D. vulgaris* and the O_2-stable (NiFe) and (NiFeSe) found in *D. vulgaris*, other SRB's and the methanogens. The structure of the metal clusters and their roles in the activation of H_2 are being investigated, and genes for the hydrogenases cloned and sequenced to obtain structural information, and have been established structural relationships among the hydrogenases. The goals are to understand the roles and regulation of hydrogenases in interspecies H_2 transfer, H_2 cycling and the generation of a proton gradient. The formate dehydrogenases have characteristics in common with the hydrogenase system: multiple enzyme species with different metal redox centers, MoSeFe, MoFe, WSeFe and pterin; cytoplasmic and periplasmic localizations and involvement in the generation of a proton gradient by vectorial electron transfer. The structures of the metal clusters and their role in the metabolism of formate will be investigated with the goal of understanding the function of formate in the total synthesis of acetate from CO_2 and its role in the bioenergetics of these microorganisms. CO dehydrogenase, a key enzyme in the new anaerobic autotrophic CO_2 fixation pathway contains Ni plus non-heme iron and the structure of its metal redox centers will continue to be investigated. Additionally, the enzyme studies will be performed using thermophiles and also the isolation of some new pertinent species. The project will also include research on the mechanism of extreme thermophily (growth over 70°) in bacteria that grow over a temperature span of 40°C or more. These bacteria exhibit a biphasic growth response to temperature and preliminary evidence suggests that the phenomenon is due to the expression of a new set of enzymes. These initial observations will be extended employing techniques of molecular biology.

University of Georgia

Athens, GA 30602

58. Characterization of Phytochrome and the Genes that Produce It In Photosynthetically Competent Avena

L.H. Pratt, Department of Botany *$63,000*

Photomorphogenic responses in plants typically result in an increase in the efficiency with which they convert solar to chemical energy via photosynthesis. Phytochrome is a photosensory pigment that controls many of these responses. Most of what is known about phytochrome is derived from study of this chromoprotein obtained from dark-grown plant tissues. Previous DOE-supported research, however, led to the discovery that the phytochrome that is most abundant in photosynthetically competent plants is different from that which predominates in dark-grown tissues of the same organism. We therefore made monoclonal antibodies directed to and specific for this newly discovered green-oat phytochrome to assist in its characterization. Somewhat unexpectedly these antibodies led to the further discovery that green-oat phytochrome is itself composed of two populations. Because each of these populations is only about 1 part in 50,000 to 100,000 of readily extractable protein, current efforts are directed towards cloning the genes that encode them. For this purpose, we have engineered a vector that permits directional, in-frame cloning of PCR-derived DNA suitable for expression screening with our antibodies to green-oat phytochrome. Cloned coding sequences thus obtained will then be expressed to a high level in a suitable organism such as tomato, such that relatively large quantities of these pools of phytochrome can be obtained for its further physicochemical characterization. Information obtained with cloned genes will supplement that obtained by continued investigation of green-oat phytochrome in light-grown *Avena* itself.

University of Georgia
Athens, GA 30602

59. Nitrogen Control of Chloroplast Development and Differentiation
G.W. Schmidt, Department of Botany *$156,000 (FY90 funds/two years)*

Our work concerns how nitrogen deprivation alters photosynthetic membranes and carbon metabolism. In addition to biochemical and biophysical analyses, we study gene expression in the chloroplast and nuclear compartments with *Chlamydomonas reinhardtii* grown in ammonium-limited chemostats. As for nitrogen-deficient vascular plants, cells adapted to such conditions are reversibly chlorotic. Chlorosis mostly is due to deficiencies of major light-harvesting complexes although most other pigment-protein complexes also are reduced by 50%. Synthesis of light-harvesting apoproteins is blocked because transcripts of their nuclear genes are virtually absent. However, products of the multigene family that normally are minor are conspicuous in limited cells; these appear to give rise to novel, efficient antenna arrays for Photosystems I and II. In N-limited plastids, synthesis of Photosystem II reaction center proteins is restricted by mRNA translation whereas apoproteins of Photosystem I are made at normal rates. Despite uncoupled synthesis of the reaction centers, chloroplasts sustain CO_2 reduction and amass starch and triglycerides. N-limited thylakoids are surprisingly active in chlororespiration such that it constitutes 30% of total cell respiration. The activity correlates with high levels of thylakoid NAD(P)H dehydrogenase and novel cytochromes. The pathway may avert photoinhibition of N-limited cells by supplementing oxidation of the electron acceptor together with oxygen consumption. Current studies include further characterization of the chlororespiratory electron transport components and their genes. Additionally, many nuclear genes that are subject to both positive and negative regulation by nitrogen have been isolated and are analyzed to understand how nutrients selectively affect transcript levels.

University of Georgia
Athens, GA 30602

60. Molecular Characterization of a Maize Regulatory Gene
S.R. Wessler, Botany Department *$178,000 (FY90 funds/two years)*

The *R* gene family controls the distribution of anthocyanin pigments in the maize plant. This sensitive, non-lethal phenotype has facilitated the isolation of over 50 naturally occurring *R* variants; each displays a distinctive pigmentation pattern. During the previous funding period we determined that the *R* allele called *Lc* encodes a polypeptide with features of a transcriptional activator including large acidic and basic domains and the *myc* DNA-binding dimerization motif.

We have proposed that all *R* genes encode proteins that are functionally equivalent to the Lc protein and that *R* gene diversity reflects promotor diversity. In support of this model we have recently demonstrated that when a 35S:Lc cDNA construct is introduced into maize tissues by particle bombardment pigmentation is induced in virtually all maize tissues, even in tissues that *Lc* does not normally pigment.

Future experiments are directed toward testing our model of *R* gene diversity and learning how the R protein activates transcription. We will (l) utilize biochemical assays coupled with the microprojectile assay to localize regions of the R protein responsible for DNA binding, transcriptional activation and nuclear localization, (2) characterize the stable derivatives of 14 *R-sc Ds* alleles (in collaboration with Jerry Kermicle) with insertions presumed to be in the R protein coding region and (3) use immunolocalization and *in situ* hybridization to localize *R* gene products and analyze their temporal appearance.

University of Georgia
Athens, GA 30602

61. Hemicellulases from Anaerobic Thermophiles
J. Wiegel, Department of Microbiology *$102,098 (FY89 funds/two years)*

The goal of this research effort is to obtain a modified anaerobic thermophilic bacterium that more efficiently than the wild type converts various hemicellulose-containing biomass to ethanol. The strategy for reaching this goal is to genetically modify the formation and regulation of rate-limiting hemicellulases in the ethanologenic, thermophilic, anaerobic bacterium *Thermoanaerobacter ethanolicus* isolated previously by us. This approach requires some knowledge of the involved hemicellulases. Thus, for this application the short term goal is to characterize the hemicellulases. This will extend the presently limited knowledge of hemicellulases in anaerobic bacteria. So far no xylanases have been purified from the extreme thermophilic anaerobes. The objective includes the following tasks: i) purification and characterization of the hemicellulases (namely xylanases) from *T. ethanolicus*, ii) purification and isolation of the special xylanases(s) and xylosidase from *Clostridium thermohydrosulfuricum* which effectively degrade 4-0-methyl glucuronic acid substituted xylans, and iii) the elucidation of the regulatory properties of these enzymes. The next step will be to synthesize gene probes of the characterized xylanases so the organism can be genetically modified towards a more efficient hydrolysis of the various xylans.

Harvard University
Cambridge, MA 02138

62. Unraveling Photosystems
L. Bogorad, Department of Cellular and Developmental Biology *$103,530*

The objective of this project is to identify and characterize protein components of the energy-transducing reaction centers in photosynthetic membranes and to understand how these components are arranged and function in the membrane. This information is essential for understanding how the photosynthetic apparatus works. One approach to determining the role of products of chloroplast genes is to identify and isolate the homologous gene in the facultatively heterotropic single celled cyanobacterium *Synechocystis* PCC 6803, to inactivate the gene in this organism and then to study the phenotype of the mutant so generated. This is being done with some chloroplast genes. Another approach to identifying, characterizing and establishing the role of proteins of the photosynthetic apparatus is to generate photosynthetic mutants of *Synechocystis* PCC 6803 and then to identify DNA sequences that correct the mutant phenotype. Cyanobacteria are relatively simple organisms that carry out the same type of oxygen-evolving photosynthesis as chloroplasts of higher green plants but they are more convenient for certain experiments. Mutants isolated to date have been very useful for revealing the paths of transfer of excitation energy within the photosynthetic apparatus; mutants with other types of genetic lesions are also being analyzed.

University of Idaho
Moscow, ID 83843

63. Genetics and Chemistry of Lignin Degradation by Streptomyces
D.L. Crawford, Department of Bacteriology and Biochemistry *$162,000 (FY89 funds/two years)*

Our research goal is to define how lignin peroxidases are involved in lignin solubilization by *Streptomyces*. Studies of *S. viridosporus* T7A and *S. badius* 252 show that each organism produces 4 lignin peroxidase isoforms differing in substrate specificities and possibly acting synergistically to oxidize lignin. The enzymes of T7A and 252 are immunogenically related. All isoforms from each organism react with antibodies prepared against purified isoform ALip-P3 of T7A. These studies also showed that the *Streptomyces* peroxidases are not immunogenically related to fungal lignin peroxidase H8 of *Phanerochaete chrysosporium.* Presently, we are comparing DNA sequences of fungal and actinomycete genes and are isolating additional peroxidase genes from *Streptomyces*. An antibody affinity purification procedure was developed for the simple purification of lignin peroxidase ALip-P3. Contrary to previous conclusions, recent studies with strain T7A show that lignin peroxidase production is associated more with stationary phase than with growth phase. However, when the ALip-P3 gene is expressed in plasmid pIJ702 in recombinant *S. lividans* strains, production becomes solely growth associated. Several new *Streptomyces* have been shown to be superior to strains T7A and 252 in lignin-solubilizing activity. These strains produce lignin peroxidases similar to those of T7A, but in greater amounts. Recently, we found that recombinant lignin peroxidase-expressing *S. lividans* strains, when released into soil, increase the rate of carbon mineralization in the soils, presumably by affecting soil lignin depolymerization rates. Overall, our research is aimed at elucidating the mechanisms of lignin degradation by *Streptomyces*, with an ultimate goal of using genetic engineering to construct superior strains for use in bioconversion of lignin into useful chemicals.

University of Illinois
Urbana, IL 61801

64. Photosynthesis in Intact Plants
A.R. Crofts, Department of Physiology and Biophysics *$208,000 (FY89 funds/two years)*

The main goals of the project are the development of instrumentation and methodology for studying photosynthesis in intact plants, but the project includes 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 have previously designed and constructed laboratory and portable versions of a flash fluorimeter, and a flash spectrophotometer. During the last year we have upgraded the portable flash fluorimeter to allow measurement of a full kinetic trace in a single experiment. This makes it possible to monitor the state of PSII donor and acceptor reactions in intact leaves from their characteristic oscillatory patterns on flash activation. In field experiments, we have concentrated in assaying activation of the ATP-ase under normal and droughted conditions, since earlier work had suggested that this enzyme was a potential control site. We have established that activation is the same under both conditions, and that it occurs at such low light intensities as never to be rate determining. In the laboratory, we have been characterizing the kinetics and thermodynamics of electron transfer through the cyt b_6/f complex. We conclude that the enzyme functions through a modified Q-cycle, and have demonstrated the functionality of two b cytochrome centers with different potentials (cyts b_H and b_L). We have also characterized the kinetics of electron transfer, quinone binding, and inhibitor binding in atrazine susceptible and resistant strains of *Amaranthus hybridus*, and shown how the thermodynamic and kinetic characteristics of the two-electron gate differ in the two strains. We have sequenced the DNA encoding the Q_B-site using PCR methods and shown that resistance is due to a change of Ser-264 to glycine in the D1 protein. The detailed kinetic, thermodynamic and structural models we have proposed for the two electron gate and the cyt b_6/f complex will further our investigations and understanding of the factors which limit photosynthetic efficiency under field conditions. We are at

present working in several areas: a) on the acceptor side of photosystem II, we are developing molecular genetics techniques for modifying the two-electron gate; we will use the computer linked fluorescence video imaging system to screen for mutants, and the flash fluorimeter and stopped-flow mixing device to characterize the physico-chemical changes in modified sites; b) we are continuing our studies on electron transfer between the photosystems, and on cyclic electron flow round PSI, and will extend these to intact plants; c) several collaborative projects are using the fluorescence video imaging system in physiological studies, screening of mutants, environmental studies, etc.; and d) in collaborative research using the portable instruments we are measuring several environmental effects on the physiology and photosynthesis of intact plants, either in the laboratory or in the field. We will continue the development of novel instrumentation; we will make use of a combined biophysical/molecular engineering approach to probe detailed mechanism, and we will continue and extend the collaborations established around the novel instrumentation developed under the program.

University of Illinois
Urbana, IL 61801

65. Mechanism of Proton Pumping in Bacteriorhodopsin
T.G. Ebrey *$64,090*

The purple membrane of *Halobacterium halobium* probably represents the simplest biological solar energy conversion system. Light absorbed by bacteriorhodopsin, a small protein whose chromophore is retinal, directly leads to the transport of protons across the cell membrane. The resulting chemosmotic potential can be used to make ATP. An additional feature of the purple membrane is its ability to pump protons over a wide variety of salt concentration including in extreme saline environments. This project investigates the relationship between the transport of protons across the membrane and structure and conformation of bacteriorhodopsin. We have proposed experiments to study the pH dependence of proton pumping. Secondly, we are examining the role of divalent cations in the proton pumping process. We are also studying the effect of the large surface potential of the purple membrane on the proton pumping function of this membrane using the photocurrents associated with the pumping process.

University of Illinois
Urbana, IL 61801

66. Studies on the Escherichia coli Respiratory Chain
R.B. Gennis, Department of Biochemistry *$207,558 (FY90 funds/two years)*

Our primary interest is to learn how *Escherichia coli* generates a proton motive force during aerobic respiration. Previously, the cytochrome *o* complex was identified as a coupling site in the respiratory chain, where electron transfer is coupled to the electrogenic translocation of protons across the cytoplasmic membrane. The enzyme has been purified and this activity has been demonstrated in reconstituted residues. The clone of the *cyo* operon, encoding all the subunits of this terminal oxidase, reveals striking sequence homology between the bacterial ubiquinol oxidase and the well characterized mitochondrial cytochrome *c* oxidase. During the past year we have confirmed this similarity using biophysical techniques, and we have exploited the similarity to design site-directed mutagenesis experiments. Electron spin resonance as well as low temperature Fourier transform infrared spectroscopy have clearly shown that this enzyme contains a heme-copper binuclear center which is virtually identical to the oxygen-reactive center in cytochrome *c* oxidase. Work on the eukaryotic enzyme implicates histidines within subunit I as heme and copper ligands, and we have altered each of the seven totally conserved histidines to identify which are metal ligands. So far, each of the histidines has been shown to be essential for function. One (His 334) is almost certainly a copper ligand. Work during the next year will complete and extend these studies.

University of Illinois
Urbana, IL 61801

67. Regulation of Cell Division in Higher Plants
T. Jacobs, Department of Plant Biology *$158,000 (FY90 funds/two years)*

Cell division is arguably the most fundamental of all developmental processes. In higher plants, mitotic activity is largely confined to foci of patterned cell divisions called meristems. From these perpetually embryonic tissues arise the plant's essential organs of light capture, support, protection and reproduction. Once an adequate understanding of plant cell mitotic regulation is attained, unprecedented opportunities will ensue for analyzing and genetically controlling diverse aspects of development, including plant architecture, leaf shape, plant height, and root depth. The mitotic cycle in a variety of model eukaryotic systems is under the control of a regulatory network of striking evolutionary conservation. Homologues of the yeast *cdc2* gene, its product p34, and other components of the MPF complex have emerged as ubiquitous mitotic regulators. Higher plant genomes encode a p34-like gene product as well. As in other eukaryotic model systems, p34 of *Pisum sativum* appears to be a phosphoprotein with *in vitro* histone H1 kinase activity. Pea p34 also appears to occur in a high molecular weight complex, formally analogous to MPF of *Xenopus*. Our primary objective in this study is to gain baseline information about the regulation of the higher plant *cdc2*/MPF cell division control complex in non-dividing, differentiated cells as well as in synchronous and asynchronous mitotic cells. We are investigating *cdc2* expression and p34 activity at the levels of protein abundance, protein phosphorylation and quaternary associations.

University of Illinois
Urbana, IL 61801

68. Genetics of the Methanogenic Bacterium, Methanococcus voltae With Attention to Genetic Expression Mechanisms
J. Konisky, Department of Microbiology *$130,000 (FY90 funds/two years)*

The objective of this research program is to study the genetics, physiology and molecular biology of *Methanococcus voltae*, a marine archaebacterium. This obligate anaerobe produces methane from the reduction of carbon dioxide by hydrogen. For this study, we have chosen to study the molecular biology of the gene encoding a membrane associated ATPase which we believe plays an important role in methanogen energetics.

We intend to examine the molecular biology of the ATPase structural gene by using a multifaceted approach. The mRNA encoding the enzyme will be characterized to determine whether it is polycistronic. In addition, possible regulation of production and turnover of ATPase encoding mRNA will be examined under various physiological conditions.

Those DNA sequences which function to control ATPase gene expression will be examined. These include studies to identify DNA-dependent RNA polymerase binding sites as well as transcription start sites. Our ultimate goal is to determine the mechanisms by which gene expression is influenced by changes in cell physiology. Such studies may reveal novel mechanisms of gene expression in this group of important organisms.

University of Illinois
Urbana, IL 61801

69. Genetic and Molecular Studies on Cytoplasmic Male Sterility in Maize
J.R. Laughnan and S. Gabay-Laughnan, Department of Plant Biology

$170,000 (FY90 funds/two years)

The objective of this project is to determine the basic mechanisms of cytoplasmic male sterility (CMS) in maize and to understand the genetic and molecular bases for both nuclear and cytoplasmic reversions to male fertility. Genetic studies involve attempts to identify cases of insertion of transposable controlling elements into nuclear *cms-T* and *cms-S* restorer gene sites and, in the case of restorers of *cms-S*, also a search for cases in which a transposable restorer-of-fertility (*Rf*) gene is inserted into a wild-type maize gene. A number of spontaneous *Rf* genes has arisen in inbred nuclear backgrounds and these fall into two classes--functional and nonfunctional. Plants heterozygous for a newly-arisen *Rf* gene of either class are phenotypically male fertile, having normal anther exsertion and exhibiting the 50% pollen abortion expected of gametophytic restorers of *cms-S*. When fertile plants carrying function *Rf* genes are crossed as male parents onto *cms-S* male-sterile testers, or onto male-fertile isogenic maintainer plants with normal cytoplasm, there is seed set on the ears. When plants carrying nonfunctional *Rf* genes (*Rf-nf*) are handled similarly, there is no seed set on the ears. To date, eight independently-occurring spontaneous *Rf-nf* genes have been identified. The allelic relationships between all newly-arisen *Rf* genes is under study. Molecular analysis will be conducted in which the protein profiles of germinating pollen grains from *Rf-nf* strains will be compared with normal pollen grains from isogenic controls. *Rf-nf* genes have been found to be functional in hybrid backgrounds and the genetic nature of this hybrid vigor effect is under study. In *cms-S* plants, a number of newly-arisen *Rf* genes has been shown to be transposable, as has the standard *Rf* gene. Efforts to characterize these transposition events continue. Genetic studies will indicate whether *Rf* elements transpose to a large number of different sites or whether there are preferred sites for insertion. The molecular determination of CMS is in the mitochondrial DNA (mtDNA). We have shown that in *cms-S* the organization of the mtDNA is controlled by the nucleus of the cell, that significant changes in mtDNA organization occur when one nuclear genotype is substituted for another, and that similar reorganizations of mtDNA accompany cytoplasmic reversion of *cms-S* to male fertility. We would expect reversion to somehow correct the defect in S-type cytoplasms that causes male sterility. We are characterizing cytoplasmic revertants from different inbred backgrounds in an effort to find mtDNA alterations common to all reversion events. Additional molecular studies are aimed at characterizing the control the nucleus has over the organization of the mitochondrial genome.

University of Illinois
Urbana, IL 61801

70. Hydrogen-Independent Methanogenic Systems
R.S. Wolfe, Department of Microbiology *$86,000*

Methanogenic bacteria are found in diverse habitats wherever active anaerobic biodegradation of organic matter occurs. A new approach to the study of methanogenesis concerns the oxidation of alcohols by methanogens. This system, recently discovered by F. Widdel, provides a hydrogen-independent approach to the generation of electrons, enabling us to fractionate the system so as to by-pass the hydrogenases and their electron carriers. This approach provides another parameter through which to study the biochemistry of methanogenesis. The deazaflavin, F-420, has been found to be the coenzyme for a secondary alcohol dehydrogenase which was purified to homogeneity from *Methanogenium thermophilum*. Cells grown on alcohol and CO_2 oxidized secondary alcohols to ketones. These cells when exposed to hydrogen reduced ketones or aldehydes to alcohols. In the four strains studied, these reactions were inducible; cells grown only on hydrogen and carbon dioxide did not catalyze these reactions. We are studying the role of methanofuran in methanogenesis especially in cells grown in the absence of hydrogen and where the source of methyl groups is not by the reduction of carbon dioxide.

University of Illinois
Urbana, IL 61801

71. Structure and Expression of Nuclear Genes Encoding Rubisco Activase
R.E. Zielinski, Department of Plant Biology *$72,964*

Rubisco activase consists of two soluble chloroplast polypeptides, which catalyze the activation of rubisco, the enzyme which initiates the photosynthetic carbon reduction cycle, to catalytic competency. Activase polypeptides of 45 and 41 kDal are independently capable of activating rubisco *in vitro*. We have isolated and completely sequenced 7.3 kb of barley genomic DNA containing two, tandemly oriented activase genes, and a full-length cDNA encoding the 41-kDal activase polypeptide. Primer extension and northern blot analyses indicate that the second barley activase gene is also expressed, but we do not yet know the nature of the mRNA or the activase polypeptide encoded by this gene. Our goals are to answer the following specific questions: (1) what is the nature of the *cis*-acting DNA sequences that regulate activase gene expression; (2) what are the relative contributions of the two activase structural genes to the ultimate production of activase polypeptides; (3) at what stages of gene expression are the expression of activase and rubisco subunit genes coordinated to maintain the constant stoichiometry observed between rubisco and activase; and (4) what is the cause and physiological significance of the diurnal fluctuations in activase mRNA content of barley leaves.

Iowa State Univeristy
Ames, IA 50011-3223

72. Characterization of Mitochondrial hsp60 Function in Corn and Yeast Mitochondria
R.L. Hallberg, Department of Zoology *$75,286*

In order to understand the role which the yeast mitochondrial chaperonin, hsp60, plays in the molecular assembly process within the mitochondrial matrix, we have generated new yeast strains which express HSP60 genes coding for altered hsp60s. These temperature sensitive mutant strains display a phenotype at the non-permissive temperature in which a number of mitochondrial assembly processes are inhibited. In addition, a number of other molecular phenotypes have been identified which are not necessarily shared by all the mutant strains. These include: temperature-induced dissociation of the native hsp60 complex, inability to correctly replicate and/or maintain a normal mitochondrial genome, and loss of maintenance of the mitochondrial protein synthetic machinery. We have also been able to show that at least some of the proteins synthesized within corn mitochondria form a physical association with the native hsp60 complex. One of these proteins was shown to be the alpha subunit of the F1-ATPase, the gene for which is contained in the mitochondrial DNA of plant mitochondria. The associated proteins were released from the hsp60 complex when such complexes were treated with ATP.

University of Iowa
Iowa City, IA 52242

73. Molecular Biology of Anaerobic Aromatic Biodegradation
C.S. Harwood, Department of Microbiology *$34,510*

Our laboratory is investigating the molecular basis of anaerobic benzoate and 4-hydroxybenzoate biodegradation by the bacterium *Rhodopseudomonas palustris.* Aromatic compounds like these are abundant on earth; they occur naturally - mainly as components of the plant polymer lignin - and they also comprise a large proportion of the synthetic organic molecules that are released into the biosphere. Although a great deal of aromatic material accumulates in anaerobic environments, the enzymatic steps taken in the anaerobic degradation of even the simplest aromatic compounds are poorly understood and the molecular genetics of anaerobic aromatic breakdown are almost completely unexplored. We have recently started to analyze a 26 kb cloned fragment of *R. palustris* DNA which complements a series of mutants blocked in aromatic utilization. Because the cloned fragment is so large and because it complements all the catabolic mutants in our collection, we think it is likely that it encodes many of the enzymes required for benzoate and 4-hydroxybenzoate breakdown. We are identifying regions of interest on the clone by transposon mutagenesis and subcloning. Where possible, functions will be assigned to cloned genes based on the ability of expressed gene products to catalyze known reactions. The eventual goal is to use proteins that have been expressed from cloned genes to better characterize the enzymatic steps mediating anaerobic aromatic degradation. This work will be coordinated with ongoing biochemical studies by Dr. Jane Gibson at Cornell University. We also plan to determine how aromatic utilization genes are organized physically on the *R. palustris* chromosome. This information will be valuable should it at any point become desirable to transfer these genes to other bacteria with characteristics that may be particularly well suited for specific applications.

Johns Hopkins University
Baltimore, MD 21218

74. Hydrogen/Sulfur Metabolism in the Hyperthermophilic Archaebacterium
R.J. Maier, Department of Biology *$60,303 (FY90 funds/18 months)*

The mechanisms by which hyperthermophilic archaebacteria grow and carry out metabolic functions at elevated temperatures have yet to be determined. Progress along these lines requires some understanding of the roles that molecular hydrogen and elemental sulfur play in their metabolism. The objectives of the work include developing an understanding of the metabolic characteristics of, and the enzymes involved in, hydrogen/sulfur transformation by hyperthermophilic archaebacteria. Efforts will focus primarily on the autotrophic bacterium, *Pyrodictium brockii*, which has a reported optimum growth temperature (105°C) in pure culture. Biochemical and genetic characterization of enzymes involved in hydrogen/sulfur transformations for these organisms will be pursued. These include the H_2-activating hydrogenase enzyme, a ubiquinone, a c-type cytochrome, and the S-reducing complex. For example, the H_2 activating hydrogenase and electron-carrying cytochrome in the H_2 oxidizing pathway will be studied in cell-free preparations. Comparisons of the biochemical and genetic properties of these electron transport components will be made with mesophilic counterparts. Characterization of both purified hydrogenase and the cloned hydrogenase gene will receive a major research effort. The long-term goal is to understand the biochemical basis of extreme thermophily.

University of Kentucky
Lexington, KY 40546-0091

75. Photoinhibition of PS2 Reaction Centers; Photoactivation, Reconstitution and Resynthesis of PS2 Reaction Center/Water Oxidizing Complex Components

G.M. Cheniae, Department of Agronomy *$192,000 (FY89 funds/two years)*

Analyses were made of the effects of preillumination of PS2 membranes devoid of the water oxidizing complex (WOC) on their capacity to generate $Z^+/D^+/P_{680}^+$, to photooxidize Mn^{2+}/I^-/diphenylcarbazide or to assemble functional WOC via photoactivation. The loss of photooxidation of the donors showed two first-order components (half-times of 2-3 min and $\geq$1 h), suggesting two photodamageable sites of electron donation, a conclusion confirmed by analyses of velocity curves of electron donation by donors. The loss of capacity to generate Z^+ and D^+ occurred with half times of 2-3 minutes and >1 h, respectively, without decrease in P_{680} abundance. Formation (<0.8 min) of a radical; (1/Reaction Center) correlated with a decrease in the rate constant of Z --> P_{680}^+ and formation of a quencher of variable fluorescence. Loss of photoactivation also exhibited biphasic kinetics with components having half-times of 0.8 min and 2-3 min. No photoactivation was observed following loss of capacity to generate Z^+. The K_m (10 μM) for Mn^{2+} photooxidation and for Mn^{2+} in photoactivation is $\geq$30 times that for photooxidation of Mn^{2+} in the presence of $NH_2OH/NH_2NH_2/H_2O_2$ at concentrations insufficient to support photooxidations of their own species, yet inhibit photoactivation. Moreover, the steady-state quantum efficiency of Mn^{2+} photooxidation is only ~1/10th that of Mn^{2+} photooxidation in the presence of chemical reductants of $Mn^{\geq 3+}$. We conclude: only Z^+ directly contributes to photoactivation of WOC; 2) $Mn^{\geq 3+}$ ligated near Z^+ on the first photoact (K_m ~300 nM) inhibits Mn^{2+} photooxidation on the second photoact (K_m ~10 μM); 3) chemical reduction of the first formed/ligated $Mn^{\geq 3+}$ eliminates the competition, inhibits photoactivation and increases the quantum efficiency of Mn^{2+} photooxidation.

University of Kentucky
Lexington, KY 40546-0091

76. The Role of Purine Degradation in Methane Biosynthesis and Energy Production in Methanococcus vannielii

E. DeMoll, Department of Microbiology and Immunology *$64,090*

Purine degrading metabolic pathways have been discovered in *Methanococcus vannielii*. We are currently studying the relationships between purine degradation, methane biosynthesis, and tetrahydromethanopterin (MPT) biochemistry in *M. vannielii*. In so far as it has been characterized, the purine degrading pathway of *M. vannielii* resembles that of the clostridia. Preliminary results show that *M. vannielii* can use certain purines as partial carbon and energy and sole nitrogen source. The 2-, 6-, and 8-carbon atoms of xanthine may all be eventually converted by *M. vannielii* to methane. The 2-, 6-carbons enter the methane biosynthetic pathway at the beginning as CO_2, however the 8-carbon apparently enters the methane pathway in the form of 5, 10-methenyl-MPT. We are specifically trying to determine exactly how this carbon is converted to methane. Glycine is a product of clostridial purine degradation. Its further metabolism by clostridia generates one mole of ATP. Another goal of our studies is to examine possible further metabolism of glycine by *M. vannielii*. Preliminary results and work by others indicate that methanogens probably use MPT as other organisms use tetrahydrofolic acid. We are also examining whether various forms of MPT freely exchange between enzymes of the methane biosynthetic pathway and other enzymes that likely would use one of these forms of MPT, such as thymidylate synthetase, or whether an MPT molecule remains more or less dedicated to the methane biosynthetic pathway. A final objective of our work is to measure the extent to which carbon atoms released during purine degradation appear in biosynthetic and energy producing pathways in *M. vannielii*.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

77. Enzymatic Synthesis of Material
M.D. Alper, M. Bednarski, H.W. Blanch, D. Clark, J.F. Kirsch, P.G. Schultz, P. Smith, D. Soane, and C.H. Wong, Center for Advanced Materials *$148,000*

The goal of this research (jointly funded by the Division of Materials Sciences) is the use of the natural, engineered and "created" enzymes to synthesize new materials. The unique stereochemical control exerted by enzymes and their ability to catalyze reactions at low temperature will allow the synthesis of materials with structures and therefore properties that cannot be achieved using conventional synthetic routes.

Initial 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; characterization and processing of the polymer products of these reactions and understanding the structure/function relationships of this new class of materials. Work is also progressing on the synthesis of organic thin films on metal and semiconductor surfaces to alter adhesive and other interfacial properties and to fabricate sensor devices. Genes have been cloned and expressed to allow production of natural and engineered enzymes. Enzyme thermal stability has been explored. Active sites have been altered through site-directed mutagenesis and other techniques to help understand enzyme/substrate binding and provide a rational basis for modifications required for binding and reaction with unusual substrates. A system was developed (funded jointly with the Office of Naval Research) to incorporate synthetic, unnatural amino acids at specific positions in an enzyme active site. Catalytic antibodies capable of catalyzing the Diels-Alder reaction have been produced, demonstrating that enzyme catalysis can be used for "non-biological" reactions.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

78. Characterization of Carotenoid Biosynthesis Genes from a Photosynthetic and a Non-Photosynthetic Bacterium
J. Hearst *$286,000*

We have completed the nucleotide sequences of both the first and the second *crt* gene clusters ever sequenced. The *Rhodobacter* cluster contains seven open reading frames corresponding to previously identified genes (*crt*A, *crt*B, *crt*C, *crt*D, *crt*E, *crt*F, and *crt*I) as well as a new gene, *crt*K, which appears to act with *crt*C in the hydration of neurosporene. RNA-DNA hybridization of these genes confirms the postulated direction of transcription. The remaining *crt* gene, *crt*J, is located 8 kb from the rest of the *crt* cluster amongst *bch* genes. Analysis of the sequence of this cluster has revealed previously unknown potential transcriptional control signals as well as some of the properties of the proteins. All of the *crt* gene products except *crt*B contain runs of hydrophobic amino acids consistent with potential membrane spanning domains, although only the amino-terminus of *crt*C is hydrophobic. *crt*B codes for the first enzyme specifically on the Crt pathway, the substrate and product of which are both soluble pyrophosphates. The *crt*K gene product has a high overall hydrophobicity similar to that of integral membrane proteins, and it may serve to anchor the *crt*C gene product to the membrane.

Kinetic analysis of mRNA accumulation from 8 of the *crt* genes indicate that mRNAs for 6 of the 8 *crt* genes examined are induced roughly 2 to 10-fold during a shift from aerobic to photosynthetic growth, while mRNA for crtI is expressed constitutively. In addition, the time of maximum accumulation occurs 45-60 minutes after the shift, as opposed to 90 minutes for the LH I and RC mRNAs. This has led us to propose that there is a program of gene expression in which pigmentation genes, or at least the *crt* genes, are expressed before the structural genes.

The sequence of the 12.4 kb *crt* gene cluster from the non-photosynthetic phytopathogen *Erwinia herbicola* suggests the presence of 11 open reading frames. Three of these bear a striking degree of similarity with *Rb. capsulatus crt* genes at the amino acid level: *crt*I, which codes for phytoene dehydrogenase, and *crt*B and *crt*E, which direct the synthesis of phytoene and its immediate precursor prephytoene pyrophosphate. This is especially significant considering the wide evolutionary divergence between *Rhodobacter* and *Erwinia*. Several sizeable regions of perfect amino acid homology were selected for use in the design of oligonucleotide probes for locating the corresponding genes in cyanobacteria (see below), which are much more closely related to the chloroplasts of plants than are the other two bacteria.

We have partially characterized the carotenoid biosynthetic pathway *Erwinia herbicola* by dehydrogenation of phytoene. Phytoene is the first C_{40} carotenoid precursor and is a common precursor to many carotenogenic organisms. Its use resulted in the accumulation of phytoene. Nicotene blocks the reaction of the terminal double bonds of carotenoids, either hydration in the family *Rhodospirillaceae* or cyclization in plants, some nonphotosynthetic bacteria and fungi. The use of nicotene resulted in the accumulation of less polar, red-shifted compounds (*versus* wild type Crt), consistent with a block in either a cyclization or hydration reaction (or both). Our evidence from the use of these two inhibitors is consistent with the DNA sequence results above and with the earlier suggestion by Tuveson *et al.* that the carotenoids produced in *Erwinia* are xanthophylls with ß-carotene-like (i.e., cyclic) structures that are synthesized via a pathway that includes phytoene.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

79. Mechanism of Water Splitting and Oxygen Evolution in Photosynthesis
M.P. Klein, Chemical Biodynamics Division *$227,000*

Oxygen evolution in photosystem II of green plants is thought to involve reactions through a cycle of four states by which electrons are removed from two H_2O and donated to the oxidized P680 reaction center. The most successful interpretation of the data regarding this cycle is Kok's S-state scheme, which postulates a series of five (S_0-S_4) states through which electrons are cycled during oxygen evolution. Manganese is thought to play a central role in these reactions. X-ray absorption spectroscopy using synchrotron radiation is used to determine the structural and electronic state(s) of the manganese sites. In photosystem II particles of both spinach and the cyanobacterium *Synechococcus sp.*, we have determined that the manganese occur minimally as a binuclear complex with Mn-Mn separation of ~2.7Å. We observe a Mn K-edge shift of ca 1 eV to higher energy upon advancing from the S_1 to the S_2 state, implying an oxidation state increase of Mn. Creation of an S_0-like state produces a K-edge shift in the opposite direction indicating a reduction of Mn between S_1 and S_0. There is no further oxidation of Mn upon advancing from the S_2 state to the S_3 state although the EPR signal characteristic of the S_2 state disappears. The structures of the Mn complex in the S_1, S_2 and S_3 states, determined by EXAFS, are similar and well described as pairs of di-μ-oxo binuclear centers with Mn-Mn distances of 2.69Å and 2.79Å containing Mn(III) and Mn(IV). The distance between these centers is 3.3Å. Recent data of higher quality indicate small differences between the structures in the S_1 and S_2 states. The structures in the S_0-like state are more heterogeneous because of the longer bond distances attributable to the Mn(II) content. Electron spin echo (ESE) spectroscopy on the S_2 multiline EPR signal provided the first direct evidence that the Mn centers are accessible to solvent water. Illumination at 190K followed by brief warming of PS II particles prepared with $^{14}NH_3$ or $^{15}NH_3$ produces an altered EPR signal whose ESE signals show

modulation characteristic of ^{14}N and ^{15}N, respectively, providing the first direct evidence that NH_3 binds to Mn during the S_1 to S_2 state transition. Differences between the ESE of PSII particles from *Synechococcus sp.* grown respectively on ^{14}N or ^{15}N shows that nitrogen is liganded to the Mn centers. The same ^{14}N feature is observed in both spinach and *Synechococcus* adding further evidence for the similarity of the Mn centers in these evolutionarily distant species.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

80. Photochemical Conversion of Solar Energy
L. Packer, R.J. Mehlhorn, I.V. Fry, and J. Maguire, Applied Science Division *$72,000*

This project seeks to understand mechanisms of electron transport components in energy conversion by microbial systems. Using cyanobacteria and *B. subtilis*, the role of photosynthesis and respiration, in cell energetic conditions is investigated.

The use of stress has proven to be an excellent tool for elucidating the roles of photosynthesis and respiration in maintaining cellular viability. Electron spin resonance for the analyses of bioenergetics parameters in several compartments of intact cells have been applied to resolve the contributions of alternative energy sources to pH and volume regulation, to characterize restoration of transmembrane chemical gradients after membrane depolarization and to elucidate cellular adaptations to altered ionic and non-electrolyte environments. The adaptations that occur in membrane structure are being investigated with ESR probes of lipid fluidity, ordering and thermal transitions in cytoplasmic and thylakoid membranes isolated and purified from control and stress adapted cells. NMR studies in intact cells of high energy phosphates, fixed carbon species and sodium gradients will provide more detailed information about the bioenergetic status accompanying stress responses.

Studies on the membrane subunits of succinic dehydrogenase from *B. subtilis* mutants particularly that of the flavin and iron-sulfur redox centers are being conducted by spectroscopic and low temperature electron paramagnetic resonance techniques to determine the role of these components in assembly of the protein and catalytic function.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

81. Chemistry of Phycobiliproteins and Phytochrome
H. Rapoport *$36,0000*

A complete understanding of the chemistry and stereochemistry of phycobiliproteins and of phytochrome is sought to facilitate full understanding of the role of light in regulation of gene expression in green plants. The chemical structures of phycobiliproteins and phytochrome are being determined, including the detailed nature of the covalent attachments of chromophore to protein, by stereospecific synthesis of model chromophores. Chromophore peptides also are being synthesized to ascertain the effect of the peptide on solution conformations and energy transfer.

Knowledge of this mechanism is required for future improvement in plant growth and quality based on genetic engineering at the molecular level.

Lawrence Berkeley Laboratory
Berkeley, CA 94720

82. Photosynthetic Membrane Structure and Photosynthetic Light Reactions
K. Sauer, Chemical Biodynamics Division *$324,000*

Excitation transfer and trapping in reaction centers of photosynthetic membranes occurs in less than a nanosecond following the absorption of visible light photons. We are investigating the kinetics and energetics of this process using wavelength-resolved transient absorption change and fluorescence decay measurements applied to well defined preparations of antenna pigment proteins or reaction center complexes. Recent studies using X-ray crystallography from several laboratories have provided detailed structural information for several of these proteins, which have enabled us to carry out excitation transfer calculations using exciton theory and/or Förster inductive resonance transfer applied to pigment arrays of known geometry. For the cyanobacterial pigments Cphycocyanin (PC) and phycoerythrocyanin (PEC), coordinates based on X-ray crystallographic structure determinations make it clear that some delocalization should be occurring by the exciton mechanism. As a consequence there should be some signficant excited state relaxation that is faster than had been resolved in previous measurements. Using a recently completed picosecond pulse/probe spectrometer, we have obtained evidence that the relaxation is not so fast in PC, although we do observe it at the limit of time resolution for the supposedly related molecule allophycocyanin (AP). We are investigating the possibility that our measurements are detecting a slower relaxation between excitonically coupled states. This process has not previously been time-resolved for any molecular system to our knowledge. Reaction center complexes often incorporate or are associated with antenna pigments that have lower energy excited states than that of the charge-separated donor-acceptor state of the reaction center itself. It appears that in these cases there is a thermal activation character to the excitation trapping process that results in a pronounced temperature dependence of competing processes, such as fluorescence. We have characterized the nature of the temperature-dependent fluorescence in a variety of complexes derived from Photosystem I. It appears that the presence of the reaction center itself is not required for the phenomena to be observed. We are investigating the role that low lying excited states play in photosystem architecture and in the mechanism of energy conversion.

Lehigh University
Bethlehem, PA 18015

83. Post-Transcriptional Regulation of Chloroplast Gene Expression by Nuclear Encoded Gene Products
M.R. Kuchka, Department of Biology *$162,000 (FY90 funds/two years)*

The major objective of this research is to understand the molecular mechanisms by which nuclear gene products participate in chloroplast gene expression. An analysis of photosynthetically deficient mutants of the unicellular green algae *Chlamydomonas reinhardtii* has shown that nuclear gene products are required at every major step in the synthesis of chloroplast encoded proteins. This project focuses on nuclear mutations which block the expression of the chloroplast gene psbD which encodes the D2 polypeptide of photosystem II. Nuclear mutations specifically affect the synthesis of this protein at two distinct post-transcriptional stages - the stabilization of psbD mRNA and the translation and/or turnover of the D2 polypeptide. To understand the involvement of nuclear gene products in D2 translation, we will determine whether the psbD message is properly processed in mutant cells, whether this message is translatable *in vitro*, and whether the psbD mRNA is associated with thylakoid bound polysomes as it is in wild type cells. Pseudoreversion analysis will be used to extend the genetics of this system. We seek to identify additional gene products and sequences with which the original mutant gene products interact. *In vitro* RNA binding studies will be used to characterize trans-acting factors which specifically associate with psbD mRNA. We will likewise identify the exact sequences on this message to which these factors bind to confer stability or promote translation. Ultimately, our goal is to clone and sequence these nuclear genes and understand how the products of these genes work in chloroplast gene expression.

Los Alamos National Laboratory
Los Alamos, NM 87545

84. Structural Biology of the Plant Cell Wall
L.O. Sillerud and N.H. Fink, Division of Life Sciences *$105,000*

We propose to study the structure, dynamics, metabolism and self-assembly of suspension-cultured *Panicum miliaceum* cell wall components by means of a combination of nuclear magnetic resonance spectroscopy (NMR), and x-ray and neutron scattering. NMR provides unique and valuable information about sugar residue composition, linkages, degree of polymerization, metabolism, and dynamics of cell wall carbohydrates. The x-ray and neutron scattering experiments provide data with respect to the size distribution and shape of the wall components, both in solution and *in situ* in the wall. These methods generate data from an examination of unmodified, intact polymers, and intact or reconstituted cell walls. Molecular details of the interpolymer interactions that act to stabilize the hemicelluloses and the cellulose in the cell wall will be probed in a series of reconstitution experiments. The pH and calcium-binding behavior of the extracted molecules will be determined, and then the same behavior will be monitored as the components are mixed. The assimilation of isotopes from labeled precursors into wall biopolymers will be used in order to observe cell wall biogenesis *in vivo*, in real time, and to monitor specific polymers. These methods of analysis are non-destructive and non-invasive so that no extractions or chemical derivatizations need to be done to the material prior to study, once the basic features of the extracted molecules are understood. We will continue with experiments designed to characterize individual wall components with the aid of NMR with respect to residue composition, three-dimensional structure, degrees of polymerization, polymer size, and dynamics, utilizing both high-resolution and solid-state ^{2}H and ^{13}C NMR methods. The scattering experiments will be designed to reveal shape and size factors, and modes of aggregation and interaction of both the extracted polymers and their counterparts *in situ* within the wall.

Los Alamos National Laboratory
Los Alamos, NM 87545

85. Carbon Metabolism in Methylotrophic Bacteria
C.J. Unkefer, Isotope and Nuclear Chemistry Division *$105,000*

Methylotrophic bacteria are able to grow using simple one-carbon compounds such as methane, methylamine, or methanol as their sole source of carbon. The ability to grow on one-carbon compounds gives them considerable industrial potential because they could be used to produce useful substances from these inexpensive precursors. In addition, methylotrophs are potentially useful in environmental clean-up of trichloroethylene, alcohols, and amines.

We are studying the gram-negative *icl-* serine-type methylotroph *Methylobacterium extorquens* AM1. This organism derives energy from the oxidation of methanol to formaldehyde catalyzed by methanol dehydrogenase (MDH). This enzyme requires the tightly bound cofactor pyrroloquinoline quinone (PQQ) and donates electrons derived from the oxidation of methanol directly to the respiratory chain used for energy transduction. *M. e.* AM1 assimilates carbon by condensing formaldehyde with glycine to form serine. This organism lacks the enzyme isocitrate lyase, and the complete pathway used to regenerate the glycine required for carbon assimilation is unknown. Our work is focused on two aspects of the carbon metabolism of *Methylobacterium extorquens* AM1. 1) How is PQQ, the novel cofactor of methanol dehydrogenase, biosynthesized? 2) By what pathway is acetate effectively oxidized to glyoxylate? This function is required to complete the cycle of carbon assimilation.

Our approach to these metabolic questions is to incubate growing cells and cell extracts with ^{13}C-labeled substrates and identify the labeled products by ^{13}C NMR spectroscopy. Information obtained from tracing one- and two-carbon units yields insight into metabolic conversions. For example, by isolating PQQ from *M. e.* AM1 cultured on labeled ethanol, we demonstrated that the amino acids glutamate and tyrosine are the precursors for the biosynthesis of PQQ. Results from our experiments will lead to a greater understanding of the biochemistry and physiology of methylotropic bacteria and will provide a foundation for the industrial application of these organisms.

University of Maryland
College Park, MD 20742

86. Organization of Photosystem I and Photosystem II in the Photosynthetic Membranes of Phycobilisome-Containing Plants
E. Gantt, Botany Department *$73,640*

Acclimation of plants with changing light conditions is being studied in phycobilisome-containing plants. The study is an integrated approach combining quantitative assessments of principal proteins of photosystem I, photosystem II, and phycobilisomes with physiological activity. The structural arrangement of the photosystems within the thylakoids is being probed by use of cross-linking reagents. Correlations are being made with specific antisera and immuno-gold labelling. The stoichiometry of reaction centers and the antennae is being determined by photochemical techniques. Results to date indicate that the stoichiometry of PS I (P700) and PS II (Q_A) is little affected over a photon flux range (white light) of 5 to 280 $\mu E \cdot m^{-2} \cdot s^{-1}$ in *Porphyridium cruentum* which encompasses the effective growth range. Light quality, however, resulted in variable stoichiometries of photosystem I and II. Cells grown in green light had a decrease of photosystem II centers, with a Q_A/P700 ratio of only 0.26. Cells grown under red light contained five times as many Q_A per P700. The chlorophyll antennae size of each photosystem appears to remain constant with about 70 Chl/PSII and 140 Chl/PSI whether grown in red, green or white light of low intensity. The phycobilisome size and composition remained relatively unaffected by light quality. Of the total carotenoid content, zeaxanthin accounted for just over 50%, beta-carotene about 40%, and cryptoxanthin about 4% in all cases. Zeaxanthin did not increase even in high light conditions. Results from the integrated approach being pursued will provide insight into the basic structure of photosynthetic membranes and regulation of energy transfer under varying environmental conditions.

University of Maryland
College Park, MD 20742

87. Active and Passive Calcium Transport Systems in Plant Cells
H. Sze, Department of Botany *$70,000 (FY89 funds/17 months)*

Many stimulus-response systems in plant cells are coupled to transient changes in cytoplasmic [Ca]. The role of Ca as a messenger in cells necessitates regulation of cytosolic ion activity. Cytoplasmic Ca concentration is determined by the coordination of passive Ca fluxes which increase cytosolic Ca concentration and active Ca transport systems that lower cytosolic Ca. The transport mechanisms by which plants achieve this is poorly understood. Using isolated tonoplast vesicles from oat roots, we have shown that Ca^{2+} accumulation into vacuoles depends on a proton motive force generated by the vacuolar H^+-pumping ATPase. As a first step towards the identification of the Ca^{2+}/H^+ exchange the tonoplast proteins have been solubilized, and the Ca^{2+} uptake into liposomes was detected by generating a pH gradient (acid inside). The similar properties of the Ca^{2+}/H^+ exchange activity in the liposomes relative to the native vesicles indicate the antiporter has been reconstituted in active form. This provides an assay essential for the identification of the antiporter. We found that a calmodulin-stimulated ca-ATPase is associated with the E.R. and have been testing whether this activity is stimulated directly by calmodulin or indirectly via phosphorylation of a regulatory polypeptide. The molecular identity of the Ca pump(s) is being established by purification and formation of a phosphoenzyme intermediate.

Massachusetts Institute of Technology
Cambridge, MA 02139

88. Genetic and Biophysical Analyses of the Photosynthetic Reaction Center
D.C. Youvan, Department of Chemistry *$110,000*

The reaction center protein from purple nonsulfur bacteria mediates the efficient transduction of light into chemical energy. The structure of this membrane protein has been determined to atomic resolution; we have developed methods to express genetically modified reaction centers in *Rb. capsulatus*. Mechanistic models of the light reactions of photosynthesis will be tested through *in vitro* mutagenesis of the reaction center structural genes, followed by spectroscopic analyses of the genetically altered proteins. This research is aimed at understanding how critical amino acid residues (and peptides) interact to modify the chemistry of the prosthetic groups, for example: (1) in determining whether bacteriochlorophyll (BC) or bacteriopheophytin (BP) is bound at particular sites, (2) in facilitating electron transfer between prosthetic groups either by direct mechanisms or superexchange, (3) in modifying redox or spectral properties of the prosthetic groups, (4) in imparting resistance to certain quinone inhibitors, such as the triazine herbicides, and (5) in directing the path of the photoelectron down only one of two apparent pathways in the reaction center. A principal engineering goal of this research will be to construct "wrong-way" electron transfer mutants. This will be accomplished either by changing the specificity of tetrapyrrole binding sites (BC <-> BP interconversion) or by "swapping" entire helices through the pseudo C2 symmetry axis. Such mutants are important for understanding pigment-protein assembly as well as mechanistic aspects of photochemistry.

University of Massachusetts
Amherst, MA 01003

89. Cellulose Fermentation by Nitrogen-Fixing Anaerobic Bacteria
E. Canale-Parola, Department of Microbiology *$80,852*

The project's objective is to conduct studies aimed at increasing our understanding of i) the physiology of anaerobic cellulolytic N_2-fixing bacteria, ii) the N_2-fixing system of these bacteria, iii) the effects that N_2 fixation has on cellulose degradation, and iv) the physiological interactions between non-cellulolytic, N_2-fixing, commensal bacteria and cellulose fermenters in nitrogen-poor environments. Strains of obligately anaerobic cellulolytic N_2-fixing bacteria that we have isolated from freshwater environments, as well as known cellulolytic species that we have shown to fix N_2, will be investigated. Additional N_2-fixing cellulolytic strains with diverse properties will be isolated from terrestrial, freshwater, and marine environments. Studies of these strains, including their morphology, fine structure, general physiology, and N_2-fixing systems, are intended to provide an understanding of the interrelations between N_2 fixation and the fermentation of cellulose in biological systems in which these two complex physiological processes coexist within the same cell. In addition, biologically defined, diazotrophic, anaerobic consortia consisting of cellulolytic bacteria and non-cellulolytic commensal bacteria will be established. These consortia will be used to study the process of cellulose degradation under conditions in which consortium members compete for the products of cellulose depolymerization.

Meharry Medical College
Nashville, TN 37208

90. Enzymes of Respiratory Iron Oxidation
R.C. Blake II, Biochemistry Department — *$155,000 (FY90 funds/two years)*

Aerobic respiration on reduced iron is a principal metabolic activity exhibited by certain chemolithotrophic bacteria that inhabit iron-bearing geological formations exposed to the atmosphere. Despite the environmental and possible economic importance of these organisms, very little basic information is available concerning the identities of the respiratory enzymes responsible for this activity. The aim of this research is to continue the systematic isolation and characterization of the respiratory enzymes expressed by these bacteria when grown at pH 1.5 on soluble ferrous ions. The principal organisms currently under investigation include, but are not limited to, *Thiobacillus ferrooxidans, Leptospirillum ferrooxidans*, and three strains of moderately thermophilic iron-oxidizing eubacteria, strains BC, ALV, and TH3. Three major experimental goals are in progress: (1) to develop an improved procedure for the routine large scale culture of iron-oxidizing chemolithotrophs based on the *in situ* electrolysis of the soluble iron in the growth medium; (2) to perform iron oxidation kinetic studies on whole cells using the oxygen electrode; and (3) to identify, separate, purify, and characterize the individual cellular components that comprise each individual iron respiratory electron transport chain. The project is expected to generate a broader picture of the apparent diversity in bacterial iron respiratory chains. It can also provide useful information toward manipulating *T. ferrooxidans* and related organisms for commercial use.

Michigan Biotechnology Institute
Lansing, MI 48909

91. One Carbon Metabolism in Anaerobic Bacteria: Regulation of Carbon and Electron Flow During Organic Acid Production
J.G Zeikus and M.K. Jain — *$200,000 (FY90 funds/two years)*

Our project deals with understanding the fundamental biochemical mechanisms that control and regulate carbon and electron flow in anaerobic chemosynthetic bacteria that couple the consumption of single carbon compounds and hydrogen to the production of organic acids and alcohols. Fermentation, enzyme, and electron carrier studies in *Butyribacterium methylotrophicum, Anaerobiospirillum succiniciproducens* and a syntropic butyrate degrading co-culture are used as model systems. In *B. methylotrophicum* the metabolic pathway for conversion of CO into butanol and butyrate is elucidated in relation to regulation of alcohol dehydrogenase and hydrogenase. In *A. succiniciproducens* the mechanism of formate production from pyruvate and CO_2 fixation-reduction to succinate are investigated by purification and characterization of key electron carriers and oxidoreductases. In the syntrophic butyrate degrader the biochemical mechanism of formate production is studied in relationship to analysis of membrane linked ATP synthesis. These studies may yield information on improvement of anaerobic fermentations for production of organic acids or biomethanation of wastes.

Michigan State University
East Lansing, MI 48824-1319

92. Structure of Surface Glycoconjugates of Rhizobium Species and Their Function in Nitrogen Fixation
R.I. Hollingsworth, Department of Biochemistry *$140,300 (FY89&90 funds/two years)*

In our laboratory we are trying to determine what structural features of the LPS or LPS-associated glycoconjugates on the surface of *Rhizobium* may be conserved between strains of any given species of this genus. This is important for understanding the specificity of the relationship between the plant host and bacterial symbiont. Purified bacterial surface glycoconjugates are partially hydrolysed to generate fragments which are used in NMR analyses. The structures of the fragments are also studied using classical analytical techniques. The oligosaccharides are conjugated to a protein carrier and used to raise antibodies in rabbits. A fluorescent-labeled goat anti-rabbit antibody is used to locate rabbit antibodies on cells of a wide cross section of strains following incubation with the rabbit antibody. This indirect immunofluorescence assay will be applied to a wide variety of strains using antibodies to a wide variety of fragments. This study should identify which fragments are common to strains of any one species and so identify structural features which are species specific.

This study will be extended to include bacterial strains in which the nodulation genes have been induced. These results will answer the question of the fate of the bacterial cell surface and the LPS structure on nod gene induction.

Michigan State University
East Lansing, MI 48823-1312

93. Role of Acyl Carrier Protein Isoforms in Plant Lipid Metabolism
J.B. Ohlrogge, Department of Botany & Plant Pathology *$74,813*

Our long term goal is to understand how plants control the activity of the fatty acid synthesis (FAS) pathway and how its products are channeled into their diverse roles and locations within the plant cell. Acyl carrier protein (ACP) is the central cofactor required for at least 10 reactions of plastid fatty acid metabolism. We have recently discovered the occurrence and tissue specific expression of two isoforms of ACP. Our preliminary evidence indicates that the two forms of ACP have different activity in reactions which direct the distribution of acyl chains within the plant cell.

The general objective of this project is to further examine the biochemical significance of ACP isoforms to plant lipid metabolism. Specifically, we are a) examining the distribution of ACP forms in a variety of photosynthetic species, in various tissues and under different environmental influence; b) preparing monospecific antibodies to each spinach leaf isoform to use as probes of their individual function; c) examining the amount and type of acyl groups esterified to ACP which will reflect the status of the fatty acid biosynthetic pathway. We have found that approximately 10 percent of both ACP-I and ACP-II contain long chain acyl groups in actively growing spinach leaves. We will examine the controls on the size of this long chain acyl-ACP pool. In addition we are developing methods to examine the composition of the short chain acyl-ACP pool *in vivo*. The relative levels of ACP-SH, acetyl-ACP and malonyl-ACP will be determined in order to evaluate potential rate limiting steps in plant fatty acid biosynthesis.

Michigan State University
East Lansing, MI 48824-1101

94. Physiology and Molecular Biology of Extracellular Peroxidases and H_2O_2 Generating System of Phanerochaete chrysosporium

C.A. Reddy, Department of Microbiology & Public Health *$149,500 (FY90 funds/two years)*

Phanerochaete chrysosporium is a white-rot basidiomycete which has become the focus of numerous studies on lignin biodegradation, on detoxification of environmental pollutants such as dioxins and benzo(a)pyrenes, and decolorization of pulp mill effluents. This organism produces two classes of extracellular glycosylated heme proteins designated lignin peroxidases (LIPs) and manganese-dependent peroxidases (MNPs), and H_2O_2 generating enzymes as the major components of the lignin-degrading enzyme system. We have previously isolated and characterized cDNA and genomic clones encoding several distinct LIP enzymes and have described a novel non-integrative transformation system for this organism. We will study the possibility of expressing and eventually over-producing the cloned *LIP* genes in a *lip* mutant of *P. chrysosporium* that has recently been described. This system should also allow us to study the regulation of expression of *LIP* genes and the study of the function of individual LIP isozymes. We will also study the relative importance of an extracellular enzyme, glyoxal oxidase (GLY), vis a vis a cell-associated enzyme, glucose oxidase (GOX), as a source of hydrogen peroxide required for the activity of LIPs and MNPs. For this, we will employ mutants lacking glucose oxidase, glyoxal oxidase, or both and study their hydrogen peroxide production levels as well as their lignin degradation abilities in comparison to the wild type. The relative contributions LIPs vs MNPs to lignin degradation as well as degradation of chlorophenols will be studied using selected mutants and culture conditions that affect the levels of different extracellular peroxidases. From the standpoint of comparative biology, we will also initiate studies on the molecular biology of lignin peroxidase of other well characterized white-rot fungi such as *Trametes versicolor*.

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

95. Molecular Basis of Symbiotic Plant-Microbe Interactions

F.J. de Bruijn *$130,000*

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 fine-tuned interaction between the bacteria and their host. We wish to understand the regulatory signals, between rhizobia and plants, that play a role in the symbiotic control of gene expression in both partners, during nodule establishment and in the mature nodule. Specifically we are characterizing the physiological signals and regulatory circuits which play a role in controlling free-living versus symbiotic expression of rhizobial nitrogen-fixation, assimilation and heme-biosynthetic genes. In addition, we are studying the bacterial- and plant-derived signals which are involved in the nodule-specific expression of plant genes encoding nodulins.

Michigan State University DOE Plant Research Laboratory

East Lansing, MI 48824

96. Enzymatic Mechanisms and Regulation of Plant Cell Wall Biosynthesis

D. Delmer *$117,000*

The major goal of this task is to elucidate the mechanism and regulation of synthesis of the plant cell wall with special emphasis on the biosynthesis of cellulose [1,4-ß-glucan] and callose [1,3-ß-glucan]. A major effort this year has been placed upon identification of polypeptides which comprise the subunits of these enzymes. Using affinity labeling techniques, we have identified polypeptides of 46, 50, and >200 kD as being UDP-glucose-binding polypeptides which are likely components of the callose synthase. Characterization of a monoclonal antibody which can specifically immunoprecipitate detergent-solubilized callose synthase has identified an additional 60 kD polypeptide which shows Mg^{2+}-dependent association with this enzyme. These, and several other UDP-glucose binding polypeptides are being purified for further study. We have also continued our characterization of the structure of the unique cell walls possessed by tomato cells adapted to growth on the herbicide 2,6-dichloro-benzonitrile (DCB, a specific inhibitor of cellulose synthesis). These unusual walls virtually lack a cellulose-xyloglucan network, have reduced levels of hydroxyproline-rich glycoproteins, and appear to be largely composed of cross-linked homo- and rhamno-galacturanans. We have also prepared a highly specific antibody against an 18-kD DCB-binding protein from cotton fibers; the antibody will be used to study localization of the protein *in vivo*, and to attempt to clone the gene coding for the protein.

Michigan State University DOE Plant Research Laboratory

East Lansing, MI 48824

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

P. Green *$201,000*

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. Another goal is to understand how certain components respond to plant growth regulators and to environmental stimuli so as to alter selectively the rates of mRNA degradation. We have found that degradation rates of specific transcripts can be measured directly in stably transformed tobacco cells grown in suspension cultures. This system is presently being used to identify sequences that act as stability or instability determinants within natural plant transcripts or those encoded by reporter genes. 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 identify 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

98. Resistance of Crop Plants to Environmental Stress
A.D. Hanson *$140,000*

Plants have biochemical or metabolic adaptations to environmental stresses, as well as adaptations expressed at higher levels of organization. If biochemical adaptations to stress were better understood, they could be used in crop improvement, via conventional or recombinant DNA technologies. This project aims (1) to identify adaptive biochemical responses of plants to stress, (2) to find the enzymes and genes involved, and (3) to explore the effect on the whole plant of genetically modifying biochemical adaptations. We are working on two topics: betaine accumulation and lactate glycolysis. During water- and salt-stress, certain plants accumulate betaine. Much evidence indicates that betaine acts as a non-toxic cytoplasmic osmolyte during stress. Betaine is synthesized in the chloroplast by a two-step oxidation: choline → betaine aldehyde → betaine. The second step is catalyzed by a stromal, NAD-linked dehydrogenase. We have isolated cDNA clones for this enzyme (betaine aldehyde dehydrogenase, BADH) from spinach and sugar beet, and shown that salinity stress causes the BADH message to accumulate. We have identified the first enzyme of betaine synthesis as a ferredoxin-dependent choline monooxygenase (CMO). CMO has been partially purified, and shown to have a native mol wt of 100,000. Lactate dehydrogenase (LDH) is induced by hypoxia in roots and catalyzes lactate glycolysis. We have isolated LDH cDNA clones from barley. These clones fall into two classes which probably correspond to the 2 *Ldh* genes postulated from genetic evidence. Hypoxia greatly increases the abundance of LDH mRNA.

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East Lansing, MI 48824

99. Action and Synthesis of Plant Hormones
H. Kende *$205,000*

The objective of this project is to gain knowledge on the synthesis and mode of action of the plant hormones ethylene and cytokinin. We have studied the enzymes that mediate the synthesis of ethylene from S-adenosyl-L-methionine. The first enzyme in this pathway, 1-aminocyclopropane-1-carboxylate (ACC) synthase, is usually the limiting enzyme in ethylene synthesis. It can be induced by a variety of chemicals and conditions, including stress. We have purified this important enzyme in plant development, have obtained amino-acid sequences from it and have isolated a partial clone of the gene that encodes it. Our goal is to understand, at the molecular level, how different stimuli regulate ACC synthase activity. We are also investigating the ethylene-forming enzyme (EFE) which catalyzes the oxidation of ACC to ethylene. We found that it is localized, at least in part, in the vacuolar membrane and that its activity requires membrane integrity. Since this enzyme does not survive homogenization, we are trying to identify it using affinity labels. In support of our experiments on ACC synthase and EFE, we are also isolating mutants of *Arabidopsis thaliana* that are blocked in either of these two steps of the ethylene-biosynthetic pathway. The role of stress ethylene is being investigated in deepwater rice where low-oxygen stress during submergence induces ethylene biosynthesis. Ethylene, in turn, mediates the growth response of submerged plants. We are investigating the effect of ethylene on a number of biochemical processes that are related to the growth response, e.g., cell wall biosynthesis. We have also returned to earlier work on cytokinin- and substrate-induced nitrate reductase in *Agrostemma* in attempts to understand how cytokinins regulate this enzyme.

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East Lansing, MI 48824

100. Cell Wall Proteins

D.T.A. Lamport — *$185,000*

This program is an approach to primary cell wall function from the perspective of the wall as an organelle possessing structural integrity and enzymic autonomy. The structure and function of cell wall glycoproteins in general and the hydroxyproline-rich structural wall protein extensin in particular are the focal points of this research. Thus, our recent isolation of several types of extensin monomer suggests an extensin network involving covalent intermolecular crosslinks. Additional evidence based on isolation of monomeric extensin precursors to the putative covalently linked extensin network, suggests that extensin is a transmural protein. Hence the "warp- weft" hypothesis: a crosslinked extensin network ("weft") of defined porosity is penetrated by a "warp" of cellulose microfibrils creating a true molecular fabric. This simple model based on interpenetrating networks is analogous to an "angle-interlock" composite and has a profound implication - it could mechanically couple load-bearing wall polymers thereby distributing stress among the coupled wall components. Is it possible that cell extension, so often described as "biochemically controlled creep", can be explained as a slippage of cellulose microfibrils through the pores of an extensin network?

Current work seeks to test the major premises of the warp-weft hypothesis. Do extensin networks of defined porosity exist? If so, do cellulose microfibrils penetrate the pores? We propose four general lines of enquiry: (1) structural elucidation of extensin crosslink sites and network porosity; (2) enzymic generation of the network *in vitro*; (3) electron microscopy of extensin monomers, oligomers, and network; and (4) comparative biochemistry of newly isolated extensins from graminaceous monocots (Maize), nongraminaceous monocots (*Asparagus*), primitive dicots (Sugarbeet), and the gymnosperms (primitive and advanced) *Ginkgo, Pseudotsuga, Pinus, Gnetum* and *Ephedra*.

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East Lansing, MI 48824

101. Interaction of Nuclear and Organelle Genomes

L. McIntosh — *$201,000*

Our objective is to elucidate the molecular bases of energy transduction in photosynthetic organisms. One aim is to achieve a better understanding of the regulation of electron flow in photosynthesis: specifically, within the reaction centers of photosystem I and photosystem II. A model genetic organism, the unicellular cyanobacterium *Synechocystis* sp. PCC 6803, is being employed in combination with site-specific mutagenesis to "unravel" electron flow in the two reaction centers. Selected genes encoding the reaction center proteins of photosystem I and photosystem II have been isolated and sequenced. The regulation of expression of these genes is being investigated. In parallel, mutations encoding amino acid replacements, deletions and insertions are being introduced into these genes. They are then used in transformation experiments to replace the "wild-type" gene(s) present in *Synechocystis*. Genetic, biochemical and biophysical analyses are employed to determine the effect of specific amino-acid modifications within the reaction centers.

Higher plants contain *two* terminal oxidases within their inner mitochondrial membranes, thus providing a branched pathway for mitochondrial electron flow. An "alternative" pathway, one which branches at ubiquinone and does not contribute to a proton gradient, is present in addition to the "normal" cytochrome pathway. There is little understanding of (1) how electron flow is partitioned between the two pathways or (2) the physiological function of the alternative pathway. It is critical to our understanding of energy balance in plants that we determine the mechanism by which electron flow is divided between the two pathways, especially as one of them is not linked to energy production. In order to approach this question we have isolated antibodies to the alternative oxidase and, employing these antibodies, we have cloned alternative oxidase "genes" from an aroid, *Sauromatum guttatum*, potato, and rice. These physical probes are being employed to study the biochemistry of the alternative oxidase as well as the control of its expression in plant tissues.

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East Lansing, MI 48824

102. Sensory Transduction in Plants

K.L. Poff

$185,000

The primary objective of this project is to understand the mechanisms for the acquisition of environmental information via light and gravity reception. We are studying the blue light photoreceptor pigment system(s), which control(s) numerous light responses such as phototropism in flowering plants, and in addition, we are studying the mechanism for the perception of gravity in gravitropism. For these studies, we are developing 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 shoot phototropism, altered shoot gravitropism, and/or altered root gravitropism. Based on these strains, one can conclude that shoot phototropism and shoot gravitropism share many common elements, but that shoot gravitropism and root gravitropism are substantially separate pathways. The shoot photo-minus, gravi-normal phenotype should represent an alteration early in the phototropism pathway and could arise from an altered photoreceptor pigment. High resolution analysis of the fluence response relationship for phototropism shows that at least two different photoreceptor pigments are involved. In addition we have identified a strain with its threshold fluence for phototropism increased by a factor of 50. The fluence response relationship for this strain shows that the threshold fluence for one of the photoreceptor pigments has been altered. Based on these data, this strain represents a probable candidate as a photoreceptor pigment mutant. Under conditions of long term irradiation, the plant has the capacity to adapt to the irradiation, such that its responses at the beginning and end of a long irradiation are not equal. We have begun an analysis of adaptation in order to understand the plant's response to long term irradiations. Our approach to the study of the pathways for phototropism and gravitropism includes mutant isolation, and genetic, physiological and biophysical characterization. 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.

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East Lansing, MI 48824

103. Molecular Mechanisms of Trafficking in the Plant Cell
N.V. Raikhel — *$185,000*

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. There is increasing evidence that proteins destined for subcellular organelles contain a "sorting signal" which dictates the compartment to which the protein is targeted. We are interested in understanding the molecular determinants of differential protein compartmentalization and in identifying the components of the molecular machinery which carry out the sorting process. Our goals include the analysis and characterization of protein sequences responsible for sorting to the vacuole and to the nucleus and the identification and isolation of one or more putative receptors which recognize these sorting sequences and subsequently mediate protein transport to vacuoles and to nucleus.

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East Lansing, MI 48824

104. Physiological and Molecular Genetics of Arabidopsis
C.R. Somerville — *$205,000*

The overall objective of this task is to develop genetic methods for the analysis and modification of specific physiological processes in plants. Our research is primarily concerned with a genetic analysis of membrane lipid composition in *Arabidopsis*. We have identified a series of mutants of *Arabidopsis* in which the fatty acid composition of leaf and seed lipids has been altered by specific deficiencies in fatty acid desaturases, G3P acyltransferase or elongases. Detailed analysis of lipid metabolism in the mutants has provided new insights into the regulation of membrane lipid acyl group composition. The ability of the mutants to compensate, in several cases, for the loss of specific enzyme activities suggests that the composition of membranes is at least partially regulated in response to functional properties of the membranes. The mutants have also been useful for examining the roles of lipid composition in determining membrane structure and function. In general, it appears that under growth conditions in the laboratory, large changes in lipid acyl group composition have relatively slight effects on the function of membrane associated processes such as photosynthetic electron transport but may have significant effects on the overall organization of the membranes. We are currently exploring ways of exploiting the availability of the mutants to clone the genes affected.

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East Lansing, MI 48824

105. Molecular Basis of Disease Resistance in Barley
S.C. Somerville — *$183,000*

Plant diseases are considered a major limitation to crop yields. However, little is known of the molecular basis of disease development or host resistance mechanisms. The long term goal of this project is to develop a description of the biochemical events of the infection process in compatible and incompatible combinations of barley lines and *Erysiphe graminis* f. sp. *hordei* races. *E. g. hordei* is the causal agent of powdery mildew disease.

The results of mutational analyses suggest that more than one component distinguishes resistance from susceptibility in barley, and similarly, more than one component determines avirulence in *E. g. hordei*. Our conclusion is that the "gene-for-gene" hypothesis, in its simplest formulation, does not adequately describe barley-*E. g. hordei* interactions. We will continue the mutational analysis of the barley *Ml-a* locus as a means of clarifying the apparent genetic complexity of this disease reaction locus.

As a complement to the extensive cytological descriptions of the infection sequence, we have begun to characterize biochemical changes in barley following inoculation with *E. g. hordei*. The specific activity of two extracellular peroxidase isozymes increased between 8 and 16 hours post-inoculation. We will prepare isozyme-specific antibodies so that these isozymes can be localized in the infection court relative to structural features, such as papillae, which are correlated with resistance.

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East Lansing, MI 48824

106. Biochemical and Molecular Aspects of Plant Pathogenesis

J.D. Walton *$184,000*

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 the biochemistry and molecular genetics of the enzyme polygalacturonase, a putative general pathogenicity factor made by most if not all pathogenic fungi. It has been proposed to be necessary for the fundamental processes of tissue penetration and nutrient assimilation, and also, via its products, to act as a trigger of host plant defenses. We are testing its role in pathogenicity by cloning the gene from the maize pathogen *Cochliobolus carbonum* and then using this cloned gene to selectively knock out the wild-type copy by transformation-mediated gene disruption. Like many pathogenic fungi, *C. carbonum* also shows host- specificity: race 1 infects only maize that is homozygous recessive at the nuclear *Hm* locus. This is due to its ability to make a host-selective toxin, a cyclic tetrapeptide called HC-toxin. We have identified enzymes involved in the biosynthesis of HC-toxin and are cloning the corresponding genes, known as the TOX genes. We are also studying the metabolism of HC-toxin by maize to test whether differential metabolism can explain resistance and susceptibility to the toxin and hence to *C. carbonum* race 1.

Michigan State University DOE Plant Research Laboratory

East Lansing, MI 48824

107. Developmental Biology of Nitrogen-Fixing Cyanobacteria

C.P. Wolk *$201,000*

Anabaena and related cyanobacteria (blue-green algae) utilize solar energy to fix nitrogen gas within cells called heterocysts under anaerobic as well as aerobic conditions. We seek to elucidate the metabolism of heterocysts. We have developed a system for efficient transposon-mutagenesis of *Anabaena* which incorporates *Vibrio* luciferase as a reporter of genetic transcription. Using this system, we have identified ca. 150 Nif mutants as well as transposon-insertions into genes that are greatly activated at various times after deprivation for fixed nitrogen, or are activated in response to addition of nitrate or reduction of temperature from 30° to 20°C. Preliminary evidence suggests that we should be able to map the sites of transposition within a few kb in the chromosome by restriction and by use of the PHOGE system of pulsed field gel electrophoresis. We continue to develop a system for amplification of the activity of weak promoters so that the cells in which they are active may be identified. We are developing tools for genetic and physiological analysis of photoautotrophic, nitrogen-fixing cyanobacteria. This work will facilitate understanding of cellular differentiation and construction of modified strains particularly suited for applied, biological conversion of solar energy.

Michigan State University DOE Plant Research Laboratory

East Lansing, MI 48824

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

J.A.D. Zeevaart *$187,000*

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 objective of this project is to characterize the hormones involved, how their synthesis and catabolism are regulated by the environment, and how these hormones act. In the long-day (LD) rosette plant spinach, the following members of the C-13 hydroxylation pathway have been identified: GA_{53}, GA_{44}, GA_{17}, GA_{20}, GA_1, GA_{29}, and GA_8. In feeding studies, conversion of GA_{53} to GA_{44} and of GA_{19} to GA_{20} take place in plants in light, but not in darkness. On the other hand, GA_{44} is converted to GA_{19} under both light and dark conditions. There is a close correlation between *in vivo* metabolism of these GAs in spinach leaves, and activity of the respective oxidases in cell-free systems from leaves. GA_{53}-oxidase and GA_{19}-oxidase activities quickly disappear when plants are transferred from light to darkness, whereas the activity of GA_{44}-oxidase is not affected. Thus, the biochemical basis for photoperiodic control of stem growth in spinach upon transfer from short to long days is the increase in enzyme activities in the later part of the GA biosynthetic pathway which leads to production of the bioactive GA which in turn causes stem growth.

Studies on the biosynthesis of the hormone abscisic acid (ABA) have demonstrated that three of the four oxygen atoms are derived from molecular oxygen. In stressed leaves incubated in $^{18}O_2$, one ^{18}O atom is rapidly incorporated in the carboxyl group, whereas isotopic enrichment is much less in the oxygen atoms of the ring. This indicates that ABA is derived by oxidative cleavage from a larger precursor (a xanthophyll) with the ring oxygens already present. Apple fruits, unlike water-stressed leaves, synthesize the majority of [^{18}O]ABA with the label in the 1'-hydroxyl position and unlabeled in the carboxyl group. A possible explanation is that exchange of ^{18}O with water occurred at the stage of an aldehyde intermediate, resulting in ABA unlabeled in the side chain. In apple fruit ABA-aldehyde is an endogenous metabolite. ^{18}O-Labeling patterns of ABA-aldehyde and ABA demonstrate that the aldehyde is a precursor to ABA. ABA biosynthesis is much higher in leaves than in fruits. In fruits, the majority of ABA is conjugated to the glucose ester, whereas in leaves ABA is mainly hydroxylated to phaseic acid.

University of Minnesota

Navarre, MN 55392

109. Genetics of Bacteria that Utilize One Carbon Compounds

R.S. Hanson, Gray Freshwater Biological Institute *$68,034*

Methylotrophic bacteria that grow on the one-carbon compounds; methane, methanol, methylamines and dichloromethane are a morphologically and physiologically diverse group of eubacteria. The 16S rRNA molecules of several gram-negative methylotrophs have been sequenced. Two phylogenetically related groups containing type I and type II methylotrophs have been identified. Each group contains two subgroups of bacteria.

DNA probes homologous to 16S rRNA's of each group of methanotrophs have been synthesized and have been shown to hybridize only to the 16S rRNA's from target bacteria.

We have mapped the positions of 15 genes controlling the synthesis of methanol dehydrogenase, cytochrome C_L and other functions required for the oxidation of methanol to formaldehyde in three species of type II methanotrophs. We have isolated a DNA-binding protein that binds to a cloned 172 bp sequence that is located upstream from the MDH structural gene. The function of this protein in the regulation of MDH synthesis will be investigated.

The gene encoding the methane monooxygenase B component of *Methylosinus trichosporium* OB3b has been cloned and expressed in *Escherichia coli*. We intend to clone and map all five genes required for the expression of soluble MMO activity in *M. trichosporium* OB3b and to study the regulation of their synthesis.

University of Minnesota
Minneapolis, MN 55455

110. The Biochemistry and Enzymology of Halomethyl Corrinoids
H.P.C. Hogenkamp, Department of Biochemistry *$77,894*

Trifluoro-, difluoro- and monofluoromethylcobalamin have been synthesized. Reaction of cob(I)alamin with trifluorobromomethane gave trifluoro- and difluoromethylcobalamin, which are readily separated by HPLC on a C8 column. Difluoromethylcobalamin was also prepared from cob(I)alamin and difluorochloromethane. Monofluoromethylcobalamin was formed from cob(I)alamin and fluorodichloromethane, indicating that under the reaction conditions, the second chlorine atom was replaced by hydrogen.

We have been unable to prepare trichloro- and dichloromethylcobalamin from cob(I)alamin and carbon tetrachloride and chloroform respectively. Indeed, reaction of cob(I)inamide with ^{13}C-enriched chloroform gave two corrinoids which were again tentatively identified as the two monochloromethyl-cobinamides, indicating that one of the chlorine atoms was replaced by hydrogen.

Reaction of cob(I)alamin with methylene chloride gave chloromethylcobalamin in moderate yield. All the corrinoids are being characterized by UV-visible spectroscopy and where applicable by ^{1}H, ^{13}C and ^{19}F NMR spectroscopy.

University of Minnesota
Minneapolis, MN 55455

111. The Mechanism of Switching from an Acidogenic to a Butanol-Acetone Fermentation by Clostridium acetobutylicum
P. Rogers, Department of Microbiology *$78,880*

Clostridium acetobutylicum is an obligate anaerobic bacteria that ferments sugars to acetic and butyric acids during exponential growth, and, following accumulation of acids, switches the fermentation to production of butanol, acetone, and ethanol. This research is designed to examine the molecular mechanism by which these bacteria regulate the synthesis and activities of the key enzymes catalyzing the reaction sequences of this dual fermentation. We have purified and characterized the butyraldehyde dehydrogenase and determined the kinetic constants for the other key enzymes in the two pathways branching from butyryl-CoA producing either butyric acid or butanol. Prior to solvent formation the synthesis of five or six enzymes is induced 40 to 100 fold. During solvent formation these enzymes recycle acetate and butyrate continuously to butanol and ethanol.

We have isolated three classes of regulatory mutants that either under-produce or over-produce the enzymes for solvent formation. The *C. acetobutylicum* genes for an NAD-dependent alcohol dehydrogenase, and lactate dehydrogenase have been cloned and expressed in *E. coli* from a pBR322 DNA library. Sequencing of these genes will determine control regions and other characteristics. Complimentation of *C. acetobutylicum* mutants by DNA transformation will be studied. We have transferred into *C. acetobutylicum* conjugative plasmids bearing the transposon, Tn916. Insertional inactivation of regulatory genes of *C. acetobutylicum* is under study. The regulatory genes for the fermentation and for sporulation will be identified.

University of Missouri
Columbia, MO 65211

112. Genetics of the Sulfate-Reducing Bacteria
J.D. Wall and B.J. Rapp-Giles, Biochemistry Department *$148,000 (FY90 funds/two years)*

Progress in the development of genetic exchange procedures for the sulfate-reducing bacterium *Desulfovibrio desulfuricans* has been made. Conjugational transfer of Inc-Q and P plasmids from *Escherichia coli* to *D. desulfuricans* strain G100A has been documented. Plasmid DNA from exconjugants was visualized on agarose gels and was used to transform *E. coli* to the appropriate antibiotic resistances. Optimal conditions for the conjugational transfer were examined.

Analysis of exconjugants revealed with strain G100A possesses a small endogenous plasmid, pBGI. It has been stably subcloned into a sequencing vector, pTZ18U, nested deletions of this plasmid have been prepared and sequencing is in progress. Its ability for development as a shuttle vector will be explored.

In collaboration with G. Voordouw (University of Calgary), the cytochrome c_3 gene from *Desulfovibrio vulgaris* Hildenborough has been introduced into *D. desulfuricans*. The Hildenborough cytochrome c_3 is properly exported to the periplasm, correctly processed and has four covalently inserted hemes per polypeptide. In contrast, *E. coli* could synthesize and export the apo-cytochrome c_3 polypeptide but could not insert the hemes [Pollock *et al.*, (1989) J.Gen.Microbiol. 135:2319-2328]. Thus our conjugation procedure may allow *D. desulfuricans* to serve as a functional expression host for genes from other sulfate-reducing bacteria or other anaerobes.

Mount Sinai School of Medicine
New York, NY 10029

113. The Respiratory Chain of Alkalophilic Bacteria
T.A. Krulwich, Department of Biochemistry *$166,000 (FY89 funds/two years)*

The long-term goal of this project is to study the respiratory chain complexes of the extremely alkalophilic bacilli in order to gain insight into the basis for alkalophily, and the specific role(s) therein of the high concentration and multitude of redox species. The focus during the current period is on the terminal oxidases. Recent data have confirmed that the major focus of cytochrome increase during growth of facultatively alkalophilic *Bacillus firmus* OF4 at pH 10.5 vs pH 7.5 is in the caa_3-type oxidase. Attempts to clone the genes encoding this complex for studies of their structure and pH regulation have been initiated. We have further found that the organism possesses to other terminal oxidases--a *d*-type oxidase that is found in log phase cells grown at pH 7.5 or in stationary phase cells at higher pH, and an *o*-type oxidase whose electron donor is not yet established and which shows modest increases in activity at high vs low growth pH. Mutants with altered patterns of terminal oxidases are being sought. An ongoing effort to understand the role of specific membrane lipids in alkalophily is currently focused upon the differences between the facultative and obligate strains, showing that the latter have an advantage during growth at very high pH values.

University of Nebraska
Lincoln, NE 68588-0118

114. Physiology and Genetics of Metabolic Flux Regulation in Zymomonas mobilis
T. Conway, School of Biological Sciences *$151,400 (FY90 funds/two years)*

The enzymology of glycolytic pathways is now well established, but the molecular mechanisms that control carbon flux are only beginning to be understood. The proposed research addresses two fundamental biological questions. First, how do the enzymes of glycolytic pathways act in concert to regulate metabolic flux? Second, what is the role of gene expression in regulation of glycolytic enzyme levels and flux? This investigation requires a model organism that is metabolically simple and amenable to genetic studies, such that the variables involved in regulation of flux can be easily manipulated. The metabolically simple bacterium, *Zymomonas mobilis,* has been chosen for this work. This organism uses the Entner-Doudoroff pathway exclusively for conversion of carbohydrates to the sole fermentation products ethanol and carbon dioxide.

In the proposed study, cloned genes encoding the enzymes of the *Z. mobilis* glycolytic pathway will be used as tools to manipulate individual enzyme levels in order to assess which steps in the pathway serve to limit carbon flux. The genes for glucokinase, glucose-6-phosphate dehydrogenase, phosphogluconate dehydratase, 2-keto-3-deoxy-phosphogluconate aldolase, enolase, phosphoglucose isomerase and the glucose facilitated diffusion protein have been cloned and characterized in our laboratory. We are examining the mechanisms controlling expression of these genes at the transcriptional and post-transcriptional levels. This integrated approach of physiology and genetics will lead to a greater understanding of the molecular mechanisms that control the relative expression of these enzymes in accordance with the biochemical and physiological needs of the cell.

University of Nebraska
Lincoln, NE 68583-0722

115. Viruses of Eukaryotic Green Algae
J.L. Van Etten, Department of Plant Pathology *$153,999 (FY89 funds/two years)*

We have isolated and partially characterized a number of large polyhedral, dsDNA containing (300 kbp), plaque forming viruses which infect a unicellular, eukaryotic, *Chlorella*-like green alga. The plaque assay, the ability to synchronously infect the host, the short life cycle, and the ability of the virus to undergo homologous recombination make them excellent model systems for studying gene regulation and expression in a photosynthetic eukaryote. These are the first plant-virus systems amenable to standard bacteriophage technology. At least some of these viruses, whose genomes contain various levels of methylated bases (0.1 to 47% 5-methylcytosine and 0 to 37% N^6-methyladenosine), encode for DNA modification and restriction systems. The virus infected algae are a new source of site specific (restriction) endonucleases and the first source from a nonprokaryotic system. Some of these endonucleases recognize and cleave at the same position as bacterial enzymes, whereas others have specificities and properties not previously observed.

The primary objective of this proposal is to continue to isolate and characterize some of the site specific endonuclease and methyltransferase enzymes. An additional objective is to establish if the virus encoded site specific endonucleases are involved in host DNA degradation *in vivo* as we have hypothesized.

New York University Medical Center
New York, NY 10016

116. Anaerobic O-demethylation of Phenylmethylethers
A.C. Frazer, L.Y. Young, Department of Microbiology — *$73,950*

Anaerobic O-demethylation (AOD) of phenylmethylethers is a process of both basic and applied significance. The aryl-O-methyl ethers are abundant in natural products, particularly as components of lignin. They are present as methoxylated lignin monomers in anaerobic environments and can be completely degraded there by mixed microbial populations. AOD is an essential early step in this process, and it is also a key reaction in the utilization of the O-methyl substituent as a C-one substrate by acetogens. The biochemical mechanism for the anaerobic cleavage of the aryl-O-methyl ether bond is an intriguing, but relatively unexplored process. In contrast to aerobic O-demethylating enzymes, AOD appears to involve methyl group transfer. An understanding of the AOD reaction mechanism might suggest new ways in which chemicals could be derived from the aromatic constituents of lignin, or new ways to process lignocellulose to make cellulose and hemicellulose more available. A major goal of this project is to purify the AOD enzyme from the acetogen *Acetobacterium woodii* in order to characterize the component proteins and the enzymatic reaction. Structure-activity relationships of methoxylated aromatic acids are being studied, and possible methyl group receptors for the AOD reaction will be evaluated. For comparison, AOD pathways and activity in extracts will be examined in other kinds of anaerobes.

Ohio State University
Columbus, OH 43210

117. The Molecular Characteristics of the Lignin-forming Peroxidase
L.M. Lagrimini, Department of Horticulture — *$61,184*

Many peroxidases are synthesized in response to physical, chemical, or biological stress, and have long been used as a marker for the physiological and developmental state of a plant. The most abundant and best characterized peroxidase in tobacco is the anionic lignin-forming isoenzyme. I have recently isolated and characterized a cDNA clone for the anionic peroxidase from tobacco. This cDNA was joined to the CaMV 35S promoter and transformed into tobacco. This construct promotes the synthesis of the anionic peroxidase in all tissues throughout the plant, and at all developmental stages. The peroxidase cDNA was also inserted in the antisense configuration behind the 35S promoter to suppress the synthesis of the endogenous anionic peroxidase. Regenerated transgenic plants were assayed for peroxidase activity, isoenzyme synthesis, and tissue specific expression. In addition, these plants are being assayed for lignin content and distribution, cell wall morphology, growth and photosynthetic rate, wound-healing capability, and disease susceptibility. Both sense and antisense plants exhibit dramatic alterations in phenotype. The overproduction of the anionic peroxidase results in chronic wilting, excessive browning of tissue, and poor seed germination. The suppression of peroxidase activity results in larger leaves due to larger cell size, inadequate wound healing, and poor seed set. The anionic peroxidase gene including the promoter/regulatory sequences has been isolated. The gene is being sequenced, and the promoter will be joined to the ß-glucuronidase reporter gene. This POD/GUS chimeric gene will be transformed into tobacco, and the expression of this gene will be observed in tissue sections, and in the presence of auxin. Auxin has been shown to inhibit the expression of the anionic peroxidase in tissue explants. Deletions and point mutations will be made in the promoter/regulatory region to identify sequences which confer auxin suppression and/or tissue specific expression of this gene.

Ohio State University
Columbus, OH 43210

118. Structure and Regulation of Methanogen Genes
J.N. Reeve, Department of Microbiology *$207,500 (FY90 funds/two years)*

The long-term goals of this project are to characterize the genetic organization and mechanisms of regulation of gene expression in methanogens, to use gene cloning and the techniques of molecular biology to dissect and understand the biosynthesis of methane and to develop genetic exchange systems for methanogens. The regulations of synthesis of methyl coenzyme M reductase (MR) in *Methanococcus vannielii* and of methyl-viologen reducing hydrogenase (MVH) in *Methanobacterium thermoautotrophicum* are being studied. Although five, closely-lined genes (*mcrBDCGA*) form the MR operon only the products of the *mcr*B, *mcr*G and *mcr*A genes are components of the purified MR holoenzyme. We have subcloned and expressed the *mcr*D and *mcr*C genes in *E. coli* and are now using antibodies raised against these polypeptides to investigate the locations and functions of the *mcr*D and *mcr*C gene products in *M. vannielii*. The MVH subunits are also encoded by linked genes (*mvhDGA*). The product of an additional gene, *mvh*B, located immediately downstream of *mvh*A is predicted to contain 48 Fe atoms in six tandemly-arranged ferredoxin-like domains; a structure we have termed a polyferredoxin. The role of this molecule as an electron conduit in methanogenesis is being investigated. The cloned *mcr* and *mvh* genes are being used as hybridization probes to characterize and quantify transcripts of these genes synthesized *in vivo* in methanogens growing under a variety of physiological conditions. The effects of environmental and physiological changes on the expression of these genes is being determined. Development of antibiotic resistance conferring determinants and a transformation system for methanogens is an additional major component of this project.

Oklahoma State University
Stillwater, OK 74078

119. The Structure of Pectins from Cotton Suspension Culture Cell Walls
A. Mort, Department of Biochemistry *$75,922*

The overall goal of the project is to characterize as much of the structure of the pectins of the cell walls of cotton suspension cultures as possible. We are solubilizing the pectins from the walls by selective cleavage by either HF solvolysis, endopolygalacturonase hydrolysis or a combination of the two. An additional endoglucanase or strong alkali treatment can be used to solubilize the residual RGI.

In the homogalacturonan regions, we will be determining the distribution of the interspersed rhamnose residues by specifically cleaving at rhamnose residues and determining the length of the homogalacturonan fragments on a Dionex Carbohydrate System.

The distribution of methyl esterification will be determined by conversion of the methyl esterified galacturonic acid residues to galactose and then specific fragmentation of resulting mixed polymer and characterization of the fragments.

The sidechains (attached to rhamnose residues) of RGI will be released by treatment with lithium metal in ethylenediamine to destroy the galacturonic acid residues in the backbone of the polymer.

The oligosaccharides we generate in the experiments described above will be characterized using liquid secondary ion mass spectrometry (LSIMS) and NMR spectroscopy.

University of Oklahoma
Norman, OK 73019

120. Effect of Community Structure on Anaerobic Aromatic Degradation
M.J. McInerney, Department of Botany and Microbiology *$70,992*

Aromatic compounds are degraded to methane and carbon dioxide by consortia of interacting bacterial species. The importance of the kinetics for hydrogen (or formate) and acetate use on the rate of benzoate degradation will be determined using defined consortia containing known species. Cocultures of the anaerobic, syntrophic benzoate degrader, *Syntrophus buswelii*, with the hydrogen and formate user, *Desulfovibrio* strain G11 metabolized benzoate at a maximum rate of 50 μmol per liter per hour. At low benzoate concentrations, the rate of reaction deviated from that predicted by a first order decay process and reached a threshold benzoate concentration of 2 μM. These data suggested that the fate of aromatic compounds in anaerobic environments may depend on the thermodynamics of the reaction.

A novel anaerobic bacterium was isolated in pure culture which metabolized phenoxyacetate and 2-chlorophenoxyacetate to the corresponding phenol. This is the first anaerobe known to catalyze and aryl ether cleavage reaction. The bacterium is a gram-negative rod that uses a variety of sugars and crotonate as the energy source.

Oregon Graduate Institute of Science and Technology
Beaverton, OR 97006-1999

121. Biochemical Genetics of Lignin Degradation by Phanerochaete chrysosporium
M.H. Gold, Department of Chemical and Biological Sciences *$120,000 (FY89 funds)*

Lignin, the most abundant renewable aromatic polymer, comprises 20-30% of woody plant cell walls. White rot fungi are primarily responsible for the initiation of the decomposition of lignin in wood. The best-studied lignin-degrading organism, *P. chrysosporium*, secretes two extracellular heme enzymes - lignin peroxidase (LiP) and manganese peroxidase (MnP) - which catalyze the oxidative degradation of lignin. The objective of this project is to understand the enzymology of the degradation of lignin and related aromatic compounds on molecular biological and biochemical levels.

During the past year we have characterized genomic clones for LiP and MnP. We have found that the promoter region of the MnP gene contains putative *cis*-acting metal response elements and heat shock elements. Northern blot analysis indicates that MnP gene transcription is regulated by both MnII ion and by heat shock. To study these regulatory systems in detail we have developed the first DNA transformation system for *P. chrysosporium*. This transformation system uses adenine auxotrophic marker strains and the corresponding homologous and heterologous biosynthetic genes. We are preparing promoter-reporter constructs to examine the role of these putative metal response and heat shock elements in the MnP gene transcriptional regulation we have observed.

We have also continued our spectroscopic and kinetic studies of native LiP and MnP enzymes and their oxidized intermediates to characterize the structure and catalytic mechanism of these peroxidases. During the past year we have elucidated in considerable detail the role played by the *P. chrysosporium* secondary metabolite veratryl alcohol in protecting LiP from inactivation by excess hydrogen peroxide. Finally, we are studying the pathways by which *P. chrysosporium* and its peroxidases degrade lignin, lignin model compounds and a variety of persistent aromatic pollutants.

Oregon Graduate Institute of Science and Technology

Beaverton, OR 97006-1999

122. Expansion of Bioconversion Technology at the Oregon Graduate Center

M.H. Gold, Department of Chemical and Biological Sciences *$300,000 (FY89 funds)*

This expansion is establishing a multidisciplinary program for bioconversion research at the Oregon Graduate Institute. During the initial period four new faculty have been added; brief descriptions of their work are included here.

1. Characterization of laccases from lignin-degrading fungi
   N.J. Blackburn

Many wood-rotting fungi secrete extracellular laccases as a component of their lignin-degrading system; however, the role of these enzymes in lignin degradation is not well understood. A major goal of this project is to elucidate the mechanisms of fungal laccases and their role in lignin degradation. We have characterized the laccase from the white rot fungus *Pycnoporus cinnabarinus* as a 60 KD thermostable glycoprotein (16% carbohydrate) exhibiting a low pH optimum of 3.5. The catalytic center of the protein contains 4 copper atoms which have been studied by EPR spectroscopy and resemble those of the *Polyporus versicolor* enzyme. These studies are being coordinated with ongoing biochemical studies on laccases in M.H. Gold's lab.

2. Molecular and cellular biology of peroxisomes in methanol-utilizing yeasts
   J.M. Cregg

Among eukaryotic organisms, the ability to grow on methanol as sole carbon source is limited to a few yeast and fungal species. Methylotrophic yeasts are often found on rotting wood and are thought to oxidize methanol that is released during the degradation of wood by other microorganisms. Key enzymes in the methanol metabolic pathway are known to be compartmentalized in a subcellular organelle called the peroxisome. This organelle exists in all eukaryotes and is the site of hydrogen peroxide-generating oxidative reactions in cells. We are utilizing the methylotrophic yeasts *Hansenula polymorpha* and *Pichia pastoris* as model systems to investigate peroxisome biogenesis and function. We have established that peroxisomes are essential for growth on methanol and have isolated mutants that are completely devoid of the organelle. Future plans call for the detailed genetic, biochemical and cellular biological analysis of our peroxisome-deficient mutants and the isolation and analysis of genes required for peroxisome function.

3. Oxidative enzymes involved in fungal cellulose degradation
   V. Renganathan

Interest in biotechnological conversion of cellulose to glucose has led to the detailed examination of fungal cellulase systems. Many cellulolytic fungi produce extracellular oxidative enzymes in addition to cellulases, but the role of these enzymes in cellulose degradation is unclear. Cellulolytic cultures of the lignocellulose-degrading white-rot fungus *Phanerochaete chrysosporium* produce two extracellular cellobiose oxidizing enzymes: cellobiose oxidase (CBO), a hemoflavoenzyme; and cellobiose:quinone oxidoreductase (CBQase), a flavoenzyme. The object of this project is to understand the structure, function and mechanism of CBO and CBQase and the specific role(s) of these enzymes in lignocellulose degradation by *P. chrysosporium*.

4. Regulatory pathways in filamentous fungi
M.S. Sachs

The phenomenon of multipathway gene activation in response to amino acid imbalance or limitation, known as cross pathway control (CPC), in *Neurospora crassa*, has been an exciting area of research in recent years. The regulation of arginine biosynthesis is under cross pathway control, and regulation of *arg-2* gene expression addresses central issues in gene regulation, since there is evidence for both transcriptional and translational control of ARG2 polypeptide synthesis. We have been studying the structure and regulation of the *arg-2* locus and have shown that *cis*-acting sequences in the *arg-2* gene promoter, the CPC1 box, bind a *trans*-acting factor encoded by the *cpc-1* gene, which is thought to be a transcriptional activator. We have located these CPC-1 boxes to introns within a second small open reading frame upstream of the *arg-2* coding region. We are continuing to study the structure of the *arg-2* locus and the mechanisms involved in its regulation using promoter-reporter constructs containing specific *in vitro* deletions and mutations of the sequences involved.

Oregon Graduate Institute of Science and Technology
Beaverton, OR 97006-1999

123. Chemistry of Ultrathin Membranes and Films
D.H. Thompson, M. Silverberg, D.W. Grainger, and S.B. Rananavare
Department of Chemical and Biological Sciences *$500,000*

This award represents third-year support for a three-year program which has the overall objective of establishing a faculty group with research interests and requisite educational backgrounds to develop graduate- and postgraduate-level curricula and training in membrane chemistry. Four faculty positions have been created under the auspices of the grant, resulting in the addition of individuals listed in the heading. Research activities within the center are directed at elucidating the molecular forces controlling supramolecular assembly and dynamics of ultrathin membranes and films. Major interests encompassed by the group include:

I. Synthesis of novel materials patterned after archaebacterial membranes, with specific reference to understanding the unique structural elements giving rise to their exceptional thinness and durability.

2. Mechanisms of molecular recognition/binding at supramolecular surfaces, including interactions of biologically active materials with nonbiological surfaces.

3. Use of low-angle x-ray and magnetic imaging techniques as a means of developing conceptual structural models of molecular organization within microphases.

4. Theoretical modeling by stochastic methods of dynamic processes including diffusion on two-dimensional lattices, transmembrane ion and electron transport, domain formation and phase separation.

Results from these activities should impact significantly upon development of diverse membrane-based technologies in photoconversion, molecular electronics, and medical pharmaceutics/prophylaxis.

Oregon State University
Corvallis, OR 97331

124. Catalytic Mechanism of Hydrogenase from Aerobic N_2-Fixing Microorganisms
D.J. Arp, Laboratory for Nitrogen Fixation Research *$55,216*

This project aims at elucidating the catalytic mechanism of hydrogenase from aerobic N_2-fixing microorganisms. This enzyme efficiently recycles the H_2 evolved by nitrogenase. Several properties of these hydrogenases make them ideal to function in an environment in which all of the available substrate is generated *in situ* (e.g., a very low rate of the back reaction, H_2 evolution, and a low km for H_2). We are particularly interested in the enzymes from *Rhizobium*-induced root nodules and the soil microorganism, *Azotobacter vinelandii*, because of their role in improving the efficiency of biological N_2 fixation. Both enzymes are Ni- and Fe-containing dimers composed of subunits with molecular weights of 65,000 and 35,000. Their metal content and subunit composition are typical of a large group of H_2-oxidizing hydrogenases. We have focused on the catalytic functions of this enzyme. Recent efforts have dealt with the mechanisms of several inhibitors. Slow-binding and rapid-equilibrium inhibitors competitive with H_2 have been described, as have rapid-equilibrium inhibitors which are not competitive with H_2. This implies that different binding sites are present for these various inhibitors. Two additional, critical factors are the redox state of the enzyme and whether it is membrane-associated or purified. The inhibitors are now being used in conjunction with EPR and uv-vis spectroscopy to identify which redox centers are affected by inhibitor binding.

Oregon State University
Corvallis, OR 97331

125. Analysis of Potyvirus Proteolytic Processing: A Basis for Pathogen Derived Resistance?
W.G. Dougherty, Department of Microbiology *$142,000 (FY89 funds/two years)*

The RNA genome of a potyvirus is expressed as a high molecular weight polyprotein which is co- and post-translationally processed by two viral-encoded proteinases. One of these proteolytic activities is associated with a 49kilodalton (49kDa) protein the focus of our studies. The use of recombinant plasmids containing potyviral complementary DNA sequences and cell-free transcription and translation systems has enabled us to study the 49kDa protein and cleavage events associated with its formation. This protein is formed by two self-cleavage events via which the proteinase releases itself from an internal location in the polyprotein. Amino acid sequences flanking this 49kDa proteinase do not appear to effect the *cis* cleavage. The proteinase is able to cleavage itself out when placed in other regions of the viral polyprotein or in a variety of foreign proteins. Deletion mutagenesis experiments suggest that there is a cleavage site present in the middle of the 49kDa protein. Removal of the C-terminal portion of the 49kDa protein exposes a previously cryptic site which can be processed when 49kDa proteinase is added in *trans* and results in the formation of 24kDa and 23kDa proteins. This suggests with other experimental data, that the 49kDa protein is a polyprotein consisting of two separable domains; an N-terminal VPg protein and a C-terminal proteinase. The activity and specificity of the proteinase is not affected by the presence or absence of the N-terminal sequences.

Oregon State University
Corvallis, OR 97331

126. Genomic Variation in Maize
C. Rivin, Department of Botany and Plant Pathology *$60,000 (FY89 funds)*

Our long term goals are to learn how different DNA sequences and sequence arrangements contribute to genome plasticity in maize, and to investigate the role genome variation may play in plant growth and adaptation. We have described quantitative genomic variation among maize inbred lines for tandemly arrayed and dispersed repeated DNA sequences and gene families, and qualitative variation for sequences homologous to the *Mutator* transposon family. The potential of these sequences to undergo unequal cross-over, non-allelic recombination and transposition makes them a source of genome instability. We have found examples of rapid genomic change involving these sequences in F1 hybrids, tissue culture cells and regenerated plants.

In the research proposed, we will examine rapidly occurring genomic alterations at the DNA sequence level in order to understand the molecular mechanisms that create the quantitative and qualitative polymorphisms we have found. We will also look for correlations between genomic variation for specific sequence families and the relative vigor of the maize plant. These experiments will contribute to a general understanding of the balance between genome stability and genome flexibility in plants and the potential for genetic alteration of an important crop species.

Pennsylvania State University
University Park, PA 16802

127. Rapid Regulatory Control of Plant Cell Expansion and Wall Relaxation
D.J. Cosgrove, Department of Biology *$173,000 (FY90 funds/two years)*

Plant size and shape is controlled by regulation of wall relaxation, which is the biophysical starting point for cell expansion. Our efforts to measure relaxation and to characterize its relationship to growth have revealed complex dynamics in the relaxation process, suggesting the operation of a feedback-controlled system for growth. The nature and characteristics of this regulatory system are being explored. Wall relaxation is measured in rapidly growing intact plants by the pressure-block technique. We have found that internode-length mutants of peas (*Pisum sativum* L.) show reduced wall relaxation. This is largely due to an inhibition of the process that shifts the yield threshold to lower values. In a similar manner, growth effects of light, gibberellin and drought stress are mediated by changes in wall relaxation. Measurements of turgor pressure in growing cells, using the pressure probe, indicate that turgor plays a necessary, but passive, role in growth regulation. New pressure-block techniques will be used to characterize the dynamics of the growth feedback system, and spontaneous oscillations in growth rate will be analyzed for evidence of feedback. These experiments will characterize and define an aspect of plant growth (growth compensation and regulation) which is poorly understood, but which is likely involved in many growth responses and which enables plants to adapt to varying environmental conditions such as reduced supply of water or light.

Pennsylvania State University
University Park, PA 16802

128. Enzymology and Molecular Biology of Lignin Biodegradation
M. Tien, Department of Molecular and Cell Biology

$80,852

The lignin peroxidase isozyme family of the wood-degrading fungus *Phanerochaete chrysosporium* catalyze the oxidative depolymerization of lignin. These isozymes are extracellular hemeprotein peroxidase which require hydrogen peroxide for catalyzing the oxidation of a large number of organic substrates. The lignin peroxidases are unique in their low pH optimum for catalysis (pH 2.0) and in their ability to oxidize substrates of high redox potential. Our research is focused on understanding the structural aspects which confer the ligninase with these unique properties. Rapid kinetics, electrochemistry and spectroscopy are being used to characterize the heme active site. The manganese-dependent peroxidases, enzymes which oxidize Mn(II) to Mn(III) are also being characterized by similar techniques. We have also isolated the genes encoding these isozymes and are presently attempting to express these genes in heterologous systems. This research is focused on understanding the role of these different isozymes in degradation of lignin.

University of Pennsylvania
Philadelphia, PA 19104

129. DNA Sequences encoding Chlorophyll a/b Binding Polypeptides
A.R. Cashmore, Plant Science Institute, Department of Biology *$110,799*

The aim of this project is to characterize the DNA sequences and protein factors responsible for mediating the expression of the photoregulated genes encoding the chlorophyll a/b binding polypeptides. By site-directed mutagenesis studies we have explored the role of conserved DNA sequences within a light-regulatory promoter element from the *Nicotiana plumbaginifolia* Cab E gene. These studies have been carried out in transgenic tobacco plants using promoter-GUS fusions. Mutation of the Cab E G-box element that we have previously described has been demonstrated to result in dramatic loss in expression. Similarly, mutation of the conserved GATA box sequences, that reside between the CAAT box and TATA box has also been demonstrated to have a large effect on expression.

In addition to examining the role of promoter sequences, we have been studying protein factors that bind to these DNA sequences. We have previously characterized an AT-rich negative regulatory element for the Cab E promoter. We have now demonstrated that a factor binds selectively to certain AT-rich sequences in various photoregulatory promoters, including sequences within this negative element of the Cab E promoter. This factor, which we call AT-1, is of particular interest as we have shown that binding of AT-1 is modulated by phosphorylation.

In addition to AT-1, we have characterized other protein factors that bind to elements within the Cab E promoter. Included in these factors is: GBF, which we had previously characterized for binding to various RBCS genes; GT-1, a factor that binds to conserved sequences in pea RBCS genes; GA-1, a factor that binds to the GATA box sequences; and GC-1, a factor that binds to GC-rich positive regulatory elements of the Cab E promoter.

Purdue University
West Lafayette, IN 47907

130. Biosynthesis and Assembly of Cell Wall Polysaccharides in Cereal Grasses
N.C. Carpita, Department of Botany and Plant Pathology *$65,080*

We investigate the biosynthesis of mixed-linkage ß-D-glucan and glucuronoarabinoxylans which make up the hemicellulosic matrix of the primary walls of maize and other cereal grasses. The Golgi apparatus was enriched from plasma membrane and other organelles by flotation density gradient centrifugation. The Golgi membranes, a subfraction of endoplasmic reticulum separated from the Golgi after addition of chelator, and plasma membrane were each used to form radioactive glucans *in vitro* with UDP-[^{14}C]-D-glucose. Substantial amounts of glucan were made by Golgi membranes in the presence of Mn^{2+} and the absence of Ca^{2+}. A portion of the radioactive product was solubilized by protease K or a mixed-linkage glucan-specific endohydrolase from *Bacillus subtilis*. Separation of the oligomeric digestion products by high performance liquid chromatography yielded two novel radioactive glucan oligomers as well as small amounts of radioactive tri- and tetra-cellodextrin oligomers typical of native cereal mixed-linkage ß-D-glucan. We are now determining the chemical structure of the major oligomeric products by sequential enzyme digestion and high performance liquid chromatography of the products. We then will examine reaction conditions, substrate components, and membrane interactions to optimize *in vitro* synthesis of specific oligomeric sequence units of the ß-D-glucan. We have also initiated studies to determine if any of these units are attached to protein "primers" and if any of these can be identified by SDS-PAGE and radioautography.

Purdue University
West Lafayette, IN 47907

131. Ca^{++} Gated Proton Fluxes in Energy Transducing Membranes
R.A. Dilley, Department of Biological Sciences *$76,908*

Our research on chloroplast bioenergetics focuses on the proton electrochemical potential gradient that forms as an intermediary energy storage in, or across, the thylakoid membrane, during the conversion of absorbed sunlight energy into the chemical energy forms needed for plant life. The proton gradient - akin to a battery but powered by protons (H^+) rather than electrons - drives the energy-requiring synthesis of adenosine triphosphate (ATP) as the protons flow, down the energy gradient, through a special membrane protein complex that "couples" the energy-releasing H^+ flow to the energy-requiring ATP formation reaction. The molecular mechanisms for H^+ ion movement in and through membranes are not understood, and represent an important, unsolved question in biology.

We have evidence that the proton flow through the energy coupling complex is a "gated" flux, with calcium ions providing part of the mechanism for switching the H^+ flux gate between the open or closed condition. It appears that when calcium ions are tightly bound, to the 8 kDa subunit of the H^+ channel part of the coupling complex, the H^+ gradient stays within localized domains, but when the calcium ions are displaced, the H^+ ions flow out of the postulated gate into the inner volume of the thylakoid and form a proton gradient over a larger volume, thus the term delocalized proton gradient is used. We are studying the biochemical parameters that control the calcium gating action on the H^+ gradients and testing for the occurrence of other membrane-related phenomena which may be controlled by the different states of the calcium gating action.

Purdue University
West Lafayette, IN 47907

132. Regulation of Photosynthetic Membrane Components in Cyanobacteria
L.A. Sherman, Department of Biological Sciences *$162,680 (FY89 funds/two years)*

The major objectives of this proposal are to analyze gene regulation under different environmental conditions, and to determine the role of the PsbO protein (MSP, the Mn-stabilizing protein, the 33 kDa protein) in O_2-evolution. These objectives are studied in the transformable cyanobacteria *Synechococcus* sp. PCC7942 and *Synechocystis* sp. PCC6803, respectively. As a prelude to site-directed mutagenesis of *psbO* to detect a putative Ca^{+2}-binding site, we have produced a deletion strain (Δ*psbO*) of *Synechocystis* sp. PCC6803 that completely lacks the gene or the gene product. Contrary to expectations, Δ*psbO* can grow photosynthetically, although at approximately 50% the normal rate. O_2-evolution is also 50% of normal, and fluorescence kinetics in the mutant are altered. All of the other PSII components are transcribed and translated normally in the absence of *psbO*. Work in the current year will concentrate on the changes in PSII caused by the deletion of PsbO, characterization of potential Ca^{+2}-binding sites by site-directed mutagenesis, and the role of PsbO in the regulation of PSII activity.

We have also learned a great deal about gene regulation in cyanobacteria when cells are growing in iron-deficient media or under oxidative stress. In particular, we have shown that a number of cyanobacterial genes have a DNA sequence in the promoter region that is identical to the "iron-box" of *E. coli*. We are now attempting to detect the repressor protein that is responsible for gene regulation by iron.

Rockefeller University
New York, NY 10021-6399

133. Asparagine Synthetase Gene Family: Differential Expression During Plant Development
G. Coruzzi, Lab of Plant Molecular Biology *$85,782*

While asparagine is an important transport amino acid in higher plants, the enzyme responsible for its synthesis remains uncharacterized. To circumvent biochemical problems, we have directly cloned asparagine synthetase (AS) genes from plants and will use a "reverse" biochemical approach to learn more about the encoded enzyme. cDNA and genomic clone analysis has uncovered two homologous but distinct genes for asparagine synthetase (AS1 and AS2) in pea. Full length cDNAs will be used to generate protein for antibodies and also to complement *E. coli* Asn- mutants. The AS1 and AS2 polypeptides of pea both contain glutamine binding domains and appear to encode cytosolic forms of AS. AS1 mRNA accumulates in the dark in a "photophobic" fashion and moreover, we have shown that this dark-induced expression is a phytochrome mediated response which occurs at the transcriptional level. Both AS1 and AS2 mRNAs are expressed at high levels in two contexts of increased nitrogen mobilization (e.g. in cotyledons of germinating seeds, and in nitrogen-fixing root nodules). We are currently characterizing the nucleotide sequence and transcription start sites for the genomic clones encoding AS1 and AS2. The AS promoters will be used to direct the expression of a "reporter" gene in transgenic tobacco plants in experiments designed to define cis-acting elements required for "photophobic" expression or developmentally regulated expression of AS genes in plants.

Rutgers University
New Brunswick, NJ 08903-0231

134. Cellulase - A Key Enzyme for Fermentation Feedstocks
D.E. Eveleigh, Department of Biochemistry and Microbiology

$165,940 (FY90 funds/27 1/2 months)

Feedstock chemicals can be obtained through the fermentation of biomass, such as cellulose. A prime requisite is to employ a well characterized and effective cellulase in order to efficiently convert cellulosics to glucose syrups. *Trichoderma reesei* cellulase is well known. We have shown that this system has low level constitutive expression, that permits initial attack of the insoluble cellulose substrate to release soluble inducers and thereby switch on full synthesis. The proof was through initial inhibition of the constitutive cellulase components (through binding with antibodies) and subsequently measuring mRNA levels using insoluble and soluble inducers. We also study the cellulase from a thermophilic actinomycete *Microbispora bispora* which produces good yields of a thermally stable, cellulase. The cellulase is comprised of endoglucanases, cellobiohydrolases and cellobiases that act synergistically. These components have been characterized to differing degrees. The cellobiase is remarkably resistant to end-product inhibition and is being focused on. Two cellobiases were recognized following cloning into *E. coli*. Their genes are being sequenced. The cloned cellobiase bgl-1 (app. MW 66,000, opt. pH 6.5 and pI 6.85) is soluble (intracellular) in *E. coli* facilitating study of this normally membrane associated enzyme.

Rutgers University
Piscataway, NJ 08855-0759

135. Corn Storage Protein: A Molecular Genetic Model
J. Messing, Waksman Institute *$204,572 (FY90 funds/two years)*

Corn is the staple crop for animal feed worldwide. The corn kernel is rich in oils, carbohydrates, and proteins. Most of these macromolecules are digested in the gut and are the essential source of fatty acids, sugars, and amino acids. The latter ones are more complex because nonruminants are unable to interconvert certain amino acids. Therefore, the amino acid composition of the corn kernel directly determines the nutritional value of the feed. Since amino acid composition is not balanced in the seed of one plant, a diet of corn and soybean meal can be used. Still, one amino acid, namely methionine, remains too low. Therefore, a diet of animal feed is supplemented with free methionine. This supplement, however, has to be derived by fermentation, an energy costly process. Consequently, a higher level of methionine in the seed would create an important energy saving step. Recently, we have isolated a storage protein gene from corn that contains about 23% methionine and is called the 10 kD zein gene. It is a single copy gene in contrast to the multigenic zein family that we have studied previously. We have now found two trans-acting factors that are absent in most inbreds. Both aid in the overexpression of the 10 kD protein and lead to increased methionine levels in the mature seed of BSSS-53. It is interesting that, by overexpression of a single gene, methionine levels in the mature corn kernel can be significantly increased. One factor, Zpr10/(22), acts on the translational or post translational, and the other tafA, on the post transcriptional level. We are now studying the trans-acting mechanisms and their map location.

Salk Institute for Biological Studies
San Diego, CA 92138-9216

136. Genetic Analysis of Photoreceptor Action Pathways in Arabidopsis thaliana
J. Chory, Plant Biology Laboratory *$84,796*

The process of greening, or differentiation of the chloroplast, involves the coordinate regulation of many nuclear and chloroplast 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 molecular genetic information are not understood. We have identified *Arabidopsis thaliana* mutants in both signal perception and transduction elements of these pathways based on aberrant morphological phenotypes in response to light. We are using a combination of genetics, molecular biology, and biochemistry to elucidate the precise biochemical lesion in these mutants and to begin to dissect the number and interactions of signal transduction pathways involved in light regulation of plant development.

In order to identify mutants that are not dependent on a pre-determined phenotype, we are using promoter fusions to select trans-acting regulatory mutations. We have introduced an *Arabidopsis* light activated *cab* promoter fused to both screenable and selectable markers into *Arabidopsis* using *Agrobacterium tumefaciens* transformation. We have characterized the transgenic plants for the structure of the inserted sequence and shown that the transgenic lines express the marker genes correctly with respect to light and tissue specificity. Seeds from these transgenic lines are being collected in order to do a large scale mutagenesis. Seedlings from this population of mutagenized transgenic seeds will be selected which aberrantly express the marker genes with respect to light/dark regulation and tissue-specificity of expression. These mutants will be characterized and used in crosses with the morphological mutants that we have already obtained.

Solar Energy Research Institute
Golden, CO 80401

137. The Water-Splitting Apparatus of Photosynthesis
M. Seibert, Photoconversion Research Branch *$123,000*

The O_2-evolving complex of photosystem II (PSII) binds a cluster of four manganese atoms required for catalyzing photosynthetic water-splitting function. Bridging ligands hold the cluster together, but the cluster is bound by terminal ligands to at least some of the proteins comprising the PSII D1-D2-cytochrome b-559 reaction center (RC) complex. Four high affinity Mn-binding sites, located on RC proteins, can be detected in spinach and *Scenedesmus* PSII membranes when functional Mn is removed (but not when it is present). Amino acid modifiers and proteases can selectively inhibit individual sites. Two of the sites are associated with carboxyl and two other sites are associated with histidyl amino acid residues. Both types of sites are mutually exclusive, located on the lumenal side of the membrane, and protected specifically by Mn^{2+} from the effects of amino acid modifiers. The carboxyl sites are observable when two loosely bound functional Mn are removed from the membrane. The histidine sites are undetectable in *Scenedesmus* LF-1 but can be regenerated if the unprocessed carboxyl end of the D1 protein is removed by protease action. Additional evidence suggests His-337 near the carboxyl end of the D1 protein as a primary candidate for one of the histidine sites. The high affinity, Mn-binding sites are involved in Mn^{2+} donation to PSII and may be required in photoactivation of O_2 evolution. They also may be sites providing some of the terminal ligands to functional Mn.

Southern Illinois University
Carbondale, IL 62901

138. Regulation of Alcohol Fermentation by Escherichia coli
D.P. Clark, Department of Microbiology *$74,929*

The purpose of this project is to elucidate the way in which the synthesis of ethanol and related fermentation products are regulated in the facultative anaerobe *Escherichia coli*. We are also investigating the control of other genes required for anaerobic growth. We have isolated both structural and regulatory mutations affecting the expression of alcohol dehydrogenase, the enzyme responsible for the final step in alcohol synthesis. Some of these regulatory mutations also affect other anaerobically induced genes. The *adh* gene has been cloned and sequenced. The ADH protein is one of the largest highly expressed proteins in *E. coli* and requires approximately 2700bp of DNA for its coding sequence. We have also isolated mutations affecting the fermentative lactate dehydrogenase and have recently cloned the *ldh* gene. In consequence it is now possible to construct *E. coli* strains defective in the production of any one or more of their normal fermentation products (i.e. formate, acetate, lactate, ethanol and succinate). The factors affecting the ratio of fermentation products are being investigated by *in vivo* NMR spectroscopy. Examination of our collection of anaerobically controlled gene fusions has shown that many can be switched on in air upon treatment with cyanide or certain other inhibitory agents. We are investigating the basis for this effect at present.

Stanford University
Stanford, CA 94305-5020

139. Nodulation Genes and Factors in the Rhizobium Legume Symbiosis
S.R. Long, Department of Biological Sciences *$236,000 (FY90 funds/two years)*

The formation of nitrogen fixing nodules by *Rhizobium* bacteria and plants provides the major input of biologically fixed nitrogen in agriculture. It is therefore an important feature of planning to use Biological Nitrogen Fixation for energy conservation. Our work will concentrate on how the bacterium accomplishes the early steps of inducing nodule formation in plants. In particular, we are studying nodulation gene mechanism, by finding and characterizing nodulation genes in *Rhizobium meliloti*. We aim to characterize signals produced by *R. meliloti* that are active in causing early responses in its host plant, alfalfa (*Medicago sativa*). We have identified two new *nod* genes, *nodP* and *nodQ*, and have found they exist in a second copy. We plan to construct and to test the phenotype of *nodPQ* double mutants. We have purified the protein product of *nodH* and are preparing antibody, the same will now be attempted for NodP and NodQ. We have used the *nodP*, and *nodQ*. The *nodP* homology to *E. coli* genomic DNA will be used to map this gene to 58 minutes of the *E. coli* chromosome, which may provide a clue as to its function in the symbiosis. Using genetics combined with chemical analysis, we will characterize bacterial metabolites that may result from *nod* gene action. Our goal will be to elucidate at least some steps of the biosynthetic pathway of nodulation gene signals. Finally, we will pursue further the development of bioassays for nodulation gene signals.

Texas A&M University
College Station, TX 77843-2128

140. Nuclear Genes Encoding Plastid Proteins Expressed Early in Chloroplast Development
J.E. Mullet, Department of Biochemistry and Biophysics *$132,000 (FY89 funds/two years)*

The long term objective of this research is to elucidate mechanisms which regulate plastid number and composition in higher plants. This proposal will focus on the identification and characterization of nuclear genes encoding plastid proteins which are expressed early in chloroplast development. In barley, early events in chloroplast biogenesis such as plastid replication, DNA synthesis and activation of plastid transcription occur in basal cells of the developing leaf. The initiation of these processes precedes accumulation of the photosynthetic apparatus which occurs as cells mature and are displaced apically in the leaf. Spatial separation of successive phases of chloroplast biogenesisin barley leaves has been used to identify chloroplast DNA-binding proteins which are expressed early in chloroplast biogenesis. Furthermore, it has been found that inhibition of the activation of plastid transcription during early chloroplast biogenesis inhibits expression of nuclear genes such as *cab* and *rbcS*. The identity of the plastid DNA-binding proteins will be sought and the nature of the signals which coordinate plastid and nuclear gene expression further elucidated.

Texas Tech University
Lubbock, TX 79409

141. Characterization of a 1,4-ß-D-Glucan Synthase from Dictyostelium discoideum
R.L. Blanton, Department of Biological Sciences *$145,765 (FY90 funds/two years)*

The biochemical and molecular biological analysis of eukaryotic cellulose synthesis has been hindered by the absence of a reliable, fully characterized *in vitro* assay for the cellulose synthase. The objective of this project is to characterize further the 1,4-ß-D-glucan synthase we have obtained from the cellular slime mold *Dictyostelium discoideum*. *D. discoideum* presents many advantages as a model organism for studying eukaryotic cellulose synthesis: cellulose may be synthesized *in vitro* (we have shown the product to be cellulose), cellulose synthesis is induced, mutants may be easily selected, cultural manipulations are diverse and simple, and there exists a significant body of techniques and protocols for the experimental manipulation of this organism. Our expectation is that at least some of what we learn will be applicable to understanding higher plant glucan synthases.

The specific goals of the project are: (1) to study the effects of membrane morphology on the ratios and types of linkages formed and on the crystalline form of the product; (2) to search for soluble factors that may activate the synthase; (3) to solubilize the synthase and to purify it further; (4) to identify the substrate-binding polypeptide using photoaffinity labeling; and (5) to isolate and begin to characterize cellulose-minus mutants.

Texas Tech University
Lubbock, TX 79409

142. The Interaction of Ferredoxin:NADP$^+$ Oxidoreductase and Ferredoxin:Thioredoxin Reductase with Substrates
D.B. Knaff, Department of Chemistry and Biochemistry *$158,250 (FY90 funds/two years)*

Monoclonal antibodies against spinach ferredoxin:NADP$^+$ oxidoreductase (FNR) will be isolated and tested for their ability to inhibit two reactions catalyzed by the enzyme. Antibodies recognizing epitopes at the pyridine nucleotide-binding site should inhibit electron transfer from NADPH to either dichlorophenol indophenol or ferredoxin, while antibodies recognizing epitopes at the ferredoxin-binding site should only inhibit electron transfer to ferredoxin. The ability of the monoclonal antibodies to affect the binding of both of FNR's substrates will also be assayed by direct binding measurements, using spectral perturbations to monitor NADP$^+$ binding and a membrane ultrafiltration assay to monitor ferredoxin binding. If monoclonal antibodies are identified that bind to epitopes at either the substrate binding sites on FNR, these epitopes will be mapped using the technique of differential chemical modification. Differential chemical modification (i.e., the protection, by complex formation, of amino acid residues against chemical modification will also be used to study the interaction of FNR with ferredoxin. The binding site on ferredoxin for two additional ferredoxin-dependent, spinach chloroplast enzymes, ferredoxin:thioredoxin reductase (FTR) and glutamate synthase, will also be investigated by differential chemical modification. Sequencing of glutamate synthase will be conducted in an attempt to locate a likely ferredoxin-binding site, similar in structure to those on FNR and FTR.

Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

143. Enzymology of Acetone-Butanol-Isopropanol Formation
J.-S. Chen, Department of Anaerobic Microbiology *$73,950*

Acetone, *n*-butanol, and isopropanol (solvents) are important industrial chemicals and fuel additives. Several *Clostridium* species produce butanol as a major product. *Clostridium beijerinckii* (also known as *C. butylicum*) can produce all three compounds. Solvent fermentation is a complex process, which requires a metabolic switch (from acid production to solvent production) and produces C_3 and C_4 compounds in different ratios under different conditions. An understanding of these organisms and their solvent-producing abilities is required for rational improvements of this fermentation. The objectives of this project are to elucidate first the properties of solvent-forming enzymes and then to apply information gained from the enzyme study to elucidate the control mechanisms for the solvent-producing pathways and the solvent-production genes in *C. beijerinckii*. We have studied enzymes catalyzing each solvent-producing reaction and also several enzymes catalyzing related reactions. So far, we have examined the following enzymes: aldehyde dehydrogenase, a primary alcohol and a primary/secondary alcohol dehydrogenases, acetoacetate-forming enzymes, acetoacetate decarboxylase, thiolase, 3-hydroxybutyryl CoA dehydrogenase, phosphotransacetylase (PTA), and phosphotransbutyrylase (PTB). When an increase in activities of solvent-producing enzymes was used as an indicator for the onset of solvent production (the metabolic switch), this event was found to occur during the exponential phase of growth, much earlier than what was previously recognized. The conversion of acetoacetyl CoA to acetoacetate is a key reaction in solvent production and may involve alternate enzymes. Besides CoA transferase, an acetoacetyl CoA hydrolase and an acetoacetyl CoA-reacting PTB have been identified in *C. beijerinckii*, and their relative importance is being studied. Following the characterization of the primary/secondary alcohol dehydrogenase, the structural gene for the enzyme is studied to help elucidate the control of expression of solvent-production genes.

Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

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

J.G. Ferry, Department of Anaerobic Microbiology *$183,000 (FY90 funds/two years)*

Several proteins in the pathway of carbon and electron flow during acetate conversion to methane in *Methanosarcina thermophila* are characterized. Acetate kinase and phosphotransacetylase activate acetate to acetyl-CoA. A carbon monoxide dehydrogenase (CODH) complex synthesizes acetyl-CoA from methyl-iodide, CO and CoASH: the reverse reaction almost certainly is involved in cleavage of the acetyl group of acetyl-CoA during growth on acetate. Resolution of the complex yields a dimeric nickel-iron protein with CO-oxidizing activity, and a dimeric corrinoid/iron-sulfur protein. It is proposed that the nickel-iron center is involved in C-C bond cleavage with transfer of the methyl group to the corrinoid/iron-sulfur protein. Methyl-coenzyme M is an intermediate in the pathway; enzymes and cofactors involved in methyl transfer from the methyl-corrinoid/iron-sulfur protein to coenzyme M are under study. Electrons for the reductive demethylation of methyl-coenzyme M to methane derive from oxidation of the CODH-bound carbonyl group of acetyl-CoA. The immediate electron acceptor for the CODH is a ferredoxin. The methyl-coenzyme M methylreductase utilizes HS-HTP; electron carriers between ferredoxin and HS-HTP are under investigation. The genes encoding the ferredoxin and the corrinoid/iron-sulfur protein have been isolated; work is continuing on sequencing these genes and isolating additional genes which encode enzymes of the pathway.

Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

145. Unravelling Lignin Formation and Structure in Living Plants

N.G. Lewis, Departments of Wood Science, Forest Products, and Biochemistry *$84,796*

It is steadily being recognized that plant lignin structure can no longer be adequately represented by synthetic preparations, obtained by treatment of the monolignols with peroxidase H_2O^2 *in vitro*. Indeed, differences in lignin composition and structure between species, tissues, and even subcellular locations indicate that a highly orchestrated and carefully controlled process occurs in plants.

Because of their intractable nature, only crude representations of lignin structure have been obtained; these were proposed following examination of lignin-derived products. In this study, we have identified the specific bonding pattern of each phenylpropanoid side-chain carbon in lignin *in situ* for a softwood (*P. taeda*), a hardwood (*L. leucocephala*), and a grass (*T. aestivum*). These involve the positions where the majority of inter-unit linkages are found. This was achieved by administration of suitable specifically enriched C-13 lignin precursors to the plant tissue, and subsequent examination by solid-state C-13 NMR. In each case, different bonding environments were observed, and all differed substantially from artificial lignin preparations.

The exclusivity of Z-monolignols in lignification is questionable. In *F. grandifolia*, it has been demonstrated the E to Z isomerization of coniferyl alcohol occurs, and that only the Z-isomer is used in subsequent glucosylation reaction. We are now delineating the process of monlignol transport (i.e., its precise chemical identity and mechanism of transport), and how lignification is regulated/controlled.

The mechanism by which C-13 labelled lignified plant tissue is biodegraded by *P. chrysosporium* is being examined. Initial experiments with C-13 labelled lignins and monolignols revealed that fungal peroxidase(s) (lignin peroxidases) in the presence of H_2O_2, only afforded synthetic lignins, i.e., depolymerization/degradation was not observed.

Washington State University
Pullman, WA 99164

146. Regulation of Terpene Metabolism
R. Croteau, Institute of Biological Chemistry *$84,796*

Terpenoid oils, resins, and waxes from plants are important renewable resources. The objective of this project is to understand the regulation of terpenoid metabolism using the monoterpenes (C_{10}) as a model. The pathways of monoterpene biosynthesis and catabolism have been established, and the relevant enzymes characterized. Developmental studies relating enzyme levels to terpene accumulation within the oil gland sites of synthesis, and work with bioregulators, indicate that monoterpene production is controlled by terpene cyclases, the enzymes catalyzing the first step of the monoterpene pathway. As the leaf oil glands mature, cyclase levels decline and monoterpene biosynthesis ceases. Yield then decreases as the monoterpenes undergo catabolism by a process involving conversion to a glycoside and transport from the leaf glands to the root. At this site, the terpenoid is oxidatively degraded to acetate that is recycled into other lipid metabolites. During the transition from terpene biosynthesis to catabolism, the oil glands undergo dramatic ultrastructural modification. Degradation of the producing cells results in mixing of previously compartmentized monoterpenes with the catabolic enzymes, ultimately leading to yield decline. This regulatory model is being applied to the formation of other terpenoid classes (C_{15}, C_{20}, C_{30}, C_{40}) within the oil glands. Preliminary investigations on the formation of sesquiterpenes (C_{15}) suggest that the corresponding cyclases may play a lesser role in determining yield of these products, but that compartmentation effects are important. From these studies, a comprehensive scheme for the regulation of terpene metabolism is being constructed. Results from this project will have important consequences for the yield and composition of terpenoid natural products that can be made available for industrial exploitation.

Washington State University
Pullman, WA 99164-4660

147. Isocitrate Lyase and the Glyoxylate Cycle
B.A. McFadden, Biochemistry/Biophysics Program *$72,964*

Our objectives are to shed light upon the structure, regulation and catalytic function of isocitrate lyase (*icl*), an enzyme which catalyzes the first unique step in the glyoxylate cycle. In this cycle, lipids are converted to carbohydrates in a process which contributes to microbial growth on fatty acids and to the growth of oil-rich seedlings and animal embryos.

We have published data describing the cloning and sequencing of the *icl* gene of *Escherichia coli* [*J. Bacteriol., 170*, 4528 (1988)]. We have also described considerably improved purifications of *icl* from *E. coli* and watermelon cotyledons [*Prep. Biochem., 18*, 431 (1988)]. In the current project period, we have characterized the glyoxylate- and succinate-binding domains of *icl* [*Arch. Biochem. Biophys., 274*, 155 (1989)] and have alkylated the active-site residue, *cys*-195, (*Arch. Biochem. Biophys., in press*). In the coming project period, the amino acid sequences of up to five active-site peptides from the *E. coli* enzyme will be elucidated as will the flanking sequences for the phosphorylated *his*. Mutagenesis of the *E. coli icl* gene will be directed towards replacing hypothetically functional active-site residues that are conserved in the castor bean enzyme and towards replacing the *his* that is uniquely phosphorylated in bacterial *icl*.

These studies will provide basic information about *icl*. The function of this enzyme is vital to microbial growth (on fatty acids) and to the growth of varied plant seedlings and their subsequent utilization of solar energy.

Washington State University
Pullman, WA 99164-6340

148. Enhancement of Photoassimilate Utilization by Manipulation of the ADPglucose Pyrophosphorylase Gene
T.W. Okita, Institute of Biological Chemistry *$145,000 (FY90 funds/two years)*

Starch biosynthesis is controlled in large part by the gene activation and expression of ADPglucose pyrophosphorylase as well as the allosteric control of the enzyme activity by 3-phosphoglycerate and Pi. During the past year we have focused on the activity and structure of the potato tuber enzyme and its corresponding genes. The incorporation of ^{14}C-sucrose into starch can be increased at least 50% when tuber discs are incubated in the presence of 10 mM mannose, a Pi sequestering agent. This result is consistent with the *in vitro* inhibition of ADPglucose pyrophosphorylase by Pi and suggests that the *in vivo* activity of this enzyme may be enhanced for increased starch synthesis. The tuber enzyme has been purified to near homogeneity. Analysis of the structure of the enzyme by two-dimensional polyacrylamide gel electrophoresis and Western blotting revealed that unlike the homotetramer arrangement exhibited by the bacterial enzyme, the tuber enzyme, as shown earlier for the spinach leaf enzyme, is composed of a pair of non-identical subunits. Using a recently isolated tuber cDNA, the temporal expression of the ADPglucose pyrophosphorylase gene was evaluated during tuber development. In young tubers (0.1 to 2 g) no correlation was observed between the levels of mRNA transcripts or enzyme activities, and tuber weight. These results indicate that the overall expression of the ADPglucose pyrophosphorylase gene may not be regulated in young tubers by developmental control alone. We are currently pursuing studies to determine the structure-function relationships of the enzyme subunits and the molecular/biochemical basis for the control of gene expression of ADPglucose pyrophosphorylase during tuber development.

Washington University
St. Louis, MO 63130

149. Genetic Engineering with a Gene Encoding a Soybean Storage Protein
R.N. Beachy, Department of Biology *$78,880*

The genes encoding the α' and β subunits of the soybean storage protein β-conglycinin are differentially expressed during seed development. We have identified the DNA sequences that control the expression of each gene and demonstrated the capacity of the promoters from both genes to control the expression of foreign genes. Our current research emphasis is to fully characterize the DNA sequences of the elements that control the different patterns of expression of the α' and β promoters. For this purpose chimeric promoters comprised of fragments of DNA taken from each promoter were combined in different arrangements and ligated with a reporter gene. The expression of wild type and chimeric promoters will be compared in transgenic plants. We also have initiated research to characterize and purify the protein factors from soybean and other seeds that bind to promoter sequences of the α' and β gene promoters. The purpose of these studies is to better understand the nature of the factors that control the expression of these important genes and to alter their expression through genetic transformation.

Washington University
St. Louis, MO 63110

150. Processing and Targeting of the Thiol Protease, Aleurain
J.C. Rogers, Division of Hematology-Oncology *$171,000 (FY90 funds/two years)*

We have studied a barley gene that encodes a protein with unusual homologies: the C-terminal portion, about 270 amino acids, is 65% identical to the mammalian lysosomal thiol protease, cathepsin H. This degree of sequence conservation indicates that the enzyme must have some specific function in both plants and mammals that cannot tolerate further divergence. We prepared specific antibodies to this barley protease by expressing it from its cDNA as a fusion protein in *E. coli*, then using the fusion protein to immunize rabbits. In barley aleurone and in leaf tissue, the antibodies define a 42 kd proenzyme form of aleurain that has two N-linked oligosaccharide chains. As defined in pulse-chase experiments, the proenzyme is processed to a 32 kd mature form after passage through the Golgi compartment. It is targeted to specific vacuoles in aleurone cells that are defined by the anti-aleurain antibodies in immunogold electron microscopy. The protein is not secreted from aleurone cells. Results from experiments using specific inhibitors of different classes of proteases demonstrate that proenzyme processing requires two separate enzymatic activities: the first, where 8 kd of the proenzyme is "clipped" off, cannot be blocked by any of the inhibitors tested; the second, where 1-2 kd of the proenzyme is then "trimmed" away, can be completely blocked by a thiol protease inhibitor. We have expressed proaleurain in tobacco suspension culture cells and have shown what is processed normally and retained in the cells, presumably in the vacuole. Experiments are underway to define the specific amino acid sequence required to target aleurain to its intracellular destination.

Washington University
St. Louis, MO 63130

151. Hydroxyproline-rich Glycoproteins of the Plant Cell Wall
J.E. Varner, Biology Department *$89,726*

The cell walls of plants, especially of dicots, characteristically contain the hydroxyproline-rich, basic, rod-forming glycoprotein extensin. Extensin molecules are most abundant in sclereids. The developmental appearance of extensin is easily followed by tissue printing on nitrocellulose paper. Extensin is abundant in roots in those cells surrounding the origin of lateral roots. Similarly tissue print northerns show the localization of accumulated extensin mRNA. In tomato stems, roots and fruits the highest concentrations of mRNA are found in the vascular tissue and in the seed coats.

We have used differential scanning calorimetry to show that walls isolated from the growing region of soybean hypocotyls undergo a phase transition--a change in specific heat--with a midpoint at 53°C. If these walls are first treated with millimolar calcium ions the magnitude of the transition is greatly reduced and the temperature of the transition is shifted to 60°C. Walls isolated from the mature region of the same hypocotyls show no transition. It is likely that these transitions involve the uronic acid blocks of pectin and reflect some property of the wall important in growth and development.

We have begun to examine the possible roles of ascorbate and ascorbate oxidase in cell walls by examining their tissue localization. And we are continuing the study of the developmental accumulation of the solanaceous lectin (hydroxyproline containing).

University of Wisconsin
Madison, WI 53706

152. Enzymology of Biological Nitrogen Fixation
R.H. Burris, Department of Biochemistry *$94,433 (FY90 funds/two years)*

Continuation of our studies on the control of nitrogenase in *Azospirillum* spp., *Rhodospirillum rubrum* and *Herbaspirillum seropedicae* has included the effects of oxygen, NH_4^+ and amino acids. *H. seropedicae* is microaerobic and did not fix N_2 at O_2 levels over 4kPa. Ammonium ion suppressed its nitrogenase, but suppression was incomplete even at 20 mM NH_4^+. We found no clear evidence that ADP-ribosylation is involved in inhibition of nitrogenase in *H. seropedicae*. In contrast "NH_4^+ switch-off/on" in *Azospirillum brasilense* and *Azospirillum lipoferum* clearly involves functioning of dinitrogenase reductase ADP-ribosyltransferase (DRAT) and dinitrogenase reductase-activating glycohydrolase (DRAG). The DRAT system was transferred from *R. rubrum* to *Escherichia coli* and *Klebsiella pneumoniae* and it functioned in these organisms. A mutation that changed the arginine 101 site, the ADP-ribose attaching site, eliminated the inactivation that results from ADP ribosylation. ^{15}N-depleted fertilizer was used in field tests to aid in selecting the most effective plant-bacterial associations for fixing N_2 by the common bean (*Phaseolus vulgaris*). We have extended studies to add common bean, red clover, birdsfoot trefoil and crown vetch to the list of legumes that when grown on normal N_2 have higher ^{15}N concentrations in their nodules than in their other plant parts. Dinitrogenase from a *nifV* mutant of *K. pneumoniae* contains an altered form of the iron-molybdenum cofactor of dinitrogenase, and the enzyme differs from the enzyme derived from the wild type organism in its Michaelis constant and inhibitory constants.

University of Wisconsin
Madison, WI 53706

153. Molecular Genetics of Ligninase Expression
D. Cullen, Department of Bacteriology *$132,000 (FY89 funds/two years)*

Lignin depolymerization is catalyzed by extracellular peroxidases of white rot basidiomycetes such as *Phanerochaete chrysosporium*. In submerged culture, multiple isozymes of lignin peroxidase (LiP) are secreted at relatively low levels, and production is derepressed under carbon, nitrogen, or sulfur limitation. Our objectives are to elucidate the organization/regulation of the genes encoding LiP's of *P. chrysosporium* and to investigate their expression in *Aspergillus nidulans*. Toward these goals, we have cloned and sequenced six closely related LiP genes. Three of these genes are clustered within a 30 kilobase region of the genome. Further, we have separated the *P. chrysosporium* chromosomes using alternating field electrophoresis and shown that all six LiP genes are present on one chromosome. Chromosome-specific cosmid libraries are being prepared to facilitate detailed mapping of genomic regions containing LiP genes. In addition to these genomic organization studies, the regulation of LiP gene expression in *P. chrysosporium* and *A. nidulans* is being assessed. The long term goal, production of highly purified recombinant peroxidases, may aid the development of processes such as biological bleaching of pulps, effluent treatments, and in biopulping.

University of Wisconsin
Madison, WI 53706

154. Role of Transit Peptide in the Localization of Nuclear-encoded Chloroplastic Proteins
K. Keegstra, Department of Botany *$178,000 (FY89 funds/two years)*

Most chloroplast proteins are encoded in the nucleus and synthesized in the cytoplasm as precursors containing additional amino acids called a transit peptide. The precursors are post-translationally imported into chloroplasts and segregated to their proper location. The objective of our work is to understand the role of the transit peptide and other topogenic sequences in directing the import and sorting processes. These processes will be studied in an *in vitro* reconstituted system. In this system, radioactive precursor proteins are synthesized by *in vitro* expression of cloned precursor genes and the resulting precursor proteins imported into isolated intact chloroplasts. The localization of imported proteins will be examined by chloroplast fractionation studies. Past efforts have focused on the precursors for ferredoxin and plastocyanin; proteins located in the stomal space and the thylakoid lumen respectively. Genes for precursor proteins destined for the chloroplast envelope membranes have been isolated and are being characterized. The role of the transit peptides is being examined by generating hybrid precursor proteins containing the transit peptide from a precursor destined for one location fused to the mature peptide destined for a different location. *In vitro* import followed by chloroplast fractionation is used to determine whether the transit peptide influences the ultimate location of the polypeptide. We expect that targeting to the chloroplast envelope will require additional topogenic sequences; some of these may be in the mature protein.

University of Wisconsin
Madison, WI 53706

155. Organization of the R Chromosome Region in Maize
J.L. Kermicle, Laboratory of Genetics *$127,000 (FY90 funds/two years)*

Organization of the *R* region in maize is under study with a view of determining the number, kind and arrangement of components involved in the control of anthocyanin pigmentation. *R* is organized on a modular basis and is extensively polymorphic. An allele comprises one or more functionally independent units (genic elements), each distinguished by particular tissue-specific effects. Intragenic recombination serves to place differences between genic elements relative to sites of recessive mutation associated with insertion of the transposable element *Dissociation.* Molecular characterization provides detail concerning the physical structure of regions of particular functional significance. The collection of *Ds* insertion mutations also are being used to investigate, (1) the relationship of kernel spotting density and germinal mutation spectrum to location of the insert within *R*, and (2) effects of *Ds* inserts on the pattern of intragenic recombination.

University of Wisconsin
Madison, WI 53706-1569

156. Carbon Monoxide Metabolism by Photosynthetic Bacteria
P.W. Ludden and G.P. Roberts, Department of Biochemistry *$152,000 (FY90 funds/two years)*

This project focuses on the biochemistry, physiology and genetics of the carbon monoxide oxidation system found in the photosynthetic bacterium *Rhodospirillum rubrum*. The enzyme carbon monoxide dehydrogenase (CODH) is produced by *Rhodospirillum rubrum* in response to carbon monoxide in the medium. The enzyme is a nickel and iron sulfur enzyme with a molecular weight of 60,000. The enzyme carries out the oxidation of CO to CO_2 and can be produced in an apo-form lacking the nickel component of the active site. In the current grant period various forms of the enzyme will be produced with isotopic substitutions for the nickel and iron sulfur centers of the enzyme. Spin labelled substrates such as ^{13}CO, ^{13}CN will be utilized to study the interaction of ligands to the active site of the enzyme. Enzyme containing ^{61}Ni, ^{59}Co, or ^{57}Fe in the active site will be analyzed by EPR, ENDOR, Mossbauer and NMR. Substitution of ^{57}Fe in the iron sulfur centers can be accomplished by growing *Rhodospirillum rubrum* on medium lacking nickel and containing ^{57}Fe. Proton NMR of the enzyme should allow investigation of the various amino acid ligands of the various metal clusters in the enzyme. In concert with the studies described above, the gene for carbon monoxide dehydrogenase will be isolated and the sequence for that gene will be determined.

University of Wisconsin
Madison, WI 53706

157. Starch Synthesis in the Maize Endosperm as Affected by Starch-Synthesizing Mutants
O.E. Nelson, Department of Genetics *$81,838*

A *Brittle-1* allele has been cloned by transposon tagging with a defective *Spm* and sequencing is well under way. The use of a methylation-sensitive restriction enzyme has overcome the difficulties associated with the multiple copies of the transposable element in the genome since the mutable *bt* allele has a unique SalI fragment that hybridizes to a *dSpm* probe. No such fragment is found in similar digests from nonmutant revertants of the mutable allele.

The absence of an endosperm cytosolic phosphoglucomutase (PGM) from some maize inbreds has been investigated in detail. The majority of the inbreds tested have only a single isozyme present 22 days postpollination, and this is the isozyme compartmentalized in the amyloplasts. Relatively few inbreds (W64A, Mo17, and W570) of those tested have both the amyloplast and the cytosolic isozymes. No mutant tested determines the presence or absence of the cytosolic isozyme. The inbred background, in which the mutant is placed, is the determinative factor. Thus there is no discernible phenotypic consequence associated with the absence of the isozyme. A report of this investigation has been prepared as a communication to PLANT PHYSIOLOGY, and a draft is attached.

Investigations have continued on the mutants, *dull*, *sugary-2*, and *vit*-8132* for which we have tentative identifications of enzymatic lesions although most time has been spent on the lines of research noted above.

University of Wisconsin
Milwaukee, WI 53201

158. Mechanism of Formation of the Carboxyl of Acetate by Acetogenic Bacteria
S.W. Ragsdale, Chemistry Department *$77,894*

Acetogenic bacteria are anaerobes which synthesize acetate by the Wood pathway of acetyl-CoA synthesis. Acetate is formed from CO, H_2/CO_2, or organic substrates. Enzymes directly involved in the formation of the carboxyl of acetate are CO dehydrogenase (CODH) in the synthesis from CO or CO_2 and pyruvate-ferredoxin oxidoreductase and CODH in the synthesis from pyruvate. We recently, in collaboration with Ljungdahl (U. of Georgia), have cloned and determined the sequence of the genes encoding the two subunits of CODH. We also are characterizing intermediates in the conversion of various C-1 donors to the carbonyl of acetyl-CoA using magnetic resonance, Mossbauer, and vibrational spectroscopies, and electrochemical methods. We have determined that the CO binding site on CODH consists of a unique metal center which consists of Ni and Fe in the form of a [4Fe-4S] center and that CO is bonded directly either to the Ni or the Fe in the complex. Determination of the structure of this complex is extremely important since it is the site of activation of CO or CO_2 by the enzyme. We have determined the redox potentials of this Ni-Fe-C center and the other metal centers in CODH and are further characterizing the structure and function of these metal centers in CODH. A goal is to elucidate the intermediates involved in formation of the carboxyl of acetate from the carboxyl of pyruvate using magnetic resonance spectroscopies. The rates of previously unknown exchange reactions are also being determined: between the carboxyl of pyruvate and the carbonyl of acetyl-CoA and between CO and the carboxyl of pyruvate.

University of Wisconsin
Madison, WI 53706

159. Feedback Limitations of Photosynthesis
T.D. Sharkey, Department of Botany *$152,000 (FY90 funds/two years)*

The regulation of photosynthesis in intact leaves is studied by combining analytical gas exchange analyses with biochemical and biophysical measurements. Photosynthetic responses to light, CO_2, and O_2 are determined in intact leaves and compared with mechanistic models of photosynthesis. The long term goal is to understand how photosynthesis is regulated and how it can be modified.

A major emphasis has been the study of photosynthesis limited by feedback resulting from insufficient capacity for starch and sucrose synthesis. We have shown that ribulose bisphosphate carboxylase reversibly loses activity by decarbamylation under feedback conditions. We are now studying how this decarbamylation occurs. We are also studying the mechanism of feedback limitation on electron transport by non-invasive optical measurements of the status of the photosynthetic electron transport chain. In another feedback project we have shown that sucrose-phosphate synthase activity is sensitive to the rate of photosynthesis occurring in leaves. This regulation appears to have a short term component and a longer term (days) component that is sensitive to cycloheximide. The mechanism of the long term regulation is under study.

The properties of photosynthesis in a transgenic plant expressing excess phytochrome are under investigation. Leaves of these plants have higher levels of many enzymes of photosynthesis but the overall rate of photosynthesis in air is lower. We are currently testing whether this is caused by increased resistance to CO_2 diffusion using stable carbon isotope discrimination analysis.

University of Wisconsin
Madison, WI 53706

160. Molecular Mechanism of Energy Transduction by Plant Membrane Proteins
M.R. Sussman, Department of Horticulture *$75,922*

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. It's 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 enzyme's primary structure that are essential for converting chemical into electrical energy. DNA cloning and sequencing techniques are being used to obtain the complete amino acid sequence for ATPase structural genes present in *Arabidopsis thaliana*, a higher plant with facile genetic characteristics such as a small genome (ca. 70,000,000 bp) and a rapid generation time (ca. 4 weeks). The sequence of genomic and cDNA clones is being determined for each of the several ATPases gene isoforms present in the nuclear genome of this plant species. Expression of these cloned genes is being studied using Northern blots and GUS gene fusions with putative ATPase promoter sequences. These studies on expression of the ATPase gene will help to delineate the tissue-specific and environmental signals that regulate activity of the plasma membrane proton pump *in situ*. In addition, these studies provide data necessary for generating and testing hypotheses concerning the molecular mechanism of protein-mediated proton conduction and energy transduction in plants.

University of Wisconsin
Madison, WI 53706

161. Mechanism for the Selective Conjugation of Ubiquitin to Phytochrome
R.D. Vierstra, Department of Horticulture *$73,950*

The selective degradation of intracellular proteins is an important component in the regulation of plant cell physiology and development. The goal of this proposal is to determine how proteins are selectively committed to breakdown by characterizing the selective degradation of the plant photoreceptor chromoprotein, phytochrome, as a model system. Phytochrome exists in two photointerconvertible forms, a red light-absorbing form, Pr, and a far-red light-absorbing form, Pfr. Because the degradation of Pfr is approximately 100 times faster than Pr, this system offers us the unique ability to rapidly and synchronously manipulate the degradation rate of a protein by non-invasive light irradiations. We have previously shown in a variety of plant species that phytochrome is conjugated with the small protein, ubiquitin, after photoconversion to Pfr. Given the involvement of ubiquitin conjugation in the degradation of both plant and animal proteins, we proposed that Pfr is degraded via a ubiquitin- dependent proteolytic pathway. Both kinetic analyses and localization studies of ubiquitin-phytochrome conjugates support this proposal. The purpose of the research described herein is to further define the relationship of ubiquitin conjugation to phytochrome with Pfr degradation. This study will involve: (i) an identification of ubiquitin conjugation sites on phytochrome, (ii) development of an *in vitro* system capable of Pfr-specific ubiquitination and degradation, and (iii) development of a transgenic system for the analysis of phytochrome ubiquitination and degradation by site-directed mutagenesis. Successful completion of this proposal will provide new insights into the molecular mechanisms for selective ubiquitin conjugation and may generate new information on the regulation of selective protein turnover in plants.

Worcester Foundation For Experimental Biology
Shrewsbury, MA 01545

162. Novel Biomaterials: Genetically Engineered Pores
H. Bayley *$74,490 (co-funded with Division of Materials Sciences)*

Recombinant DNA technology allows the generation of new materials in microorganisms. We are constructing a selection of microscopic pores by genetic manipulation of a bacterial channel protein. They include: pores with different internal diameters, with selectivity for the passage of molecules and ions, and with gating properties (the ability to open and close in response to a physical stimulus, e.g. an electric field or light). The molecule we are remodeling is a α-hemolysin (α-HL), which is secreted by *Staphylococcus aureus*. It comprises a single polypeptide chain of 33.2 kDa capable of forming hexameric pores in membranes ~11Å in internal diameter. α-HL has several advantages over other polypeptides that might be used in this project. It is a small robust molecule that is relatively easy to reengineer. The wild-type polypeptide can be obtained in gram amounts. The hexameric pore can be assembled from the monomer *in vitro* by the addition of an inexpensive detergent. Our immediate aims are to obtain α-HL by overexpression of the α-HL gene in *E. coli*, to locate the domain of α-HL responsible for pore formation using deletion mutagenesis, to alter this domain by point mutagenesis to produce new pores with a range of properties, and to define the properties of these molecules using a combination of biochemical and biophysical techniques. Ultimately, the new pores will be used to confer novel permeability properties upon materials such as thin films. Such products have potential technological applications: for example, as components of energy conversion and storage devices, selective electrodes, electronic devices, and ultrafilters.

Yale University
New Haven, CT 06511

163. Molecular Cloning and Structural Characterization of the R Locus of Maize
S.L. Dellaporta, Department of Biology *$154,000 (FY89 funds/two years)*

The *R* locus of maize controls the deposition of anthocyanin pigments. Various alleles of *R* may pigment one or several plant tissues. The *R-r* allele pigments the hypocotyls and anthers of the plant and the aleurone of the seed. Seed and plant pigmentation are controlled by separate genetic elements, termed components. We have shown that these components correspond to specific genes by analyzing recombination derivatives of *R-r*. Derivatives lacking either plant or seed pigmentation occur frequently. One class of derivatives have been shown to be the result of displaced synapsis and crossing over between specific regions of the *R-r* locus. The loss or presence of *R* hybridizing sequences in genomic mapping experiments has been correlated with specific displaced pairing arrangements among repeated elements. We have constructed a physical map of *R-r* and have shown that the locus is large (>100kb). Both tandem and inverted repeats of *R* sequences are present at the locus. We are presently investigating the basis for non-reciprocal recombination events that also contribute to the *R-r* instability.

Yale University
New Haven, CT 06511

164. Control of Genes Encoding Catabolic Enzymes in *Bradyrhizobium*
D. Parke and L.N. Ornston, Department of Biology *$79,866*

The goal of this project is to understand energy metabolism and gene control in bacteria of the genus *Bradyrhizobium*. These bacteria fix nitrogen in symbiosis with legumes and are capable of surviving on low levels of nutrients. Although the organisms are relatively fastidious with respect to such growth substrates as carbohydrates, they grow at the expense of a wide range of monocyclic phenolics which originate from lignin and plant root exudates. Diverse phenolic compounds are broken down in bacteria by metabolic pathways that converge on the ß-ketoadipate pathway. Inducible in all other microbes studied, enzymes of the pathway are produced constitutively in *Bradyrhizobium*. One enzyme in particular, ß-ketoadipate succinyl CoA transferase, is expressed at high levels in saprophytic and symbiotic *Bradyrhizobium*. The properties of this enzyme are being studied in an effort to understand the physiological basis for its high constitutive expression. The investigation is also analyzing whether unregulated enzyme synthesis is a feature of other peripheral catabolic pathways in *Bradyrhizobium*. Mutant strains blocked in the catabolism of plant phenolics have been isolated. These strains are being used to clarify routes of phenolic breakdown and to study the mechanisms of control of the genes. In addition, the strains are being used to determine whether the ability to metabolize phenolics is a factor in survival of *Bradyrhizobium* in the soil, in its proliferation in the rhizosphere, or in competition for nodulation.

Yale University
New Haven, CT 06510

165. Mechanisms and Control of K^+ Transport in Plants and Fungi
C.L. Slayman, Department of Cellular and Molecular Physiology *$102,544*

The overall purposes of this study are to provide a detailed functional description of active and passive potassium transport in model plant/fungal cells, to identify and isolate the protein molecules which mediate K^+ transport, and ultimately to determine the intramolecular mechanisms which determine ion passage. The two major research lines now being followed are cloning three varieties of K^+ transporters in *Neurospora crassa*, and characterizing potassium channels and proton pumps in *Neurospora* and *Saccharomyces cerevisiae* electrophysiologically, largely by means of patch recording. Both types of studies will be extended to cultured plant cells (*Arabidopsis* and perhaps *Avena*) in the near future.

Systems being characterized electrophysiologically are several channels in the plasma membrane and tonoplast, especially a 120-pS calcium- and voltage-dependent channel which appears to be further regulated by metabolic changes, two channel systems which open at extreme positive and negative voltages, a non-specific leak whose temperature characteristics closely resemble those of the plasma-membrane proton pump, and a high affinity K^+ uptake system which appears to be a K^+-H^+ symport. Those being cloned are a high-affinity uptake system, a low-affinity uptake system, and a leak system. The first is being cloned in *Neurospora* by selection with a probe from the corresponding cloned yeast gene (Gaber et al., Mol. Cell Biol. 8:2848-2859) and the other two by complementation of previously isolated K^+-defective mutants.

Yale University

New Haven, CT 06511

166. Transfer RNA Involvement in Chlorophyll Biosynthesis

D. Soll, Department of Molecular Biophysics and Biochemistry *$198,000 (FY90 funds/two years)*

Chlorophyll is the major pigment in photosynthesis. One molecule of chlorophyll is synthesized from eight molecules of δ-aminolevulinic acid, the universal precursor of porphyrins. Chlorophyll synthesis is regulated at the synthesis of δ-aminolevulinic acid, which is formed in the stroma of greening plastids from glutamate. This pathway differs from δ-aminolevulinic acid formation in bacteria, fungi or mammals, where glycine and succinyl-coenzyme A are the precursors. The mechanism of δ-aminolevulinic acid synthesis from glutamate is only incompletely understood. A solid body of evidence has accumulated to show that in the chloroplasts of plants and green algae, in the cyanobacteria (e.g., *Synechocystis 6803*) and in some eubacteria (e.g., *E. coli* and *B. subtilis*) and archaebacteria it involves the reduction of glutamate to glutamate-1-semialdehyde which is subsequently converted to δ-aminolevulinate. Studies on the *in vitro* synthesis of δ-aminolevulinate in extracts from barley chloroplasts, *Chlamydomonas, Synechocystis 6803* showed the involvement of $tRNA^{Glu}$. In addition to the tRNA three enzymes are involved in this process. In the first step glutamate is attached to the tRNA by the regular glutamyl-tRNA synthetase. The subsequent reduction of glutamate to glutamate semialdehyde is carried out by Glu-tRNA reductase which required tRNA as a specific "cofactor". The barley Glu-tRNA reductase shows high specificity among different tRNA species. In the final step an aminotransferase converts glutamate-1-semialdehyde to δ-levulinic acid. We have purified the three enzymes from *Chlamydomonas* and reconstituted the *in vitro* conversion of glutamate to δ-aminolevulinic acid from three purified enzymes and a purified tRNA. The goal of our studies is the cloning and characterization of the genes encoding these macromolecules and an understanding of the regulation of this biosynthetic pathway. These studies should uncover novel principles regarding the role of tRNA as a cofactor in metabolic conversions.

INVESTIGATOR INDEX

SUBJECT INDEX

UNITED STATES
DEPARTMENT OF ENERGY
WASHINGTON, D.C. 20545

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