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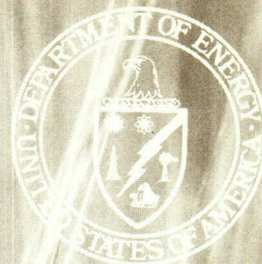

ANNUAL REPORT AND SUMMARIES OF FY 1989 ACTIVITIES

DIVISION OF ENERGY BIOSCIENCES

SEPTEMBER 1989

U.S. DEPARTMENT OF ENERGY
OFFICE OF ENERGY RESEARCH
OFFICE OF BASIC ENERGY SCIENCES
DIVISION OF ENERGY BIOSCIENCES
(FORMERLY DIVISION OF BIOLOGICAL ENERGY RESEARCH)
WASHINGTON, D.C. 20545

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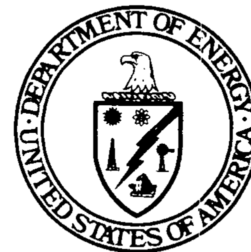
Cover photo courtesy of Kurt Stepnitz, Michigan State University

DOE/ER-0424



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PROGRAM OVERVIEW OF THE DIVISION OF ENERGY BIOSCIENCES

The Energy Biosciences program is about to enter its tenth year. The mission of the program continues to be **to provide the basic understanding** of biological phenomena relating to how organisms transform and use energy, how they produce and convert the manifold products of photosynthesis and how organisms adapt to meet their energy requirements under adverse growing conditions. Ultimately, this information will contribute to the development of biotechnologies for solving energy related problems. This includes providing a solid foundation for new or improved renewable resource production of fuels, chemicals and other materials, information about biosystems as possible alternatives for fossil energy intensive processes, and by using biological diversity and adaptability to help resolve energy related problems associated with environmental cleanup.

The Energy Biosciences program focuses on generating fundamental information that underpins applications; the program does not support optimization of processes or near term applications. A particularly important role that the program has played is in the stimulation of high quality research in selected topic areas and with representative organisms that have been neglected generally, e.g., methanogenesis, thermophiles, complex carbohydrates.

Over the period of its existence, the Energy Biosciences program has been oriented towards the support of contemporary fundamental investigations in the plant and microbiological sciences. Virtually all projects are peer reviewed at regular intervals. The program comprises a mix of projects, some of which are in areas that are rapidly moving with an abundance of workers, ideas and techniques, while other projects are in fields that are less populated because the problems may be refractory and/or the area is at a primitive level of understanding. Innovative projects are always being sought.

The program came into existence at a time when biological research had been undergoing a revolution. This was occurring as a result of the changing technology available to the researcher. The power of molecular biological technology and concepts, which is essentially an "everyman" technology, has come together with powerful analytical chemical research technologies to provide probes to answer long-standing questions. Biological molecular structures are being elaborated at rates that are astonishing by comparison to earlier research efforts. Computerized data bases for this information have become absolutely essential to maintain any sort of grasp of the information. In this milieu of change, the EB program has continued to grow and evolve with adherence to the original objective of gaining an understanding of the broad, long term basic problems relating to biological energy conversion and conservation as well as to the use of biosystems for assisting in the solution of the nation's energy problems.

The technical scope of the Energy Biosciences program reflects the growing impact of molecular biological approaches in many of the projects. One broad topic which will receive increasing attention is that of **biological metabolic diversity and adaptability**. Many individuals have warned of the loss of genetic resources around the world and this is a crucial concern. Carrying that idea forward, it is essential also to be aware of some of the implications of such losses. Not only might there be a loss of a species or a genus but along with that loss of genetic information may go codes for unusual biosynthetic reactions or a configuration of genetic factors that permit adaptation to specific niches. Thus one of the directions for increased emphasis is in the area of understanding more about metabolic diversity in plants and microbes. This is not intended to be a survey approach but rather an attempt to understand in depth the scope of mechanisms organisms possess and use. The energy

context of this is in pursuing not only how to increase the range of useful products from renewables, but also in trying to perceive how organisms deal with potentially toxic materials, inorganic heavy metals or organic compounds, in order to generate potential strategies for biotechnological clean-up measures. In the latter case we are aware of the great metabolic plasticity of microbes from past experience with the development of antibiotic resistance. To effectively use organisms in new, imaginative bioremediation techniques for cleaning up sites requires knowledge about the genetic and biochemical capabilities of the organisms involved. These efforts would complement related research supported by the Office of Health and Environmental Research and other programs within DOE. Other topic areas covered by the Energy Biosciences program include the following plant-related areas:

A. **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.

B. **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 as fixed carbon (biomass) and how that fixed carbon is used in the plant including the synthesis of additional energy trapping capacity. The mechanisms involved are perceived in biochemical and physiological terms as:

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

   2. **Metabolic Regulation**: The elaboration of biochemical intermediates and enzymes of important pathways and how they are controlled by feed-back inhibition, enzyme modification, enzyme turnover and other processes.

   3. **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.

C. **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.

D. **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.

E. **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.

F. **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.

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

G. **Lignocellulosic Degradation**: An effort that aims at understanding the genetic and biochemical regulation of the complexes of polysaccharide and lignin degrading enzymes including coordinated synthesis and function of component enzymes. This information may be used in the planning of new technologies for use of renewable resources.

H. **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.

I. **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.

J. **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.

K. **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.

L. **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.

M. **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 above represents an unusually broad scope and is a reflection of the diversity of research needs and opportunities. A program with limited resources such as Energy Biosciences will involve ultimately the making of choices. The criteria included are: quality of science, how much of the kind of work is already supported by this and other programs, innovativeness of ideas, needs within the research area, relatedness to the energy mission as well as the potential for development within an area.

Examples of recent research efforts supported by the Energy Biosciences include the following.

Regulation of Photosynthetic Capacity

The light harvesting organelles of plants, chloroplasts, undergo subtle biochemical and structural changes depending upon the quality of light they receive. This permits plants to thrive in a number of habitats with varying light conditions. Dr. W. Gruissem and coworkers at the University of California, Berkeley, have shown that the biochemical and structural changes are not the result of

regulation occurring during the reading of the chloroplast DNA during transcription. It appears that the regulation occurs after the gene has been transcribed into the equivalent polyribonucleotide messenger (m-RNA). Learning the manner in which manipulation can occur may offer a way of understanding regulatory mechanisms in plants that could lead to increased efficiencies to perform photosynthesis.

Progress In Genetic Transformation in Sulfate Reducing Bacteria

Sulfate reducing bacteria respire sulfate instead of oxygen as they grow in oxygenless environments. These organisms play critical roles in the global sulfur cycle, metal corrosion, oil well souring and sewage treatment and other anaerobic fermentation processes. While there has been considerable progress made to examine the biochemical processes occurring in the sulfate reducing bacteria, many physiological processes have not been studied. One major approach to studying both the physiology and biochemical reactions of these bacteria would be the development of genetic and molecular biological tools. Dr. Judy Wall of the University of Missouri recently described a virus that has the ability of transferring genes between various strains of a sulfate reducing bacteria. The discovery of this system constitutes a major development in genetically characterizing these organisms. While a system that would permit the genetic transformation of these organisms (the introduction of foreign DNA into the bacterial chromosome) is still being sought, the development of the viral transfer system by Dr. Wall opens the door for extensive genetic studies on these important organisms.

Unusual Gene Product in Methanogen

The analysis of the structure or sequence of a particular gene or chromosome region sometimes gives insights beyond those which were originally planned. Such an example was the discovery of a potentially fascinating protein by Dr. John Reeve and coworkers at Ohio State University. During their studies of the gene sequences encoding enzymes involved in the formation of methane in methanogenic bacteria, this group discovered a sequence for which they knew of no function. By comparison of this gene to other gene sequences using comparisons with structures in GenBank, they showed that the gene encodes a polypeptide that could best be described as polyferredoxin. The polypeptide has the capacity to form twelve iron-sulfur clusters arranged as six groups of two clusters. The physiological role of this molecule is not known, but there are several intriguing possibilities. These include behaving as a biological battery holding as many as twelve electrons, being the equivalent of a biological electric wire serving as a ferredoxin precursor molecule. Studies are now under way to determine the role of this molecule.

Every three years a workshop is convened by the Energy Biosciences program to review the program quality, balance and directions. The participants are not affiliated with the program. They come from a variety of institutions and represent different disciplines. The workshop is an opportunity to expose the program to critical scrutiny by individuals who come from different research backgrounds. Among the suggestions offered by the participants at the last workshop in 1988 was that in trying to stimulate research in less popular research areas, a major requirement is for trained investigators and efforts must be made to encourage young investigators to work in areas covered by Energy Biosciences. A modest effort in this direction was begun with the annual sponsorship of a post-doctoral fellowship through the Life Sciences Research Foundation. This year's recipient of the three-year award is Dr. James U. Bowie who is working in the laboratory of Dr. David S. Eisenberg at UCLA.

Conferences/workshops for which the EB program provided support include:

1. Summer Investigations into the Isolation, Cultivation, and Metabolism of Anaerobes Involved in Biodegradation, Marine Biological Laboratory, Woods Hole, MA, June-July 1989.
2. Fourth Annual Penn State Symposium in Plant Physiology, Pennsylvania State University, University Park, PA, May 18-20, 1989.
3. Conference on Pseudomonas '89: Biotransformation, Pathogenicity, and Emerging Biotechnology, Chicago, IL, July 9-13, 1989.
4. Symposium on The Genetics and Molecular Biology of Arabidopsis, Indiana University, Bloomington, IN, October 12-15, 1989.
5. Workshop on Trace Gas Emissions from Plants, Asilomar, California, January 21-25, 1990.

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

	Number of projects	FY 89 funding (in thousands)	Percent of total funds
University Grants & Contracts	139	$ 11,636	56%
Michigan State University Plant Research Laboratory	14	2,510	12%
Plant Science Centers at Universities	2	1,000	5%
National Laboratories Brookhaven National Lab Lawrence Berkeley Lab Los Alamos National Lab	14	2,593	12%
Solar Energy Research Institute	1	125	0.5%
Oregon Graduate Center	2	1,200	6%
Other Research Institutions	5	338	2%
Small Business Innovation Research (SBIR) contribution, Conferences & Miscellaneous	7	1,278	6%
Databases	1	100	0.5%
	185	$ 20,780	

Several items in the budget including $1.2 million for the Oregon Graduate Center, Small Business Innovation Research (SBIR), and other items are mandated by Congress or by administrative direction (11% of total), whereas the majority of the budget is discretionary. Some 73% of the budget is invested in university projects with the remainder of the funds going to national laboratories, other non-academic labs and conference support.

The discretionary budget for FY 1989 remained essentially the same as the previous year. This led to a situation where relatively few new proposals could be funded, but many highly meritorious proposals which had been received could not. The situation is not expected to improve, inasmuch as the number of proposals received for FY 90 funding increased over thirty percent with the budget not changing significantly.

As the competition for research funds intensifies, one resultant effect is the increasing traffic in research proposals. A second order effect is that more reviews are being sought for the increased proposal burden. This is not a trend towards efficiency. Nevertheless this is a situation confronting the U.S. research community. We in the granting agencies who are responsible for assuring that the funds that are available are spent as wisely as possible are deeply indebted to the hundreds of scientists in this country and abroad who perform reviews with generous commitment of their time and energies. Their efforts are greatly appreciated. The review system could not operate otherwise.

Lastly, should there be questions concerning the Energy Biosciences program, please do not hesitate to contact:

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Washington, DC 20545
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U.S. Department of Agriculture

Madison, WI 53705-5600

1. **Roman Microprobe Investigation of Molecular Structure and Oraganization in the Native State of Woody Tissue**
R.H. Atalla, Forest Products Lab

$73,100 (8 1/2 months)

The Raman microprobe has revealed evidence of a high degree of organization of cell wall constituents as well as a variability of molecular composition and orientation, within different domains of the cell walls of tissue from loblolly pine (*Pinus taeda L*) and from black spruce (*Picea mariana*). The objectives of this program are to investigate the range of variation in composition and molecular orientation, within cells and between tissue types, and to explore the implications of the findings with respect to the process of biogenesis and to the properties of the cell wall. Preliminary results using our new microprobe system confirm our earlier findings that lignin is more highly organized than had heretofore been recognized. We continue to optimize the system to enable us to carry out mappings of molecular organization and compositional variations in cell walls from a wide variety of morphological features. In assessing the roles of lignin-polysaccharide interactions we are exploring the degree to which lignin and its precursors may associate with cellulose during biogenesis. We have found evidence for an association strong enough to modify the pattern of aggregation of nascent cellulose fibrils. We are also investigating the pathways that may be available for charge transfer within the lignified cell wall structures. We have found that photoexcitation of the cell walls at UV frequencies absorbed by lignin, can produce an increment in the population of charge carriers that is sufficient to result in measurable photoconductive behavior.

**(*$2,300 awarded for three and a half months at the Institute of Paper Chemistry.*)*

Arizona State University

Tempe, AZ 85287-1604

2. **Antenna Organization in Green Photosynthetic Bacteria**
R.E. Blankenship, Department of Chemistry

$155,500 (FY88 funds/two years)

The photosynthetic unit of all chlorophyll-based photosynthetic organisms consists of a collection of pigments that act as an antenna, absorbing light and transferring the energy to a reaction center where energy storage takes place by a series of chemical reactions. The green photosynthetic bacteria have an unusually large photosynthetic unit of up to 1,500 bacteriochlorophyll/reaction center. The majority of the antenna pigment molecules are contained in chlorosomes, ellipsoidal vesicles attached to the cytoplasmic side of the cell membrane. Additional antenna pigments and reaction centers are contained in integral membrane proteins. The project objective is to determine the molecular organization and the mechanism of excitation transfer in the antenna system of green photosynthetic bacteria. The principal strategy involves isolation of the antenna system, biochemical resolution into its constituent subassemblies, and characterization using spectroscopic methods. Spectral evidence indicated that the chlorosome pigments are probably organized *in vivo* by pigment-protein and direct pigment-pigment interactions into what are essentially pigment oligomers. Current work includes time-resolved fluorescence and absorption spectroscopy on whole cells, membranes, chlorosomes, isolated pigment-protein complexes and pigment oligomers. Experimental and theoretical studies on the photophysical properties of pigment oligomers are also being carried out.

Arizona State University

Tempe, AZ 85287-1604

3. **Center for the Study of Early Events in Photosynthesis**
R.E. Blankenship, J.D. Gust, S.H. Lin, T.A. Moore, G.R. Seely, W.F.J. Vermaas 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 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

4. Specific Mutagenesis of a Chlorophyll-Binding Protein
W.F.J. Vermaas, Department of Botany

$70,000

Chlorophyll-protein interactions are critical to efficient light harvesting and energy transfer in photosynthetic systems. However, it is unknown how chlorophylls interact with pigment-binding proteins, and how such interactions lead to changes in the physical properties of the chromophores. Specific mutations are introduced into psbB, the gene encoding CP47 (a chlorophyll-binding protein in Photosystem II), to identify the protein domains and individual amino acid residues in this protein that have specific roles in pigment binding and/or energy transfer. CP47 has been chosen as the subject of study because (1) it appears to be relatively tightly associated with the proteins that bind the Photosystem II reaction center pigments, (2) its primary sequence has features in common with that of other light-harvesting proteins, (3) it is involved in assembly and/or stabilization of the Photosystem II reaction center proteins, and (4) in cyanobacteria, it may function in energy transfer between phycobilisomes (the peripheral antenna) and the Photosystem II reaction center. Both site-directed mutagenesis and construction of chimeric higher plant/cyanobacterial genes are used to introduce well-defined mutations into the experimental organism, the transformable, photoheterotrophic cyanobacterium *Synechocystis* 6803. The Photosystem II complex from the resulting mutants will be characterized in terms of electron transfer, light intensity dependence of charge separation, fluorescence properties, and protein composition and turnover. This is expected to yield a detailed insight into the role of various regions and residues of CP47 in pigment binding and in Photosystem II structure, function and assembly. This may lead to an understanding of the structural requirements for functional antenna-pigment binding to CP47 and other pigment-binding proteins.

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 architecture and developmental behavior of the plant cell wall/plasma membrane interface is central to an understanding of plant growth and development. We are studying various glycoproteins that are specifically localized at the *Nicotiana* plasma membrane. Firstly, we have constructed monoclonal libraries directed against cell surface antigens. We have identified the molecular nature of an antigen recognized by one of these antibodies as extensin, a hydroxyproline-rich glycoprotein. We have found that biosynthesis of this antigen occurs within the endoplasmic reticulum/Golgi and is correlated with a developmental switch in protoplast metabolism away from photosynthesis and towards heterotrophic growth. Using this antibody, we are currently attempting to establish conditions for the reconstitution of the endomembrane-secretory pathway *in vitro*. Secondly, we have constructed cDNA libraries in phage expression vectors in order to identify those genes whose expression is correlated with the onset of heterotrophic growth in protoplasts. Finally, we are continuing the development of techniques of *in vivo* flow cytometry and cell sorting of protoplasts, for the analysis of the expression of chimaeric genes within or at the surface of transformed and transfected protoplasts. We are employing the *E. coli* beta-glucuronidase gene reporter system in

translational fusions appropriate for the identification of targeting signals associated with the secretory process. We are also employing the vesicular stomatitis virus G-protein as a heterologous prototype for controlled post-translational modification and targeting. We intend to use these techniques for the isolation of mutant cell types defective within the endomembrane-secretory pathway. This research should lead to an improved understanding of the mechanisms that control plant cell growth and division.

University of Arizona
Tucson, AZ 85721

6. Phytoalexin Detoxifying Enzymes in the Plant Pathogenic Fungus Nectria haematococca
H.D. VanEtten, Departments of Plant Pathology and Molecular and Cellular Biology

$143,500 (FY89 funds/two years)

Production of antimicrobial compounds (phytoalexins) by plants is believed to function as an active mechanism of disease resistance. One way pathogens can overcome this resistance mechanism is by detoxifying their hosts' phytoalexins. Our studies have focused on the diseases caused by *Nectria haematococca (Fusarium solani)* on pea (*Pisum sativum*) and chickpea (*Cicer arietinum*). Detoxification of the pea phytoalexin, pisatin, and the chickpea phytoalexins, maackiain and medicarpin is required by this fungus for pathogenicity on pea and chickpea, respectively. Pisatin detoxification is catalyzed by pisatin demethylase, a substrate-inducible cytochrome P-450. Among the highly virulent isolates examined, induction of pisatin demethylase has been found to be highly specific for pisatin as the inducer, and this phytoalexin is the preferred substrate for the enzyme. One of our objectives is to further characterize this *Nectria* cytochrome P-450. In addition, recent results indicate that other pea pathogens detoxify pisatin in the same manner and we will determine whether these fungi also possess a specific cytochrome P-450 for the detoxification of pisatin. Another objective is to further characterize the gene and enzyme systems responsible for the hydroxylation of maackiain by *N. haematococca* and to determine how they relate to the cytochrome P-450's that demethylate pisatin. The results of these studies should indicate whether fungi have evolved specific cytochrome P-450's for plant pathogenicity.

Brandeis University
Waltham, MA 02254

7. Carbon and Hydrogen Metabolism of Green Algae in Light and Dark
M. Gibbs, Department of Biology

$64,000

The focus is the anaerobic metabolism in the eukaryotic green algae. Under N_2, *Chlamydomonas reinhardii* photometabolizes acetate with a rate of 22 umole per mg chlorophyll per hour and with a stoichiometry of CO_2 and H_2 evolution indicating involvement of the tricarboxylic acid and glyoxylate cycle sequences. The aconitase reaction is inferred from monofluoracetate inhibition. A cellular enzyme profile reveals that 25% of the succinic dehydrogenase, 30% NAD-malic dehydrogenase, 23% acetic CoA kinase, 40% adenylate kinase and 67% pyrophosphatase are chloroplastic while isocitric lyase and citric synthase are totally extrachloroplastic. Isolated chlamydomonas chloroplasts photo-oxidize succinate and malate in the presence of DCMU and methylviologen. The succinate reaction is blocked by malonate but not by rotenone; rotenone inhibits malate oxidation. Neither reaction is affected by cyanide. The kinetic properties of the chloroplastic and mitochondrial succinic dehydrogenases are similar. Starch is fermented by the cells to acetate, ethanol, formate, CO_2, H_2 and lactate. Enzymic profile indicates a chloroplastic glycolytic sequence. The *Chlamydomonas* chloroplast evolves CO_2 from externally supplied glucose by the oxidative pentose phosphate pathway and with extensive further metabolism by glycolysis and the oxidative pentose phosphate cycle.

Brookhaven National Laboratory
Upton, NY 11973

8. Chlorophyll-Protein Complexes of Photosystem II: Structure, Biosynthesis and Phosphorylation
J. Bennett, Biology Department

$225,000

The project studies the structure, function and formation of chlorophyllprotein complexes in photosynthetic membranes of green plants. These complexes catalyze the initial steps in photosynthesis: energy capture and photochemistry. Proteins under study include the reaction center proteins of photosystem II and the light-harvesting chlorophyll a/b protein (LHC II). Structural studies center on the characterization of post-transla-

tional modifications such as phosphorylation, acetylation and proteolytic cleavage, and on the analysis of protein-protein interactions. Functional and biosynthetic studies center on mechanisms by which light controls the organization and abundance of chlorophyll-protein complexes and the colorless proteins with which they are associated, such as extrinsic proteins involved in water oxidation. Six aspects of biosynthesis and its photoregulation are being explored: (1) transcription, (2) translation, (3) insertion of proteins into or through membranes, (4) binding of chlorophyll, (5) site-specific proteolysis, and (6) phosphorylation. The distribution of excitation energy between the two photosystems is controlled by phosphorylation of LHC II. The roles of phosphorylation of four photosystem II core proteins remain to be determined. Synthetic peptide analogs of phosphorylation sites are being used to differentiate among the protein kinases of the photosynthetic membranes and the chloroplast stroma and to assist in their assay, purification and characterization.

Brookhaven National Laboratory
Upton, NY 11973

9. Plant Molecular Genetics
B. Burr and F.A. Burr, Biology Department

$275,000

In addition to supplying energy for photosynthesis, light provides signals for many aspects of plant development. Light intensity, wave length, and photoperiod are responsible for plant growth and flowering. Likewise, anthocyanin pigment synthesis is, in part, under photo-regulation 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 as have genes that are likely to be directly involved in their coordinate regulation. pl is probably repsonsible for a light independent signal. It appears to regulate, or act in conjunction with b or its homologue r. In the absence of a functional pl, factors responding to at least three different wavelengths of light, one of which is phytochrome, either activate b, or act in conjunction with b, to regulate structural gene transcription.

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 b regulatory gene. Two mutations that enhance plant pigmentation in a light dependent fashion have also been identified. A variant that lacks response to UV-B light has been shown to be a simple recessive. These mutations are currently being mapped and tested for allelism.

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

$60,000

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 growth 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, however, exhibits a higher degree of physiological plasticity with regard to photoadaptation than 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 the level of control of protein synthesis by irradiance, namely at differentiating between transcriptional, translational and post-translational regulation of the apoproteins. A cDNA library has been constructed in λgt11 and four genes which encode for light harvesting chlorophyll a/b proteins of photosystem II have been isolated. One of the LHCII proteins was sequenced and used as a probe to assess changes in mRNA levels during transitions from high to low irradiance levels. From Southern blot analysis, we have estimated that the cab genes of D. tertiolecta are organized into a small gene family of 4 to 6 members. Probes unique to each cab gene are being prepared from the four cDNA clones and will be used in a detailed study of the cab gene expression during shift from high to low light. 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 assay. Simultaneously the effects of irradiance on pigment synthesis are assessed using specific inhibitors of chlorophyll biosynthesis and radiotracers.

Brookhaven National Laboratory
Upton, NY 11973

11. Regulation of Energy Conversion in Photosynthesis
G. Hind, Biology Department

$355,000

The project goal is to discover how energy transformation is regulated in photosynthetic membranes. Electron transport is studied in intact chloroplasts 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 evident from 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

Cyanobacteria are found in soil and waters worldwide. The structural biology of their photosynthetic energy collection system is under intensive examination. The collection system comprises an ordered assembly of phycobiliproteins attached to the outer surfaces of the thylakoids. These assemblies, called phycobilisomes, consist of two domains: a series of rods (80% of the mass) and a triangular shaped core (20% of the mass). A hydrophobic-interaction chromatographic method was developed for isolating and purifying the rods. Transmission and scanning transmission electron microscopy of the rods verified their structure, and the mass of the rods is being determined by neutron and electron scatter. The rods crystallized from ammonium sulfate, but they are spherulites and not satisfactory for crystallographic analysis. Transmission electron microscopy of small fragments of the crystals reveal the presence of stacks of closely appressed rods. Over short distances, the rods are closely parallel, but there is divergence over longer distances. The rods under examination consist of phycocyanin and phycoerythrin, and energy transfer is preserved. The biochemistry and pathophysiology of the heptapeptide toxins (microcystins) of the cyanobacterium *Microcystis aeruginosa* are being investigated. Cyclosporine a inhibits the lethality of the microcystin containing L-arginine and L-leucine with an apparent half time of 90 sec. The initial reactions of the microcystin and cyclosporine a with their target cells are rapid. The lethality of the toxins is correlated with their L-amino acid composition, although the toxicity is probably associated with their unusual amino acid composition.

Brown University
Providence, RI 02912

13. δ-Aminolevulinate Biosynthesis in Oxygenic Prokaryotes
S. Beale, Division of Biology & Medicine

$82,000

Tetrapyrrole pigments function as essential components in the processes of respiration and photosynthesis, and also exist as cofactors in other important enzyme reactions. The tetrapyrroles arise from a

branched biosynthetic pathway having δ-aminolevulinic acid (ALA) as its first committed member. ALA is known to be formed by two distinct routes: by condensation of glycine and succinyl-CoA in animal, fungal, and some bacterial cells, and by transformation of the intact carbon skeleton of glutamate in plants, algae, and other bacterial cells. 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

$87,034

The purpose of this project is to use genetic techniques to study the transcriptional regulation of C-1 specific functions in methylotrophic bacteria. The approach involves analyzing C-1 specific genes in a facultative methanol utilizer, Methylobacterium AM1 and using this organism as a host to study genes encoding similar functions in methane-utilizers. We have focused on methanol oxidation genes, and have cloned the MoxF, A1, A2 and A3 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 are currently characterizing these regions structurally and functionally.

California Institute of Technology

Pasadena, CA 91125

15. Molecular Analysis of Ethylene-Insensitive Mutants in Arabidopsis

E. Meyerowitz, Division of Biology

$80,500

The plant hormone ethylene is involved in a number of developmental processes and responses to environmental stresses in higher plants. While numerous physiological, biochemical and genetic responses to ethylene have been catalogued, virtually nothing is known about the initial molecular events which lead to this diversity of responses. The goal of our project is to use a combination of genetics, molecular biology and biochemistry to elucidate the basis for ethylene action. Mutants in *Arabidopsis* which either lack or have altered responses to ethylene are being selected, placed on both the physical and RFLP linkage maps, and physiologically and biochemically characterized. Genes important to ethylene action will be isolated based on the mutant phenotypes using the technique of chromosome walking. Ethylene receptor mutants are being identified using *in vivo* and *in vitro* ethylene binding assays.

Current efforts are focused on one dominant mutation, designated etr. which lacks a number of responses to ethylene which are present in the wild-type plant, including inhibition of cell elongation, promotion of seed germination, enhancement of peroxidase activity, and feedback suppression of ethylene synthesis by ethylene. Saturable ethylene binding in leaf tissue of the etr mutant is five-fold lower than that in wild-type tissue, indicating that the etr mutation directly effects the ethylene receptor. Through appropriate genetic crosses, the etr mutation has been placed on both the physical and RFLP linkage maps. A polymorphic lamda genomic clone which maps near the etr locus has been used to screen a cosmid genomic library. The cosmid library used was constructed from DNA prepared from the plants that were homozygous for the dominant etr mutation. This library should contain clones which are capable of conferring ethylene insensitivity when transformed into wild type plants. Cosmid clones which map near the etr locus have been isolated from this library and are currently being used to transform *Arabidopsis*.

University of California
Riverside, CA 92521

16. Catalytic Mechanism of Hydrogenase from Aerobic N_2-Fixing Microorganisms
D.J. Arp, Department of Biochemistry

$54,000

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 k_m 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 spectroscopy to identify which redox centers are affected by inhibitor binding. Further experiments deal with efforts to separate the subunits with metals and, perhaps, activity intact. This will lead to a clearer understanding of the role of each redox center in catalysis.

University of California
Los Angeles, CA 90024

17. Energy Capture and Use in Plants and Bacteria
P.D. Boyer, Molecular Biology Institute

$97,405

The main emphasis continues to be to gain understanding of the mechanism of the ATP synthase in plants and bacteria. Site-directed mutagenesis studies with the *E. coli* enzyme will probe the effects of substitution of a variety of amino acids for the tyrosine residues that are labeled by 2-azido-ATP at catalytic and noncatalytic sites of the beta subunit. Site(s) and extent of labeling of the alpha subunit by 2-azido-ATP will be assessed, particularly with the noncatalytic site tyrosine replaced by other amino acids. If the binding site is interfacial between subunits, additional alpha subunit labeling may result. Other studies show promise of considerably clarifying the nature of the Mg^{2+} inhibition of the isolated chloroplast ATPase (CF_1). Studies are planned of the important question of whether catalytic beta subunits all change their asymmetric positions during catalysis. An approach will be to prepare labeled beta subunits by growth of *E. coli* or *R. rubrum* with ^{35}S and to attempt to get one or two such subunits asymmetrically incorporated into the intact ATPase or ATP synthase, then to use methods we have previously developed to find if catalytic turnover changes the asymmetric positions of the subunits. In continuation of past approaches, we plan to find if the ATPase from a thermophilic bacterium that shows only weak binding of nucleotides will show modulation of ^{18}O exchange by ATP concentration, an important criterion of catalytic site cooperativity.

University of California
Davis, CA 95616

18. Restriction of Virus Infections by Plants
G. Bruening, Department of Plant Pathology

$90,000

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. We are taking two approaches to understanding and implementing resistance against plant viruses. In "genotypic resistance", 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 previously supported research, with two inhibitors, one of the processing of CPMV polyproteins and the other of the translation of CPMV RNAs. We are purifying proteins that exhibit these activities and which have the expected virus specificity in their action and the expected correlations in their inheritance in cowpea crosses. These will be tested for their ability to mediate

resistance to CPMV in cowpea cells. We propose to characterize the inhibitor(s) and the respective genes. 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

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

M.J. Chrispeels, Department of Biology

$85,000

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. Both extracellular and vacuolar glycoproteins have complex glycans, but do they have the same glycans or do they pass through different regions of the Golgi complex so that they will be differentially modified? Our recent work on the role of asparagine-linked glycans show that they do not have vacuolar targeting information, but that they are required for stability. These results were obtained by examining the fate of unglycosylated phytohemagglutinin in the seeds of transgenic tobacco plants and of unglycosylated invertase secreted by carrot cells.

University of California

Davis, CA 95616

20. Characterization and Expression of Clostridium Cellulase Genes

R.H. Doi, Department of Biochemistry & Biophysics

$81,000

The extracellular cellulase genes of *Clostridium cellulovorans* and *Ruminococcus flavofaciens* are being used as model systems for studying the genetics of two anaerobic mesophilic microorganisms. The current goals of this project are to (a) purify the cellulase and endo-β-glucan hydrolase activities of these organisms, (b) make immunoprobes and hybridization probes for their genes, (c) clone and sequence the genes for these enzymes, (d) determine the transcription, translation, and secretion signals present in these genes, (e) study the regulation of these genes at the transcriptional level, (f) develop plasmid transformation systems for these organisms, (g) analyze the expression of the cellulase genes in the native host and in *E. coli* and *B. subtilis*, and (h) develop hyperexpression plasmid vectors with the genes. The use of these model systems will allow us to compare the structure of the genes with other known cellulases, to compare the genetic signals used for expression and regulation of the genes with those found in gram positive aerobic *Bacilli*, and to develop bacterial strains and methods that are required for carrying out transformation and other genetic manipulations with anaerobic bacteria. Standard immuno- and hybridization probe procedures are being used to screen plasmid and lambda gt11 banks of *Clostridium cellulovorans* DNA for the cellulase genes. The isolated genes will be characterized as to their restriction map, base sequence, and derived amino acid sequence. The location of promoters will be determined by S1 nuclease mapping. Transformation procedures are being developed to allow plasmid transformation into *Clostridium*. Gene expression in heterologous microorganisms is also being analyzed.

University of California
Davis, CA 95616

21. Modifying K^+/Na^+ Discrimination in Salt Stressed Wheat Containing Individual Chromosomes of 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)

The impairment of plant productivity by salinity represents a limitation in the potential conversion of solar energy into biomass. Part of the solution is to develop crops more tolerant of salinity than our present cultivars. The project's objective is to advance our understanding of how K^+/Na^+ discrimination bears on salt tolerance, in terms of genetics and physiology. Lines of wheat, *Triticum aestivum cv.* 'Chinese Spring' (2n= 6x = 42), which like most bread wheats is rather salt-sensitive, into which has been incorporated each of the chromosomes of the very salt-tolerant wheat grass *Lophopyrum elongatum* (2n = 2x = 14) are being used.

The *Lophopyrum* X Chinese Spring amphiploid demonstrates greater K^+/Na^+ discrimination under saline stress than does Chinese Spring. Three *Lophopyrum* chromosomes (3E, 4E, and 7E) mainly contribute to enhanced salt tolerance in an additive fashion. The location(s) of the locus or loci conditioning the enhanced K^+/Na^+ discrimination is being mapped, using the set of substitution and recombinant chromosome lines, by K^+ and Na^+ analysis of plants grown under saline stress. Dry matter accumulation and grain yield, relative to unstressed, are being used as a measure of tolerance. Physiological aspects of the enhanced K^+/Na^+ discrimination are being examined using radioisotopes. Translocation and partitioning of $^{42}K^+$ (and/or $^{86}Rb^+$), $^{22}Na^+$, $^{36}Cl^-$, and $^{45}Ca^{2+}$ will be studied. The K_m and V_{max} for K^+ uptake for different genotypes over time under saline stress will be determined. Our ultimate goal is to characterize both the physiology and genetics of this enhanced salt tolerance in order to determine causal relationships.

University of California
Berkeley, CA 94720

22. Transcription Control Elements and Manipulation of Chloroplast Genes
W. Gruissem, Department of Botany

$96,000

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. Most significantly, the relative transcriptional activities of most chloroplast genes are maintained during different stages of plant development. The experimental analysis of the 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. Our work has also demonstrated that the differential accumulation of chloroplast mRNAs is, to a significant extent, regulated at the post-transcriptional level. The 3' inverted repeats flanking most chloroplast genes do not serve as efficient transcription terminators, but may have a role in the stabilization of the individual mRNAs. In addition to the structural component, our research also addresses the function of specific proteins which interact with the inverted repeat sequences, which may be important for the developmental control of mRNA levels. To support the *in vitro* analysis of regulatory elements, we continue our work to construct stable chloroplast transformants.

University of California
Los Angeles, CA 90024

23. Molecular Biology and Genetics of the Acetate-Utilizing Methanogenic Bacteria
R.P. Gunsalus, Department of Microbiology

$80,000

Acetate conversion to methane and CO_2 by the methanogenic archaebacteria is a primary rate limiting step in anaerobic biodegradative processes in nature. However, the genetic study of these organisms has not

been experimentally tractable due to the inability to grow and plate the organisms as single cells. The acetate-utilizing species, *Methanosarcina thermophila* TM-1, is being used for the proposed genetic and molecular studies because, unlike previously described acetotrophic methanosarcina that have a thick heteropolysaccharide cell wall, this species can be cultured in a unicellular form that has a protein cell wall lacking the heteropolysaccharide layer. These cells can be gently disrupted to obtain protoplasts or lysed to yield intact genomic DNA and RNA. The molecular basis for single cell growth is being examined. Experiments also are in progress to develop a gene transfer system for this species and *Escherichia coli* utilizing a recently isolated plasmid, pc2A, from *M. acetivorans*. Methods are being refined for the efficient plating of *M. thermophila* on defined media, for chemical mutagenesis, and for the isolation of mutants defective in acetate utilizations. Chromosomal DNA libraries from *M. thermophila* are being screened to isolate genes involved in the acetate utilization pathway (e.g. carbon monoxide dehydrogenase). Analysis of the genes elucidate the molecular mechanisms responsible for their regulatory control will be performed. These studies should aid our understanding of the pathway for acetate utilization in *M. thermophila* and serve as a model for elucidating regulatory mechanisms in the acetotrophic methanogens.

University of California

Davis, CA 95616

24. Physiological Genetics of Denitrification: A Route to Conserving Fixed Nitrogen

J.L. Ingraham, Department of Microbiology

$62,000

Biological denitrification, the conversion of fixed nitrogen to N_2 by procaryotes, is a cascade of anaerobic respirations for which the nitrogen oxides—NO_3-, NO_2-, NO, and N_2O—serve as terminal electron acceptors. We will study a protein, NosA located in the outer membrane, that is essential for *Pseudomonas stutzeri* to mediate the last step of this pathway, the reduction of N_2O to N_2. In the absence of NosA, N_2O reductase is made but it lacks copper which is essential for its activity. We will study the metabolic role of NosA in bringing copper to N_2O reductase and the regulation of its expression--what environmental conditions affect its synthesis and what genes mediate these effects. Using antibody prepared against purified NosA we will probe a λgt11 gene bank to identify NosA clones. With these clones we will look for equivalent functions or the lack of them in related denitrifiers. We will also determine if other proteins are needed to insert copper into N_2O reductase. Using similar techniques we plan to clone nitrate and nitrite reductase in order to determine their structure and linkage. We will determine the sequence of formation of the various denitrification enzymes following shifts of cultures of *P. stutzeri* from aerobiosis to anaerobiosis.

University of California

Berkeley, CA 94720

25. Calcium Homeostasis in Barley Aleurone

R.L. Jones, Department of Botany

$170,000 (FY88 funds/two years)

We initiated our study of Ca^{2+} homeostasis in the barley aleurone cell by developing methods to measure free Ca^{2+} in the cytosol and endomembrane system. We have developed a method to introduce the Ca^{2+}-sensitive dye indo-1 into the aleurone cell in a non-intrusive fashion. The method relies on the fact indo-1 is a weak acid and is protonated at a pH below 5. In the uncharged form the dye readily crosses the plasma membrane, but at a cytosolic pH of 7.3 the dye dissociates, and in the charged form it remains in the cytosolic compartment. The dye does not enter the vacuole although it enters the endoplasmic reticulum (ER). Using indo-1 we have shown that cytosolic Ca^{2+} is maintained at about 300 nM when external Ca^{2+} is 10 mM. The level of Ca^{2+} in the ER is about 3 μM and is maintained at this elevated level by the action of a pump located on the ER membrane having a K_m for Ca^{2+} of 0.5 μM. The activity of this pump is markedly stimulated by gibberellic acid (GA). There is a ten-fold increase in the activity of the ER Ca^{2+} pump when aleurone layers are treated with GA. To our knowledge, this represents the first report of an effect by a plant hormone on the activity of a CA^{2+} transporter in plants. We are exploring the possibility that in addition to affecting the expression of genes for secreted hydrolases such as α-amylase, GA also plays an important role in Ca^{2+} homeostasis in the aleurone cell.

University of California
Irvine, CA 92717

26. Membrane Bioenergetics of Salt Tolerant Organisms
J.K. Lanyi, Department of Physiology and Biophysics

$80,000

Ionic pumps in the cytoplasmic membrane of halobacteria are studied from the point of view of their relevance for adaption to high salt concentration. A considerable amount of knowledge on halorhodopsin, a light-driven chloride pump, has been accumulated, as methods are available for its purification and reconstitution into proteoliposomes. Present work focuses on the structure of this small membrane protein and the molecular mechanisms involved in the ion translocation. The second system under study is the halorhodopsin from an alkalophilic halobacterium, with the idea that this system, with somewhat different properties from the normal halorhodopsin, will reveal which features must be conserved for the transport function. The third system under study is the proton-translocating ATPase of halobacteria. We have recently developed a high-yield purification method for this protein, and identified its subunits. The cloning of the structural genes of the ATPase is now underway.

University of California
Berkeley, CA 94720

27. Determination of Genes and Phenotypes of Bacteria Necessary for Epiphytic Colonization and Survival on Plants
S.E. Lindow, Department of Plant Pathology

$67,000

Bacteria that live as epiphytes on the surface of healthy plants are important as plant pathogens, in catalyzing 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 strains of *Pseudomonas syringae* and *Erwinia herbicola* that are necessary for epiphytic fitness on bean leaves are being determined by an evaluation of the fitness of 5,300 individual Tn5-induced insertion mutants. The population sizes of 5,300 mutants of an ice nucleation active *P. syringae* strain were determined following successive wet and dry incubation periods on bean leaves from an analysis of the freezing point of individual leaves. One hundred twenty-six of these mutant strains exhibited a lower population size as evidenced by a decreased threshold freezing temperature. Analysis of the population dynamics of these mutants on bean leaves exposed to alternating environmental conditions have verified a reduction in population size of from 10- to 8,000-fold compared to the parental strain. Thirty-six of the 126 mutants grow as well on bean leaves as the parental strain but exhibited much more death upon drying of leaves. While four of these mutants were either nonmotile or insensitive to osmotic stress, the remaining desiccation sensitive mutants did not exhibit any *in vitro* phenotype that was correlated with this behavior. Genes affected by these Tn5 insertions will be cloned and further characterized by transcriptional "reporter genes" including a promoterless ice nucleation gene.

University of California
Davis, CA 95626

28. Transposon Tagging of Disease Resistance Genes
R.W. Michelmore, Department of Vegetable Crops

$66,000

Disease resistance genes, when available, provide the most ecologically desirable, least energetically expensive, and most predictable form of disease control. We are developing a transposon mutagenesis system for lettuce to clone genes for resistance to the fungal pathogen, *Bremia lactucae*, as a model for understanding disease resistance in plants. Our strategy involves using chimeric heterologous transposons to mutate and act as tags for resistance genes. We have generated transgenic plants containing several chimeric genes to study the movement of heterologous transposons and the efficacy of whole plant selectable markers. Southern analysis of plants containing *Tam3* from snapdragon was consistent with infrequent transposition in the T_1 generation; T_2 progeny are now being analyzed to confirm this and to determine whether transposition occurs in later generations. *Ac* from maize in the 5' region of a chimeric streptomycin gene (Courtesy of J. Jones, Sainsbury Lab., UK) was introduced; of 65 independent T_2 families, the majority clearly showed streptomycin resistance. This indicates a high rate of transposition similar to that seen by others in Solanaceous species or excision of *Ac* sequences during mRNA processing. Southern analyses are being made to distinguish between these alternatives. Transgenic plants have also been

generated containing CaMV 35S or hsp70 promoters fused to transposase coding sequences (Courtesy M. Lassner, UC Davis) to determine whether we can control the transposition of non-autonomous *Ds* elements; T_1 plants are growing and will be crossed and their progeny analyzed. We are also developing a transposition assay system involving the appearance of GUS activity in callus to test constructs more rapidly than in regenerated plants as currently.

University of California

Berkeley, CA 94720

29. 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

$77,500

Viruses represent a major class of pathogens which cause stress and yield loss in crop plants. Our research offers a unique opportunity to identify viral sequences involved in the fundamental processes of virus replication, encapsidation and events that affect the degree of disease severity. The research focuses on the molecular characterization of a unique class of symptom modulating RNAs which are associated with virus infections caused by Tomusviruses. These small RNAs are linear deletion mutants of the helper virus genome that interfere with it and reduce the severity of the disease. We have also shown that these molecules can arise spontaneously from the viral genome under certain circumstances. This discovery marks the first definitive report of defective interfering RNAs (DI RNA) in association with a small RNA plant virus. This contrasts with the common association of DI particles with animal virus infections where they have proved to be valuable models for studying symptom modulation and virus persistence. In order to develop a more detailed understanding of this newly discovered class of DI RNAs, we are investigating the molecular structure of several and developing a transcription system for producing biologically active DI RNAs *in vitro*. After completing mutagenesis studies designed to identify functional domains of the DI RNAs, we then propose to evaluate the effect of expression of engineered DI sequences in transgenic plants on host susceptibility. We predict that these studies will provide useful strategies for genetically engineering plants with viral disease resistance.

University of California

Berkeley, CA 94720

30. The Bioenergetics of Salt Tolerance in Cyanobacteria

L. Packer and I. V. Fry, Department of Physiology/Anatomy

$80,000

The sudden imposition of salt stress to a freshwater species of cyanobacteria elicits a series of complex responses, both in the short term (shock) and in the long term (recovery/adaptation). These responses occur at structural and functional levels, overcoming the physical and energetic disruptions caused by the cell's interaction with salinity. The project seeks to elucidate the mechanisms that enable photosynthetic cyanobacteria to survive under these conditions. Exposure to 0.5 M NaCl shock elicits altered gene products redirecting the energy-flow from photosynthesis towards respiration, salt extrusion, and synthesis of osmoregulatory substances. Non-invasive assays for the study of salt-stressed cells include: 1) NMR to determine the impact of salt on the initial intrusion of NaCl (^{23}-Na-NMR) and intracellular changes in Na; 2) ^{31}P-^{13}C-NMR (using $H^{13}CO_3$-enriched cells) to determine carbohydrate turnover and osmoregulatory changes; 3) ESR spin probe techniques to monitor internal volume, pH, transmembrane potential gradients and their roles in maintaining cellular function during salt exposure; 4) ESR-oximetry to measure intracellular O_2 levels due to the activity of photosynthesis and or respiration; and 5) the determination of ultrastructural changes, in conjunction with changes in glycogen and lipid composition to assess physiological responses to salinity. Major goals include elucidating the temporal sequence of the energetic, enzymatic and structural changes that accompany the inhibition and the recovery/adaptation phases encountered during exposure to salt.

University of California

Berkeley, CA 94720

31. Phytochrome from Green Plants: Assay, Purification and Characterization

P.H. Quail, Molecular Plant Biology

$86,000

Physiological, biochemical and immunochemical evidence indicates that the molecular species of phytochrome that predominates in fully-green, light grown tissue (Type 2 phytochrome) is distinct from

the well-characterized species that predominates in etiolated tissue (Type 1 phytochrome). The goal of this research is to understand the molecular properties, biogenesis and mechanism of action of Type 2 phytochrome. A variety of strategies have been employed in efforts to purify and to generate antibodies and cDNA clones for the Mr-118,000 Type 2 phytochrome polypeptide that we identified and characterized earlier in green oat tissue. These efforts are ongoing. In addition, phytochrome (phy) gene and cDNA sequences with properties consistent with the behavior of Type 2 phytochrome have recently been isolated from *Arabidopsis*. Northern blot analysis using a gene specific probe for this sequence shows that the corresponding mRNA is expressed constitutively and is not light-regulated as is observed for Type 1 phytochrome mRNA in oats. The availability of these probes should provide new avenues of approach in our attempts to investigate Type 2 phytochrome.

University of California

Berkeley, CA 94720

32. Genetic and Biochemical Basis of Race-Specific Incompatibility in Pseudomonas syringae pv. glycinea-Soybean Interactions

B.J. Staskawicz, Department of Plant Pathology

$84,000

Pseudomonas syringae pv. *glycinea* is the causal agent of bacterial blight of soybean. Resistance of the host to this pathogen is dependent on the genotypes of the interacting partners and is phenotypically manifested as a hypersensitive necrosis reaction with the concomitant inhibition of bacterial growth *in planta*. We have concentrated our efforts on the molecular characterization of the gene-for-gene interaction that occurs between the avirulence gene *avrB* in *P.s. glycinea* race O and the resistance gene *Rpg1* found in the soybean cultivar, Harosoy.

Polyclonal antisera raised against *avrB-lac* fusions has been employed to study the expression of the 36 kd protein of *avrB*. We have demonstrated that the transcription of *avrB* and the production of the *avrB* protein is induced in minimal media as measured by B-gal assays and western blot analysis, while *in planta* induction was measured by monitoring ice nucleation activity. Mutations in the *hrp* gene cluster have been identified that abolish the induction of *avrB* as measured by these two separate gene reporter systems. We have established that mutations in *hrpR*, *hrpS*, and *hrpL* inhibit the induction of *avrB* both in minimal induction media and *in planta* induction. (Huynh, Dahlbeck and Staskawicz, submitted for publication.). The *hrpR* and *hrpS* loci have been sequenced and have strong homology to each other as well as to a previously identified family of regulatory proteins that are involved in controlling bacterial gene expression in response to environmental stimuli. Research is currently being carried out to characterize the molecular interactions that may occur between the proteins encoded by *hrpR* and hrpS and the regulatory region of *avrB*.

University of California

Berkeley, CA 94720

33. Characterization of Embryo-specific Genes

Z.R. Sung, Departments of Genetics and Plant Pathology

$61,062

The growth and differentiation of a fertilized egg result in a bipolar structure, the plant embryo, which will undergo desiccation before germination into a plant. The embryo is an energy storehouse that accumulates starch, protein or lipid for germination. In order to understand the mechanism of some aspects of embryonic gene expression, we, using an antiserum enriched for antibodies that react with embryonic antigens, isolated 22 cDNA clones, and characterized the expression pattern of their corresponding RNAs (Choi et al. 1987). Several RNAs, e.g., two that correspond with cDNA clones 8 and 59, are coordinately expressed during embryo development; and these 2 genes are expressed in somatic as well as zygotic embryos of carrot (Borkird et al. 1988). In the last year, using the cDNA clones, we isolated the genomic clones for these 2 genes, named DC 8 and DC 59, and determined their DNA sequence. This analysis and *in situ* protein localization study revealed the possible functions of these two genes (Franz et al. in press). While DC 8 may be involved in embryo protection during seed desiccation (Dure et al. submitted), DC 59 encodes a lipid body membrane protein (Hatzopoulos, in preparation). Although the two genes encode proteins of widely different functions their expression is closely coordinated. In the future, we plan to analyze the cis-elements and trans-acting factors regulating the transcription of these two genes, in an attempt to elucidate the mechanism of their coordinate regulation.

University of California
Santa Cruz, CA 95064

34. Tonoplast Transport and Salt Tolerance in Plants
L. Taiz, Biology Department

$142,000 (FY89 funds/22 months)

Our research has focused on the structure, function, regulation and evolution of the vacuolar H^+-ATPase (V-ATPase), which energizes the transport of salts and other solutes into the vacuole. The V-ATPase has been localized on the tonoplast and Golgi of corn roots by EM-immunocytochemistry. Our recent immunochemical studies indicate that the V-ATPase is also present on purified plant coated vesicles. In corn roots, the level of vacuolar ATP-driven proton pumping activity is higher in the stele than in the cortex, while salinity enhances the activity of the cortex and meristem, but not that of the stele. The cDNA for the 70 kDa subunit of carrot was cloned and sequenced. The 70 kDa subunit exhibited a 25% identity to the beta subunit of F_0F_1-ATPases and a 50% identity to the alpha subunit of the sulfur metabolizing thermoacidophilic archaebacterium, *Sulfolobus acidocaldarius*. Thus, the V-ATPase of eukaryotes evolved from a common ancestor with *Sulfolobus*. The highly conserved catalytic domain of the F_1-type beta subunit is also conserved in the 70 kDa subunit. We have also obtained overexpression of the carrot 70 kDa subunit in *E. coli* using the expression plasmid pKK223-3. The purified overexpressed subunit will be useful for structure/function analyses.

University of California
Berkeley, CA 94720

35. Analysis of the Proteins Essential for Agrobacterium Mediated DNA Transfer to Plant Cells
P. Zambryski, Division of Molecular Plant Biology

$92,000

Our major goal is to understand how *Agrobacterium* transfers DNA to plant cells. This project specifically aims to characterize the protein products that mediate the transfer event. The transferable DNA is a single strand (ss) copy of the T-DNA region of *Agrobacterium* Ti plasmid, designated the T-strand. The vir region of the Ti plasmid provides the protein products that generate and ultimately transfer the T-strand to plant cells. We have focused on two types of vir proteins, DNA binding and membrane associated proteins. The DNA binding proteins should form a T-strand-protein complex that is competent for transfer, and the membrane proteins should actually facilitate the transfer of this complex through bacterial and plant cell membranes. We have shown that the VirE locus encodes a ssDNA binding protein capable of binding tightly and cooperatively to ssDNA in a nonsequence specific fashion. The VirE-ssDNA complexes are stable to high salt concentrations and resist exo- and endonucleolytic treatment. When observed under the electron microscope, VirE converts collapsed free ssDNA into extremely thin and extended structures. The properties of the VirE-ssDNA complexes predict that VirE functions to i) protect the T-strand from nucleases and ii) facilitate transfer of the T-strand through narrow channels in the bacterial membrane. The large 9.5 kbp virB locus encodes several polypeptides that fractionate to the bacterial membrane. These polypeptides are good candidates to form a specific transfer channel for the T-strand-protein complex. We are sequencing and mapping the positions of the virB specific polypeptides to allow their characterization using biochemical fractionation and immunological localization.

University of California
Santa Cruz, CA 95064

36. CO_2 and the Stomatal Control of Water Balance and Photosynthesis in Higher Plants
E. Zeiger, Division of Natural Sciences

$105,020 (FY88 funds/17 months)

Stomatal responses to CO_2 are one component of the coupling of photosynthesis and stomatal conductance to prevailing conditions in the leaf environment. A major question on the mechanism of the stomatal response to CO_2 is the nature of the first metabolic step allowing guard cells to sense CO_2, and subsequent means for sensory transduction of that signal. Characterization of photosynthetic carbon fixation in guard cell chloroplasts has defined a regulatory mechanism for the light-CO_2 interactions controlling stomatal movements. Photosynthetic active radiation activates a proton pump at the guard cell plasma membrane in a reaction that requires photosynthetic products from the guard cell chloroplast (ATP and a second, hitherto unknown product of photosynthesis). Photosynthetic carbon fixation should increase the demand for those metabolites in the chloroplast and deplete their availability at the pump sites, hence decreasing stomatal apertures in response to light, as

observed *in vivo* upon increases in intercellular CO_2. Recent results suggest that modulation of carbon fixation in guard cells is distinctly different from that characterized for mesophyll. Current investigations are evaluating conditions that switch on and off photosynthetic carbon fixation in guard cells.

Case Western Reserve University
Cleveland, OH 44106

37. Cloning and Analysis of Genes Regulating Plant Growth
C.D. Town, Biology Department

$77,000

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 they grow in the absence of hormones when transferred to culture. Individual tumors show interesting differences in morphology and degree of differentiation, in growth rate, and in their response to exogenous hormones, all suggestive of variations in hormonal status. 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. Our first molecular approach to analyzing these tumors has been to screen northern blots of polyA$^+$ RNA at low stringency with a battery of twenty animal oncogene probes to determine whether any of these highly conserved sequences were being expressed in our tumors. One probe (int-1), detects transcripts which are present two tumors, but absent from hormone-dependent normal callus. Another unrelated probe (int-2) identifies a transcript which is present in normal callus, but absent from one of the tumors. We plan to isolate these putative oncogene homologs from cDNA libraries, to characterize them and to study their relationship to the tumorous phenotype of the cultures. Other aspects of this work include a molecular karyotyping of the tumors using pulsed-field gels and sets of chromosome-specific probes to detect chromosomal rearrangements, and the application of in-gel renaturation techniques to look for local DNA amplification. Identification of genes involved in tumor formation 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

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

$172,000 (FY89 funds/two years)

We have cloned a number of fragments of DNA containing genes necessary for nitrogen fixation from the photosynthetic bacterium *Rhodobacter capsulatus*. The nif genes are locally clustered but the clusters are on non-neighboring DNA restriction fragments. So far we have identified six or seven regulatory genes among these, using a nifH::lac fusion. Four of the regulatory genes are required for expression of nifH. Two of these, nifR1 and nifR2, have sequences homologous to ntrC and ntrB of enteric bacteria. A third, nifR4, has sequence homology to the ntrA genes of *Rhizobium* and *Klebsiella*. Constitutive expression of nifR4 in *R. capsulatus*, from a plasmid clone, complemented a nifR4 chromosomal mutant but not a nifR1 mutant. Moreover, both oxygen and ammonia regulation of nitrogenase were maintained under these conditions. These results are consistent with a model requiring both nifR1 and nifR4 for nitrogenase gene expression. In contrast to the Ntr mutants of enterics, the nifR1-nifR2 mutants of *R. capsulatus* are Ntr$^+$, i.e. they appear to be mutated in a Nif-specific pathway. Nif$^+$ revertants could be selected, however, from a nifR1-nifR2 deletion strain. We believe these revertants arose by mutation of alternate ntrB-ntrC related genes, revealed as well by Southern hybridization of total genomic DNA probed with ntrBC DNA. At present we are continuing attempts to purify the nifR1, nifR2 and nifR4 gene products and to develop assays for them using defined supercoiled templates and RNA polymerase.

Clemson University

Clemson, SC 29634-1903

39. The Magnesium Chelation Step in Chlorophyll Biosynthesis

J.D. Weinstein, Department of Biological Sciences

$66,624

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 chloroplasts and the photosynthetic bacterium, *Rhodobacter spheroides.*

Previous studies have indicated that Mg-protoporyphyrin formation is dependent upon Mg^{2+}, protoporphyrin, and ATP. Both systems have an absolute requirement for membrane intactness, and the chloroplast system 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 and bacterial plasma membrane 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. Subsequent reconstitution into vesicles may be used to measure activity of partially purified components.

Cornell University

Ithaca, NY 14853

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

J. Gibson, Section of Biochemistry, Molecular and Cell Biology, Division of Biological Sciences

$70,500

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 clarify the number and specificities of these ligases, and to determine the number of enzymatic steps and cofactors involved in the next stage of their metabolism, involving reductive saturation of the aromatic nucleus. 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, now at the University of Iowa, but previously a Co-Investigator on the project. 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

41. Molecular Analysis of Cytoplasmic Male Sterility

M.R. Hanson, Section of Genetics and Development

$74,484

The ultimate aim of the project is 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. Analysis of individual plants containing recombinant mitochondrial genomes previously revealed that 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 subunit 3 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, an unidentified reading frame. Using *Agrobacterium* Ti plasmid-mediated transformation, components of the S-Pcf locus will be introduced into the nucleus of a fertile genotype under the control of an appropriate promoter and terminator, and polypeptide products of introduced genes will be directed to the mitochondrion with a transit peptide. Transgenic plants will be produced and examined with regard to pollen development and respiratory control. In this way 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 cytoplasmic male sterility.

Cornell University

Ithaca, NY 14853

42. Structure and Function of the Self-incompatibility Proteins of Brassica oleracea

M. Nasrallah, Division of Biological Sciences

$74,000

The phenomenon of self-incompatibility is known to occur in many flowering plants and is utilized in commercial hybrid seed production in economically important crops such as *Brassica oleracea*, *B. campestris* and *B. napus*. In the "Brassicaceae" we have established that self-incompatibility is a cell-cell interaction mechanism between one pollen grain and one papillar cell. The main objective of this project is to gain an understanding of the molecular events that precipitate the incompatible response when genetically identical pollen and papillar cells are brought into contact upon self-pollination for example. Having cloned the gene that encodes the stigma S locus specific glycoproteins and made fusion protein constructs, monoclonal antibodies directed against different segments of the polypeptide moiety have been raised in order to analyze aspects of structure/function relationships and establish a basis for the allele-specific recognition. The monoclonal antibody probes have identified pollen proteins that bind to the stigma S glycoproteins. Electron microscopic immunolocalization has shown that these S glycoproteins are found in the wall of papillar cells and at their surfaces where interactions with exine-held proteins of the male gametophyte are presumed to occur.

Cornell University

Ithaca, NY 14853

43. Mechanisms of Inhibition of Viral Replication in Plants

P. Palukaitis, Department of Plant Pathology

$75,000

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. Specifically, our objectives are (1) to certify that at least two mechanisms of resistance are involved in several known cases of host-resistance to viral infection, and to delimit nucleotide sequence alterations in viruses that enable them to overcome either or both resistance mechanisms; and (2) to determine which of the hypothesized mechanisms for inhibiting the cell-to-cell movement of plant viruses occurs in several well-defined systems, by studying the metabolism of both the mRNA encoding the viral movement gene (in protoplasts) and the viral movement gene product itself (in transgenic plants). In addition (3), we will compare the effects of antisense RNA derived from various parts of a viral genome on the replication of the same virus, closely related viruses and unrelated

viruses, as well as to compare different systems for delivering the antisense RNA into plant cells; i.e., optimizing and testing the limits of a 'manmade' resistance gene against virus infection in plants.

Cornell University
Ithaca, NY 14853

44. Effects of Freezing and Cold Acclimation on the Plasma Membrane of Isolated Cereal Protoplasts
P.L. Steponkus, Department of Agronomy

$86,200

The goal of our program is to provide a mechanistic understanding of the cellular and molecular aspects of freezing injury and cold acclimation in winter cereals from a perspective of the structural and functional integrity of the plasma membrane. The focus of the project is on dehydration-induced mesomorphic phase transitions in the plasma membrane that result in the loss of osmotic responsiveness of isolated protoplasts. This past year our efforts have been directed to a) the phenomenology of the loss of osmotic responsiveness during a freeze/thaw cycle, b) characterization of the lyotrophic phase behavior of the plasma membrane lipids (e.g., mono- and diunsaturated species of phosphatidylcholine and phosphatidylethanolamine, glucocerebrosides) and lipid-lipid interactions in mixtures (phospholipid-cerebroside, phospholipid-sterol, phospholipid-sterol glucoside), c) an analysis of osmotic adjustment during cold acclimation including the cellular compartmentalization of various solutes, d) development of a theory for membrane destabilization at low hydrations that is based on hydration forces and interbilayer interactions, and e) studies of ultrastructural modifications in the plasma membrane responsible for the loss of osmotic responsiveness in cold acclimated protoplasts exposed to lethal temperatures. Major findings include i) the demonstration of large differences in the hydration characteristics of the plasma membrane lipid extracts of nonacclimated and cold-acclimated rye leaves and ii) dehydration-induced lateral phase separations and hexagonal$_{II}$ phase transitions in the plasma membrane do not appear to be a consequence of L_α- to -L_β phase transitions in phosphatidylcholine species.

Cornell University
Ithaca, NY 14853-1902

45. Sorting of Tomato Chromosomes for Construction of Region-Specific Libraries
S.D. Tanksley and E.D. Earle, Department of Plant Breeding

$43,960

The overall aim of this work is to develop methods for generating region-specific libraries of plant chromosomes. These libraries will play a key role in a general strategy for isolation of important plant genes for which no protein product is known. The initial objective is to separate isolated tomato metaphase chromosomes by fluorescence activated cell sorting (FACS). Recovery of chromosome arms from telotrisomic stocks will also be attempted. Chromosome and chromosome arm-specific libraries will then be constructed. These libraries will be used for high density chromosome mapping.

As a further step in the development of the technology, chromosomes will be hybridized with one or more cloned probes known from RFLP studies to be closely linked to a gene of interest. The probes will be conjugated to fluorescent molecules, and, in some cases, also to magnetic particles. Chromosome regions containing the target gene will be obtained by FACS or by magnetic separation. DNA from these regions will then be cloned. Clones will be tested for the presence of the gene of interest via transformation into lines lacking the relevant phenotype. The strategy proposed should greatly reduce the amount of DNA that must be screened in gene isolation studies.

Tomato is currently a particularly suitable material for this work, but the approach should eventually be applicable to any species for which molecular maps and gene transfer techniques are available and for which large scale chromosome isolation is feasible.

Cornell University

Ithaca, NY 14853

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

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

$65,000

The goals of this project are to determine the molecular mechanisms regulating cellulase synthesis in the soil bacterium *Thermomonospora fusca* and to determine the molecular mechanism by which *T. fusca* cellulases degrade crystalline cellulose. The genes for two *T. Fusca* cellulases E2 and E5 have been sequenced and there is no significant homology between them even though both genes code for endoglucanases with MWs near 43,000. Both genes do contain an identical 14b inverted repeat (TGGGAGCGCCCCA) which is a short distance downstream of the translation start in each gene. The same sequence is also present in a clone that codes for an active fragment of cellulase E4. This sequence is the binding site for a *T. fusca* DNA binding protein that appears to function in the induction of cellulase synthesis.

The E4 clone codes for a protein of molecular with 58,000 which is about 50% of the 118,000 MW of E4, yet this protein has catalytic activity. The protein produced by the clone has the same N terminal sequence as E4. A set of Tn5 insertions in the E2 gene has been isolated and they are being mapped and the proteins they produced characterized to identify the location of the cellulose binding domain and the catalytic domain in the E2 sequence.

Cornell University

Ithaca, NY 14853

47. Microbial Ecology of Thermophilic Anaerobic Digestion

S.H. Zinder, Department of Microbiology

$90,000

The objective of this project is to provide an integrated understanding of the ecology of microbial populations in a thermophilic (58°C) laboratory-scale bioreactor converting a lignocellulose waste to methane. Special attention is focused on formation and breakdown of acetic acid, the precursor of two-thirds of the methane produced by the bioreactor. 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* is saturated for acetate down to concentrations near 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 carbon monoxide dehydrogenase, an enzyme implicated in methanogenesis from acetate but has little or no hydrogenase; 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 grow on H_2-CO_2 and produce acetate, the reverse of the reaction it carries out in coculture; 7) the demonstration of high levels of CO dehydrogenase activity in extracts of the AOR grown axenically on H_2-CO_2 and in the acetate oxidizng coculture, while levels of formyltetrahydrofolate synthetase and of folates were found to be extremely low; 8) 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. Current research centers on further characterization of the thermophilic *Methanothrix* and what factors allow it to compete with *Methanosarcina*.

University of Delaware

Lewes, DE 19958

48. Metabolic Mechanisms of Plant Growth at Low Water Potentials

J.S. Boyer, College of Marine Studies

$94,000 (FY88 funds/ 15 months)

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 28 kD 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. 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

49. Stable Isotope Fractionation in Photosynthesis
C.B. Osmond, Department of Botany

$174,000 (FY89 funds/two years)

This project seeks insights into the interactions between photosynthesis and respiration using the unicellular green alga *Chlamydomonas reinhardtii* as a model experimental system. Mass spectrometry of the natural abundance, stable isotopes of carbon and hydrogen is being used to explore the balance between autotrophic and heterotrophic metabolism in site-directed mutants of the pbsA and pbsD genes with varying levels of photosynthetic competence. Analysis of both site-directed mutants in PSII core polypeptides and existing mutants blocked in various steps of electron transport or CO_2 fixation permit one to assess the importance of individual steps in photosynthesis to the overall carbon and hydrogen metabolism in the organism. Thus the signatures of stable carbon and hydrogen metabolism, characterized in these mutants will serve to define the relative contributions of photosynthesis and respiration to their overall metabolic performance and serve to define the relative contributions of photosynthesis and fitness. This approach to partitioning the source of carbon and reductant has potential for rapid assessment of the functional significance of plant genotypes with differing metabolic capabilities.

Duke University

Durham, NC 27706

50. Molecular Studies of Functional Aspects of Higher Plant Mitochondria
J.N. Siedow, Department of Botany

$115,080 (FY88 funds/two years)

The sensitivity of mitochondria isolated from cms-T lines of maize to a toxin (BmT toxin) derived from the fungus *Bipolaris maydis*, race T is associated with a unique, mitochondrially-encoded 13 kDa protein (ORF-13). The goal of this research is to characterize the mechanism by which ORF-13 and bmT toxin interact to permeabilize biological membranes. This research (carried out in collaboration with C.S. Levings, N.C. State University) takes advantage of the observation that expression of ORF-13 in *E. coli* confers BmT toxin sensitivity on the resulting cells. Binding studies with radiolabeled (tritiated) toxin have established that the toxin binds to such cells in a distinctly cooperative manner. In addition, site-directed mutagenesis of ORF-13 in *E. coli* has been used to generate polypeptides that no longer respond to BmT toxin. Modified ORF-13 proteins are analyzed through their ability to permeabilize the *E. coli* plasma membrane upon toxin addition as well as their ability to bind labeled toxin. Mutants which fail to elicit a toxin response can be categorized into those which still bind BmT toxin and those which are unable to bind the toxin. Future studies will continue to develop site-directed mutants to further map those regions of the ORF-13 protein involved in toxin binding versus those associated with permeabilizing the membrane. Additional studies will involve solubilizing ORF-13 and reconstituting it into phospholipid liposomes to develop a more well-defined system for studying the ORF-13/toxin interactions. Knowing how ORF-13 and BmT toxin interact to affect membrane properties could provide new insights into membrane/protein interactions.

Florida State University
Tallahassee, FL 32306

51. Guard Cell Biochemistry - Response to Environmental Stimuli Causing Changes in Gas Exchange
W.H. Outlaw, Jr., Department of Biological Sciences

$198,000 (FY88 funds/two years)

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. Despite the presence of green plastids, which conduct linear electron transport, it is our view that guard cells lack the ability to reduce photosynthetically significant quantities of CO_2. This last statement, which has been challenged recently, is further supported by electrophoresis of proteins extracted from guard cells (in press). In 1988, we published the ABA contents of guard cells of control and water-stressed plants. (Further work indicates that other methods do not result in *in planta* values.) To elucidate the integrated functioning of various leaf cells in elevating the level of this stress-induced metabolite, we have conducted kinetic studies. These assays (requiring up to 100 individually dissected cell pairs per datum) are being repeated. Separately, we have completed a partial characterization of PEPC, which catalyzes the regulated step in anion biosynthesis. Our data on this project are the results of DOE-sponsored methods development, which allowed single-cell assays in real time with natural substrates in solution. [Additionally, as project last year, it was necessary to refine the PEPC assay (because the reaction product decarboxylates).] Our further work in this area will be to complete the characterization of PEPC.

University of Florida
Gainesville, FL 32611

52. Ethanologenic Enzymes of Zymomonas mobilis
L.O. Ingram, Department of Microbiology and Cell Science

$77,000

Our studies have focused on the ethanol pathway in Z. *mobilis*, an obligately ethanologenic bacterium. Pyruvate is converted to ethanol and carbon dioxide by pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) in this organism. We have cloned and sequenced genes encoding PDC and one of the two ADH isoenzymes, ADH II. Promoter regions were identified by primer extension mapping. Antibodies were prepared against the purified proteins expressed in *E. coli*, native ADHI, and to an N-terminal oligopeptide sequence from ADHI.

The genes for PDC, ADH, and the glycolytic enzymes are expressed at high levels in Z. *mobilis* and the basis for this high level expression is being investigated. Both genes and their respective promoter fragments have been inserted into Z. *mobilis* on multi-copy plasmids to investigate trans-active effects on the expression of each respective gene as well as on the expression of other glycolytic enzymes. Over-expression of PDC was accompanied by a reduction in ADHI, ADHII and glucokinase activities without effect on many other glycolytic enzymes. Further studies are in progress to examine the expression of lacZ' fusions, to probe the nature of the trans-active effects, and to investigate post-transcriptional control.

Based upon comparisons of the Z. *mobilis* sequences for 2 glycolytic, 2 alcohologenic genes, and those of the moderately expressed genes, a number of features have been identified which appear important for high level expression. These include highly biased codon usage, a four-base consensus Shine-Dalgarno region near the initiation codon, and tandem promoter regions. The genes, pdc and adhB, are present as single copies on the Z. *mobilis* chromosome. The two ADH isoenzymes appear to share little homology.

University of Florida

Gainesville, FL 32611

53. Gene-Enzyme Relationships in Somatic Cells and Their Organismal Derivatives in Higher Plants

R.A. Jensen, Department of Microbiology and Cell Science

$197,000 (FY88 funds/two years)

The biochemical pathway of aromatic biosynthesis is not only crucial in higher plants as a source of aromatic amino acids, but it is a point of interface with a massive biochemical network for secondary metabolism. We seek to understand physiological, biochemical, developmental and genetic interrelationships within a single experimental system. *Nicotiana silvestris* and *N. plumbaginifolia* are under study at both the tissue culture and organismal levels. Strategies for isolation of regulatory-gene and structural-gene mutants in totipotent cells are being pursued. We have shown that an intact aromatic pathway which proceeds to L-phenylalanine and L-tyrosine via L-arogenate exists in the plastic compartment. This pathway is subject to a novel sequential pattern of feedback inhibition. The extent to which all or part of a second pathway exists within the cytosol and whether cytosolic-pathway enzymes are regulated is under study. Selected comparative work will be done in order to deduce what characteristics of the *N. silvestris* system can be generalized to higher plants. 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, many secondary metabolites, and medically significant pharmacological agents.

University of Georgia

Athens, GA 30602

54. The Metabolism of Hydrogen by Extremely Thermophilic Bacteria

M.W.W. Adams, Department of Biochemistry

$73,963

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. Little is known of their metabolism and biochemistry yet they have enormous biotechnological potential. In this project we are studying the metabolism of hydrogen (H_2) by five extreme thermophiles grown under a variety of conditions *in vitro*. We are examining their hydrogenases, the enzyme responsible for catalyzing H_2 production and H_2 oxidation, and related redox proteins. Their molecular and catalytic properties are characterized using various biochemical and electron and nuclear resonance spectroscopic techniques. For example, in the last year we have purified hydrogenase and its physiological electron carrier, a novel ferredoxin, from *Pyrococcus furiosus*. This organism grows optimally at 100°C by a fermentative-type metabolism. The hydrogenase is a nickel-iron-sulfur protein which exhibits unusual electron paramagnetic resonance properties. Its optimum temperature for the catalysis of both H_2 evolution and H_2 oxidation is 95°C and catalytic rates increase up to 350-fold between 45° and 95°C. The enzyme preferentially catalyzes H_2 production at all temperatures and appears to represent a new type of "evolution" hydrogenase. The ferredoxin is also remarkably thermostable showing no loss in its electron carrier activity to the hydrogenase after 12 hours at 95°C. Since molecular H_2 plays a central role in commercial production of fuels and numerous chemicals, our overall objective is to assess the potential utility of extremely thermophilic hydrogenases in industrial energy conversions.

University of Georgia

Athens, GA 30602

55. Studies on Oligosaccharins: Carbohydrates Possessing Biological Regulatory Activities

P. Albersheim, Complex Carbohydrate Research Center

$172,000

This project is concerned with the isolation and characterization of oligosaccharins, which are naturally occurring complex carbohydrates that possess biological regulatory activities. We have hypothesized 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 microbial enzymes and a plant-derived inhibitor of microbial enzyme activity in the release of elicitor-active oligosaccharins from plant cell walls. [2] An oligosaccharin that may trigger the hypersensitive-resistance response in plants. We are purifying enzyme(s) secreted by *Pyricularia oryzae* that release this oligosaccharin

from isolated plant cell walls, and are identifying the bioactive oligosaccharide(s). [3] Oligosaccharins that are able to induce flowers and vegetative shoots and inhibit roots in isolated tobacco epidermal strips. To aid in the purification of oligosaccharins that induce specific physiological events in the epidermal strips, we are attempting to identify specific molecular markers for flowering, root development, vegetative shoot development, and callus development. At present, our studies with tobacco epidermal strips are emphasizing the oligosaccharin, released from plant cell walls by endo-α-1,4-polygalacturonase, that stimulates floral and vegetative shoot development and inhibits root development. We have purified and are in the process of characterizing a pectic oligosaccharide fragment of plant cell walls that, at $\sim 10^{-8}$ M, causes these morphogenetic effects in the tobacco thin cell layer explants.

University of Georgia

Athens, GA 30602

56. CARBBANK - A Structural and Bibliographic Database for Complex Carbohydrates

P. Albersheim, Complex Carbohydrate Research Center

$100,000

CarbBank is a computer program and database that will, for the first time, enable scientists to systematically and rapidly search for the estimated 5,000 published complex carbohydrate structures. Incorporation of carbohydrate structures linked to literature citations in a computerized database (i) allows comparison of known and 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 will enhance the research efficiency and capabilities of many scientists, not just those specializing in carbohydrates, and will make 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 that allows you 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 that will let you find and retrieve structures based on search criteria that you supply. CarbBank has facilities to export entire databases (or partial databases based on search results) to text files, and it has the ability to import text file information and transform it into a CarbBank database file. CarbBank uses a syntax checker during data entry (i.e. import or editing) to warn you if it detects entry errors or violations of carbohydrate chemistry rules. CarbBank uses structure nomenclature that is very 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 will be very rapid.

The international CarbBank Board of Overseers, with the special assistance of its Executive Committee, has selected and approved some 50 potential curators in about 20 countries. Those curators who have agreed to participate have been asked to identify and provide the database with the structures and bibliographic information of approximately 100 complex carbohydrates per year. It is anticipated that CarbBank will be initially distributed in late 1989, when it will contain the structures and bibliographic information for about one-third of all published carbohydrate structures. It is proposed that all existing carbohydrate structures will be entered into CarbBank within three years. The database will initially be distributed quarterly on magnetic media (5.25 inch floppy diskette and 3.5 inch hardshell disk). For more information about CarbBank contact: Dana Smith, CarbBank Database Manager, Complex Carbohydrate Research Center, The University of Georgia, 220 Riverbend Road, Athens, Georgia 30602, Telephone: 404-542-4484.

University of Georgia

Athens, GA 30602

57. Development of Methods to Structurally Characterize Complex Carbohydrates

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

$284,625 (FY88 funds/two years)

This research focuses on the development of methods to aid in the structural characterization of complex carbohydrates. We are using a high-mass FAB mass spectrometer and a 500-MHz NMR spectrometer

among other equipment, to develop new methods to study complex carbohydrates; this approach is aimed at obtaining the primary structures and eventually, the three-dimensional structures of these molecules. We are particularly interested in the complex carbohydrates of plant cell walls. We have also developed computer software that predicts all possible ions resulting from a particular mass spectral analysis of a complex carbohydrate of known composition. This program allows the quick, accurate and comprehensive analysis of mass spectral data. We have also developed a method for the sensitive detection and characterization of enzymes that cleave the glycosidic linkages of complex carbohydrates. Other methods we are studying that will aid in structural investigations include the development of chemical reactions and the isolation of specific endoglycanases for specifically cleaving complex carbohydrates and techniques for labeling oligosaccharides to allow rapid and highly sensitive detection during purification. For example, we have developed a method for efficiently attaching a UV-absorbing tag to the reducing end of oligosaccharides.

University of Georgia

Athens, GA 30602

58. Establishment of the University of Georgia/Department of Energy Complex Carbohydrate Research Center (CCRC)
P. Albersheim and A. Darvill, Complex Carbohydrate Research Center

$1,000,000

The CCRC, with its multidisciplinary faculty and staff, was formed to serve as a national resource for basic research in complex carbohydrates. The CCRC will actively assist in defining the structures and studying the functions of plant and microbial carbohydrates. Research, training, and service activities are components of this program. The research focuses on various aspects of carbohydrate science, including methods development, structural characterization, and function. The education function involves the training of graduate students, post-doctoral associates, and visiting scientists in the analytical methods used for studying carbohydrate structure. It is anticipated that several week-long laboratory training courses and workshop symposia will be offered in the winter of 1990 to scientists from other institutions who are interested in learning more about carbohydrate structure analyses. The services offered include conducting routine analyses of carbohydrate samples provided by scientists from other institutions. These analyses will include determination of glycosyl-residue and glycosyl-linkage compositions and acquisitions and interpretation of one-dimensional NMR and FAB-MS spectra. The Center 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

59. Structural Studies of Complex Carbohydrates of Plant Cell Walls
A. Darvill, Complex Carbohydrate Research Center

$207,000

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. These polysaccharides have been shown to possess unexpectedly complicated structures. For example, RG-II contains at least 12 different glycosyl residues, including apiosyl, aceryl (3-C-carboxy-5-deoxy-L-xylosyl), KDO (3-deoxy-D-manno-2-octulosonic acid), and DHA (3-deoxy-D- lyxo-2-heptulosaric acid). The complete structures of oligosaccharide fragments of RG-II have been determined that, among the oligosaccharides, contain all of the known glycosyl residues of RG-II. Elucidating the arrangement of these oligosaccharides in RG-II is a goal of our present work. Although RG-I contains only five different glycosyl residues, like RG-II it exhibits tremendous structural complexity. RG-I is composed of a backbone of alternating rhamnosyl and galacturonosyl residues with side chains attached to 50% of the rhamnosyl residues. At least 30 structurally different side chains consisting predominantly of

arabinosyl and galactosyl residues have already been identified. We are investigating the possibility that discrete families of side chains are attached to the RG-I backbone. We have recently isolated a cell wall fraction containing RG-I where greater than 90% of the rhamnosyl residues have side chains attached. We have also identified new structures in xyloglucan polysaccharides that add to the complexity of this hemicellulose, which is found in the primary walls of all higher plants. So far, our studies indicate that all the known polysaccharides of primary cell walls are present in dicots, monocots, and gymnosperms, and we are even obtaining evidence of their presence in fern gametophytes, although the quantities of the polysaccharides in these cell walls of widely divergent plants vary greatly.

University of Georgia

Athens, GA 30602

60. Molecular Biology of LEA Genes of Higher Plants

L. Dure, Department of Biochemistry

$72,000

This research is to study the LEA (Late Embryogenesis Abundant) genes/proteins of cotton seed from two perspectives. The first involves identifying and purifying nuclear DNA binding proteins that function to up-regulate the expression of these genes in late embryogenesis. From this, the genes for the regulatory proteins themselves are to be isolated and the regulation of their expression investigated. Should they be constitutively expressed, the possible covalent modification of their proteins and/or the possible change in their mRNA stability/ translation rate, either of which may lead to an increase in LEA proteins, will be investigated.

The second facet of research involves the determination of the 3D structure of several LEA proteins, via computer modeling and biophysical measurements, that may lead to an understanding of their function in cells. Preliminary indications implicate these proteins in preparing cells for desiccation tolerance.

University of Georgia

Tifton, GA 31793

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

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

$39,117

The objective of this project involves the (1) development of techniques for transferring germplasm from wild species to cultivated species to demonstrate the wealth of germplasm in the primary, secondary, and tertiary gene pools that can be transferred to cultivated species, (2) evaluation of cytoplasmic effects on agronomic characteristics, and (3) development of an obligate apomictic pearl millet. 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, *P. glaucum*. Cytoplasm from wild *Pennisetum* species have been identified that affect forage yields, days to anthesis, head length, seed weight, and cytoplasmic-genic male sterility (cms). A number of new cytoplasms for cms are being identified. The A' genome from the secondary gene pool in *Pennisetum* is proving to be a valuable source of genes that can be rapidly used to improve cultivated pearl millet. The discovery and use of this germplasm may have a significant impact on developing pearl millet as a new drought tolerant grain crop for the U.S. Significant progress is being made in transferring gene(s) controlling apomixis from wild *P. squamulatum* to cultivated pearl millet for the purpose of producing true-breeding hybrids. The overall impact is an increased, more efficient, and more reliable production of food, fiber and forage.

University of Georgia

Athens, GA 30602

62. 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. The studies emphasize analysis of heat shock genes including structure and regulation of expression. The physiological/biochemical role(s) of heat shock proteins in cellular function is also under study, emphasizing the phenomenon of acquired thermotolerance. Treatment with arsenite and cadmium lead to HS gene expression; thermotolerance is also induced by arsenite but not by cadmium. Amino acid analogues induce HS gene transcription, but thermotolerance is not acquired. Cycloheximide treatment does not inhibit HS gene transcription.

Short term HS regimes including superoptimal HS (e.g. 45°C for 10 min) have separated the phenomenon of induction of HS gene transcription from that of sustained synthesis. Run-off transcription studies indicate that HS gene transcription is rapidly activated following 40°C treatment, but transcription slows and is not detected by 4 hr. Following an initial HS and return of the tissue to 30°C, HS gene transcription is activated rapidly upon subsequent HS, but synthesis slows after about 1 hr.

Initial experiments demonstrate the efficacy of using a HS promoter cassette to regulate expression of a reporter gene and indicate that the HS promoter is very much more active than the constitutively expressed caMV 35S promoter. This is important because in theory any gene can be cloned into this cassette and be thermally regulated by this strong, inducible promoter. Other genes have been cloned into the HS cassette including a cytokinin biosynthesis gene; heat treatment of transgenic tobacco plants caused phenotypic changes suggestive of cytokinin production.

University of Georgia

Athens, GA 30602

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

R.B. Meagher, Department of Genetics

$81,000

The goal of this work is to elucidate the mechanisms and determinants behind the light induced turnover and general chemical instability of Rubisco small subunit (SSU) RNA and generalize these results to other RNAs. Three approaches are being used. (1) We are examining the molecular physiology surrounding RNA turnover to help define the phenomena and identify the possible cellular machinery involved in these processes. SSU RNAs in soybean leaves are preferentially associated with the polysomal fraction in proportion to the increase in transcription in the light and in low levels in nonpolysomal fractions relative to control RNAs. We propose that as newly transcribed SSU RNAs bind preferentially to polysomes that increased turnover in the light occurs as a result of translation. (2) We are examining RNA stability for various SSU constructs in transgenic plants to help identify those sequences most responsible for turnover and preferential binding of SSU RNA to polysomes over other RNAs. Initial data suggests that SSU RNA structure is similar in the light and in darkness and thus the turnover control does not act directly at the level of RNA sequence. It appears more likely that the preferential loading on polysomes is controlled by SSU RNA sequence. (3) *In vivo* RNA structure is being examined via chemical modification of RNA. We have had tremendous success in assaying *in vivo* RNA structure of 18S rRNA and SSU RNA by DMS modification. The validity of this technique has been established by examining the conserved structure of the IV domain of 18S rRNA in soybean leaf. We will continue to develop a DMS modification map of 18S rRNA and portions of Rubisco SSU RNA and predict a potential secondary structure. In the next year of this project we will combine all these approaches to examine the degradation pattern and structures of RNAs in various polysomal fractions and in transgenic plants and examine the effects of light on the polysomal distribution of SSU and control RNAs. Ultimately, we plan to confer light regulated stability on foreign RNAs in transgenic plants.

University of Georgia

Athens, GA 30602

64. Microbiology and Physiology of Anaerobic Fermentations of Cellulose

H.D. Peck, L.G. Ljungdahl, L. Mortenson, and J.K.W. Wiegel, Departments of Biochemistry and Microbiology

$817,670 (FY89 funds/31 months)

This project involves the biochemistry and physiology of four major groups (primary, secondary, ancillary and methane bacteria) of anaerobic bacteria, that interact and convert cellulosic materials industrially to important feedstock chemicals and ultimately also to methane. The primary bacterium, *Clostridium thermocellum*, produces cellulolytic enzyme complexes designated cellulosomes and polycellulosomes with M_r ranging from 2 to 80 million. The cellulosome consists of at least 14 different polypeptides, some of which have endoglucanase activity or an activity resembling exoglucanase. Individually the polypeptides do not hydrolyze crystalline cellulose as does the intact cellulosome. The latter contains from 6 to 13% carbohydrates and some of the polypeptides appear to be glycoproteins. The polypeptides are now being separately purified, their composition, physical chemical, and enzymatic properties determined. *C. thermocellum* during growth secretes a carotenoid-like yellow pigment (YAS = yellow affinity substance), that facilitates the attachment of cellulosomes to cellulose. YAS has now been purified and its structure is being determined. Research on the secondary and ancillary bacteria includes clostridial acetogens and other clostridia, sulfate reducing bacteria (SRB) and *Thermoanaerobacter ethanolicus*. Aspects of metabolism are being studied which appear relevant for bioenergetics and the interactions of bacteria in consortia. Special attention is given to enzymes involved in the metabolism of hydrogen, formate, CO, and CO_2, the molecular basis of interspecies H_2-transfer and H_2-cycling, electron-transfer proteins, ATPase systems and enzymes of one-carbon metabolism. A common property of many of these enzymes is their contents of metal redox centers consisting of inorganic sulfur and one or more combinations of (Fe), (NiFe), (NiFeSe), (MoFe), (MoFeSe), and (WFeSe). The structures of the metal clusters and their roles in the catalytic processes of hydrogenases, formate dehydrogenases and carbon monoxide dehydrogenases are determined using several spectroscopic methods that involve also substitutions with different isotopes. One very recent result is the demonstration for the first time of a selenocysteine coordination to the active site nickel in the (NiFeSe) hydrogenase from *Desulfovibrio baculatus*. Another development of considerable importance is the finding that many bacteria produce two or more different proteins with e.g. hydrogenase or formate dehydrogenase activity. Thus 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*; the 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. Although, the enzymes have different metal centers which determine to a large extent the catalytic properties, it is clear that the protein part of the enzymes strongly influence these properties. Therefore, it is necessary to obtain the primary structures of the proteins. This is being pursued using genetic techniques involving cloning and sequencing of DNA. The (NiFeSe)-hydrogenase from *D. baculatus* has been sequenced and work is in progress on other hydrogenases, formate dehydrogenases, and CO dehydrogenases. The enzymes involved with H_2, CO, CO_2 and formate metabolism are being localized in the bacterial cells using electron microscopic and immunological techniques. It is evident that they interact with membrane bound electron transfer proteins and ATPase systems. This work is especially directed toward the understanding of the generation of ATP coupled to vectorial electron transfer in acetogenic bacteria, which recently have been found to have the capacity to grow autotrophically using the newly discovered acetyl-COA pathway. *T. ethanolicus*, that ferments many hexoses, pentose and also xylan to ethanol as a major product is investigated with regards to physiology and content of thermostable enzymes. This bacterium and other extreme thermophiles grow over a temperature span of 40°C or more. A biphasic growth response to temperature is observed. Preliminary evidence suggest that this phenomenon is due to the expression of enzymes at different temperatures. This is investigated using gel electrophoresis techniques and isolation of the enzymes.

University of Georgia
Athens, GA 30602

65. Molecular Characterization of Phytochrome from Green Avena
L.H. Pratt, Department of Botany

$63,000

Plants respond both to the quantity and the wavelength distribution of incident radiant energy in ways that maximize the efficiency with which they utilize ambient light for the conversion of solar to chemical energy via photosynthesis. Phytochrome is the pigment that performs this sensory function. Until recently, virtually all that was known about the molecular properties of phytochrome derived from the study of this chromoprotein as isolated from dark-grown, etiolated plant tissues. Previous DOE-supported research, however, led to the discovery that the phytochrome that is most abundant in light-grown plants is markedly different from that which predominates in etiolated tissue. Thus, the phytochrome that has been best characterized is likely not that which is operative in photosynthetically competent plant tissues. The overall goal of this research program is therefore to learn more about this newly discovered type of phytochrome, utilizing *Avena* as a model system. Both mouse monoclonal and rabbit polyclonal antibodies to phytochrome from green *Avena* leaves have been prepared. They are being used (i) to help purify this exceedingly low abundance and labile chromoprotein, (ii) to quantitate it in crude plant extracts by ELISA, (iii) to visualize it *in situ* by immunocytochemistry, if the methodology can be made sufficiently sensitive, and (iv) to assist in its general physiochemical characterization. Using these newly developed antibodies, as well as information about the primary structure of the protein obtained from microsequencing, efforts will be made to identify the gene(s) that encode(s) this unique phytochrome.

University of Georgia
Athens, GA 30602

66. Nitrogen Control of Chloroplast Development
G.W. Schmidt, Department of Botany

$82,000

Nitrogen deficiency acutely affects chloroplast structure and function. Our work has concerned the role of this nutrient and/or its assimilation products on the organization of photosynthetic complexes and gene expression in the nuclear and chloroplast compartments. For this work, *Chlamydomonas reinhardtii* is grown in an ammonium-limited continuous culture system. When nitrogen provision is limited so that cell division occurs once every four days, contrasting with normal generation times of ten hours, cultures with a 60% deficiency of chlorophyll and, in most cases, 50% deficiency of photosynthetic proteins are maintained. The apoproteins of light-harvesting complexes are more severely affected qualitatively and quantitatively. Measurements of protein synthesis rates as well as rRNA, mRNA and organellar DNA levels, indicate that chloroplast protein synthesis generally is attenuated at the translational level. However, there is a minor effect on the synthesis of the D1 subunit of Photosystem II and the apoproteins of the Photosystem I reaction centers. Chloroplast DNA levels per cell are reduced somewhat in parallel with the amounts of chloroplast transcripts. In contrast, nuclear transcripts for light-harvesting apoproteins are severely reduced and the relative abundance of transcripts from the two ribulose 1,5-bisphosphate carboxylase small subunit genes is altered. The nitrogen-limited phenotype is reversed within 12 hours of ammonium provision. During this time, unusually high amounts of mRNAs for the light-harvesting apoproteins are accumulated. Coincidental changes include rapid metabolism of massive amounts of starch and triglycerides that are produced in the nitrogen-limited cultures. Further studies are in progress on the nitrogen effects on photosynthetic gene expression, the status of thylakoid membrane complexes and the enzymes of the carbon assimilation pathways for starch and triglyceride deposition.

University of Georgia
Athens, GA 30602

67. Transcriptional Analysis of the R Locus Maize
S.R. Wessler, Botany Department

$76,002

The R Locus controls where and when the anthocyanin pigments will be present in the maize plant. It not only controls in what organ pigment will be expressed but also the temporal appearance of the pigment in a particular organ. This sensitive, non-lethal phenotype has facilitated the isolation of over one hundred naturally occurring R alleles; each displays a distinctive pigmentation pattern.

Our lab is interested in the organization of the R transcription unit, how it is expressed in different alleles of R, and what is the function of the protein encoded by R. To this end we have analyzed transcrip-

tion of three R alleles (Lc, P and S) that condition diverse pigmentation patterns. We have correlated the appearance of the R transcript with transcripts encoded by other genes in the anthocyanin biosynthetic pathway. Finally, we have isolated a full-length cDNA from the Lc gene.

Preliminary results suggest that the R protein encoded by all R alleles may be identical. Allelic differences may simply reflect differences in when the R gene is expressed. Experiments are underway to test this hypothesis.

University of Georgia

Athens, GA 30602

68. 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 xylanase(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

69. Unraveling Photosystems
L. Bogorad, Department of Cellular and Developmental Biology

$102,000

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 identifying components is to determine whether open reading frames found in chloroplast DNA sequences in fact encode proteins of the photosynthetic apparatus and, if so, to determine where the proteins function. A new protein subunit of the cytochrome b_6/f electron transport complex has been identified in the course of this work. Another approach to identifying, characterizing and establishing the role of proteins of the photosynthetic apparatus has been to generate photosynthetic mutants of the facultatively heterotrophic single-celled cyanobacterium *Synechocystis* PCC6803 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 studied to date in this part of the program have had deficiencies in chlorophyll-proteins associated with the reaction center of Photosystem II. Such mutants have been very useful in revealing the sources of certain fluorescence emission bands in the photosynthetic apparatus and thus helping understand the transfer of energy within these systems. Sets of mutants with other genetic lesions are being analyzed.

University of Idaho

Moscow, ID 83843

70. Genetics and Chemistry of Lignin Degradation by Streptomyces
D.L. Crawford, Department of Bacteriology and Biochemistry

$162,000 (FY89 funds/two years)

The goal of this research is to define the roles of extracellular lignin peroxidases and cellulases in the depolymerization and solubilization of lignin by *Streptomyces.* Studies of the substrate specificity and en-

zyme reaction biochemistry of purified lignin peroxidase ALip-P3 from *S. viridosporus* T7A show that the enzyme is similar to known fungal lignin peroxidases, but that it has unique properties as well. The peroxidase is produced during primary growth of *S. viridosporus*, not during secondary metabolism, and its substrate specificity differs from the fungal enzymes. Purified peroxidase positively affects the rate of lignocellulose hydrolysis by cellulases, presumably by depolymerizing the lignin component to make cellulosic fibers more available to cellulases. We are now able to produce the lignin peroxidase in large amounts, using a defined medium devoid of lignin and containing sucrose as the primary carbon source for growth. We have also simplified the procedure for large scale purification of the enzyme. We are carrying out hybridization and sequencing studies of a gene coding for lignin peroxidase, cloned from the *S. viridosporus* chromosome into *S. lividans* on plasmid pIJ702, to determine if it shares sequence homology with a cDNA lignin peroxidase gene from the white-rot fungus *Phanerochaete chrysosporium*. This research is aimed at elucidating the mechanisms of lignin degradation by *Streptomyces*, with an ultimate goal of using genetic engineering to construct commercially useful lignin bioconversion strains.

University of Illinois

Urbana, IL 61801

71. Photosynthesis in Intact Plants

A.R. Crofts, Department of Physiology and Biophysics

$208,000 (FY 89 funds/two years)

The main goals of the project are the development of instrumentation and methodology for studying photosynthesis in intact plants. 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 fluorescence spectrophotometer, and have been conducting experiments both in the laboratory and in the field to characterize electron transfer, and coupling to the proton gradient and to ATP synthesis. In the laboratory we have developed a stopped-flow apparatus to measure the binding of herbicides to photosystem II, and have made a comprehensive study through experiment and computer modeling of the catalytic site involved. We will make use of this instrumentation, the methods and expertise developed in the lab, and the detailed kinetic, thermodynamic and structural models we have proposed, to further our investigations of the factors which limit photosynthetic efficiency under field conditions. We are at present working in three main areas: a) donor side, and b) acceptor side, of photosystem II; and c) collaborative research using the portable instruments in the field. In the first two of these, a major emphasis will be on use of a spectrophotometry, EPR and fluorescence under laboratory conditions, to establish parameters for measurement of specific photosynthetic reactions. We will continue the development of novel instrumentation, including new applications of computer linked fluorescence video imaging, and will make use of a collaborative biophysical/molecular engineering approach to probe detailed mechanism. The third area is somewhat open-ended; we have already started collaborations on field studies using the portable instrumentation previously developed; the fluorescence video imaging apparatus will introduce an additional facility.

University of Illinois

Urbana, IL 61801

72. Mechanism of Proton Pumping in Bacteriorhodopsin

T.G. Ebrey, Department of Physiology and Biophysics

$66,000

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 chemiosmotic 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 help resolve the long standing confusion about the photocycle of BR and in particular about the relationship between the intermediates of the photocycle and the transport of protons across the membrane both as regards temporal correlation and quantum efficiency. We are also studying the effect of the large surface potential of the purple membrane on the proton pump-

ing function of this membrane. We have also developed a method for accurately recording the photocurrents associated with the pumping process.

University of Illinois

Urbana, IL 61801

73. Studies on the Escherichia coli Respiratory Chain

R.B. Gennis, Department of Chemistry and Biochemistry

$100,000

The aerobic respiratory chain of *E. coli* is responsible for providing the energy required by the cell for oxidative phosphorylation and for driving active transport. The respiratory chain essentially directs electron flow from the oxidation of organic substrates (e.g., succinate) to oxygen, which is reduced to water. The electron flow is coupled to proton translocation across the cytoplasmic membrane, thus generating a proton motive force. A central component of the electron transport chain which is responsible for the generation of a proton motive force is the cytochrome o terminal oxidase complex. This enzyme has been purified in our laboratory and the operon encoding the polypeptide subunits has been identified, mapped, and cloned. The project supported by this grant is to determine the structure of this enzyme in the membrane and define the mechanism of proton translocation. The cloned operon has been sequenced and the polypeptide sequence of each of the four subunits has been deduced from the DNA sequence. Three of those subunits are strikingly homologous to corresponding subunits in the mitochondrial cytochrome c oxidase, an enzyme that has been very extensively studied. Subunit I, the largest, is identical in 40% of the amino acid residues to the corresponding subunit in bovine cytochrome c oxidase. Biophysical studies have confirmed this unexpected finding, that the bacterial quinol oxidase (cytochrome o) is structurally similar to the eukaryotic cytochrome c oxidases. Various mutations will be made to identify the role of each subunit within the complex. For example, individual subunits will be cloned separately to identify those which bind to the heme or copper prosthetic groups. Extensive use of genetics techniques will localize the amino acids required for catalysis and define their locations in the membrane. Biophysical and biochemical methods will be used in conjunction with this approach. One focus will be to identify amino acid residues involved in ubiquinol binding. Work has been initiated on another enzyme, succinate dehydrogenase, which also interacts with ubiquinone. A similar molecular genetics approach will be used to define the quinone binding site in this enzyme and search for similarities with the quinone binding site in the cytochrome o complex.

University of Illinois

Urbana, IL 61801

74. Cellular Energy Metabolism

M. Glaser, Department of Biochemistry

$76,000

The adenine nucleotides (ATP, ADP, and AMP) are central to the energy metabolism of all cells and also have important roles in regulating the rates of major metabolic pathways. Adenylate kinase (Mg ATP + AMP $\rightleftharpoons$ Mg ADP + ADP) catalyzes a reaction involving all three adenine nucleotides and it is the only reaction in the cell for converting AMP to ADP. The goals of this research are to determine the structure and dynamic properties of adenylate kinase and to understand its physiological role in regulating the concentrations of the adenine nucleotides. Studies will be carried out to further investigate the basis for the phenotype of temperature-sensitive adenylate kinase (adk) mutants of *E. coli*. This class of mutants is unique in that the rates of DNA, RNA, protein and phospholipid synthesis coordinately decrease at the nonpermissive temperature. This appears to be due to changes in adenine nucleotide concentrations as a result of the inactivation of adenylate kinase. Thus, adenylate kinase may be one control point for determining the rates of macromolecular synthesis and cell growth. Adenylate kinase will be isolated and characterized from wild type and mutant strains. The structure and dynamic properties of the enzyme will be determined by x-ray crystallography and fluorescence spectroscopy. The cloned adenylate kinase gene will be used to vary the level of adenylate kinase in the cell in order to determine the effects on cellular metabolism and cell growth.

University of Illinois

Urbana, IL 61801

75. Genetics of the Methanogenic Bacterium, Methanococcus voltae With Attention to Genetic Expression Mechanisms and the Development of Genetic Transformation Techniques

J. Konisky, Department of Microbiology

$65,000

The objective of this research program is to study the genetics, physiology and molecular biology of the marine archaebacterium, *Methanococcus voltae*. Since our understanding of methanogens at the molecular level is quite primitive, a multifaceted approach is being used with a major emphasis on developing a gene transfer system and understanding expression of methanogen genes.

Although low level natural transformation occurs in *M. voltae*, it would be advantageous to develop a gene vector system. To this end, methanococci plasmids and virus-like particles are being characterized with the intent of determining their suitability as gene vectors. The availability of such vectors would provide a means to introduce appropriate functional and mutant genes into *M. voltae* which would greatly expedite biochemical and physiological studies.

Our studies also involve the development of an *in vitro* DNA-dependent coupled transcription/translation system. Such a system would be quite useful in the characterization of methanogen plasmid, viral and cloned genes.

University of Illinois

Urbana, IL 61801

76. The Roles Played by Mitochondrial DNA and Nuclear Genes in Reversions to Fertility in S-Type Male-Sterile Maize

J.R. Laughnan, Department of Plant Biology

$103,760 (FY88 funds)

The underlying mechanisms of the S-type of cytoplasmic male sterility (cms-S) in maize are still unknown. The objective of this project is to elucidate the cause of cms-S and to determine the molecular-genetic basis for both cytoplasmic and nuclear reversions to male fertility. Studies of the cytoplasmic reversion events involve the genetic and molecular characterization of the organization of the mitochondrial DNA (mtDNA) genome. Cytoplasmic reversion to fertility is being characterized at the mtDNA level in the inbred line backgrounds M825, WF9, 38-11 and H95. These revertants are being analyzed using mtDNA gene probes. In all cases studied, reversion is correlated with mtDNA reorganization although the reorganization is not detected by all the mtDNA probes employed. Studies are complicated by the fact that, even in the absence of reversion, substitution of one nuclear genotype by another leads to reorganization of the mtDNA genome detectable with the same gene probes as is reversion. The organization of the cms-S mtDNA genome differs, therefore, in the different inbred lines studied and there is major reorganization of certain regions of the genome upon reversion. These facts complicate the determination of the specific mtDNA alteration responsible for cms-S. Recently a single restriction endonuclease band was detected that is present in the inbred line sterile versions and absent in their fertile revertants. Efforts are underway to clone, and then subclone, this band. New cases of cytoplasmic reversion have been established in the inbred line backgrounds R839, R853N and in a sister line of M825. When the sterile-specific band is cloned, it will be used to probe the fertile revertants and their sterile progenitors from these lines as well. Two classes of spontaneously-occurring restorer-of-fertility (Rf) genes arising in inbred lines have been found. One class restores fertility to the plant and pollen carrying the new Rf gene functions in fertilization. A second class of Rf gene produces plants which are phenotypically male fertile but the pollen produced is nonfunctional. A number of studies involving these new Rf genes are underway. Plants which carry both classes of Rf genes from the same inbred line background have been produced and are being analyzed in experiments akin to the lambda "helper" experiments. The poller proteins present in these two classes of Rf gene will be studied this summer. It has been observed that crossing the nonfunctional Rf genes into a hybrid background enables them to function. The basis of this phenomenon is also under investigation. Studies on transposition of spontaneously-occurring and standard Rf genes are being continued.

University of Illinois
Urbana, IL 61801

77. Hydrogen-Independent Methanogenic Systems
R.S. Wolfe, Department of Microbiology

$86,000

Methanogenic bacteria are widespread in nature, and 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. We are isolating thermophilic alcohol-utilizing methanogens, mass culturing them, purifying and characterizing the 2-propanol dehydrogenase. We are defining the electron acceptors and are elucidating the role of the cofactors methanofuran and tetrahydromethanopterin as well as the enzymology of this system. The recent determination of the structure of component B of the methylreductase system provides a new approach to an understanding of methanogenesis.

University of Illinois
Urbana, IL 61801

78. Structure and Expression of Nuclear Genes Encoding Rubisco Activase
R.E. Zielinski, Department of Plant Biology

$75,000

Rubisco activase consists of two soluble chloroplast polypeptides, which catalyze the activation of rubisco to catalytic competency. By using cloned cDNA probes and antibodies specific for the activase polypeptides, we have determined that the expression of total activase mRNA and protein is (a) exclusively restricted to leaf tissue; (b) qualitatively coordinated with that of the mRNAs and polypeptides of two rubisco subunits in the acropetal developmental gradient of barley leaves; and (c) strongly and rapidly regulated by light, mediated at least in part by phytochrome. The activase polypeptides are encoded by a single copy nuclear gene, which is alternatively spliced to yield two distinct mRNA species. The two activase mRNAs are translated to produce activase polypeptides of 45 and 41 kd. Both polypeptides are independently capable of activating rubisco *in vitro*. Our goals are to answer the following specific questions: (1) 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; (2) what are the sequence of events in alternative splicing of activase mRNAs and to what extent does this alternative splicing result in differential expression of the two activase polypeptides; and (3) what is the cause and physiological significance of the diurnal fluctuations in activase mRNA content of barley leaves.

Iowa State University
Ames, IA 50011-3223

79. The Study of a Heat Shock-Induced Mitochondrial Matrix Protein Essential for Mitrochondrial Biogenesis and Thermostabilization
R.L. Hallberg, Department of Zoology

$74,500

We have identified an evolutionarily conserved protein found in a wide variety of eukaryotic mitochondria that is moderately abundant at normal growth temperatures and whose level is increased several fold during hyperthermal stress. The *E. coli* homolog of this protein has been shown to be the product of groEL gene which is one of the major heat shock induced proteins in this organism. Using antiserum directed against the purified *Tetrahymena* mitochondrial protein, we were able to clone the homologous gene in yeast. This gene, which we call hsp60, encodes an essential protein of about 60,500 daltons which at the amino acid level is 54% identical to the *E. coli* groEL protein and 43% identical to the wheat chloroplast rubisco-binding protein, another protein recently shown to be a groEL homolog and one already known to be necessary for macromolecular assembly in chloroplasts. In genetic studies using yeast, we have shown that a conditional mutation in an allele of hsp60 causes a general inhibition of macromolecular assembly in mitochondria of affected cells at the non-permissive temperature. We are currently attempting to better characterize and define the biochemical properties of the native hsp60 complex isolated from both yeast and corn.

University of Iowa

Iowa City, IA 52242

80. Molecular Biology of Anaerobic Aromatic Biodegradation

C.S. Harwood, Department of Microbiology

$36,145*

Organic compounds with an aromatic nucleus are abundant. 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. Much of this 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 completely unexplored. We are investigating the molecular basis for anaerobic benzoate and 4-hydroxybenzoate degradation by the bacterium *Rhodopseudomonas palustris*. These two aromatic compounds are the starting points for two major anaerobic ring-fission pathways. We have isolated a series of mutants blocked in aromatic utilization and have established systems of genetic exchange by conjugation in *R. palustris*. At present we are working to clone the gene for benzoate-CoA ligase, an enzyme required for benzoate degradation which we have purified and characterized in collaboration with Dr. J. Gibson. We plan to clone additional genes based on their ability to complement blocked mutants. This work will be coordinated with ongoing biochemical studies by Dr. Gibson and, where possible, functions will be assigned to cloned genes based on the ability of the gene products to catalyze known reactions. In addition, we 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.

*(Additional funding provided by the U.S. Department of the Army.)

The Johns Hopkins University

Baltimore, MD 21218

81. Hydrogen/Sulfur Metabolism in the Hyperthermophilic Archaebacterium Pyrodictium brockii

R.J. Maier, and R. Kelly, Department of Biology

$46,558

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 proposed here 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 the highest 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. For example, the H_2 activating hydrogenase and electron-carrying cytochrome in the H_2 oxidizing pathway will be studied in cell-free preparations. Initial studies will focus on hydrogenase activity expressed by membrane preparations from *Pyrodictium brockii*, along with characterization of both purified hydrogenase and the cloned hydrogenase gene. Further work will address the characterization of other enzymes involved in hydrogen/sulfur transformations by these bacteria. The long-term goal is to understand the biochemical basis of extreme thermophily, therefore, comparisons to mesophilic enzymes/-genes will be made.

University of Kentucky

Lexington, KY 40546-0091

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

G.M. Cheniae, Department of Agronomy

$192,000 (FY89 funds/two years)

Analyses were made of the presumed Ca^{2+} requirements in photoactivation of the water oxidizing complex (WOC) and the Ca^{2+} functions in electron transfer in the PS2 Reaction Center (RC)/WOC system. Photoactivation of NH_2OH-PS2 in Mn^{2+}/Ca^{2+} led to

O_2-evolving Mn/Ca containing WOC displaying typical period-4 B-band oscillations (in collaboration, RIKEN Institute). With omission of Ca^{2+}, the Mn-WOC complex was still formed but did not evolve O_2 or show oscillatory B-band behavior yet a high yield of B-band emission was observed after a single flash. Post-addition/incubation in darkness of these inactive Mn-WOC complexes with Ca^{2+} released 1-2 Mn/RC and permitted O_2 evolution as well as period-4 B-band oscillations. Thus, photoligation of Mn^{2+} into WOC does not require Ca, but the binding of Mn^{2+} to PS2 Ca^{2+} sites created during photoactivation inhibits the $S_2 \rightarrow S_3$ or $S_3 \rightarrow S_0 + O_2$ transitions. Inhibition can be overcome by Ca^{2+} displacement of Mn^{2+} from the Ca sites. Analyses of the abundances of S_2-multiline Y_D^+ (fast), Y_D^+ and a Chl radical (Chl^+) were made with O_2-evolving PS2 Cores (~2 Ca^{2+}/RC) and Cores depleted of Ca^{2+} (~0.2 Ca/RC) (in collaboration, Dr. G. Brudvig). Non-depleted Cores yielded normal abundance of S_2 yet O_2 evolution was enhanced 3-5 fold. The depletion of Ca^{2+} did not diminish Y_Z^+ (fast) or Y_Z^+ but abolished O_2 evolution and S_2-formation. Brief illumination of depleted Cores generated Chl^+ reflecting a photoinhibition (Thompson/Brudvig). Reconstitution with Ca^{2+} restored O_2 evolution and S_2-formation, converted Y_Z^+ (fast) to Y_Z^+ (very fast), and eliminated formation of Chl^+. Kinetic evaluation gave evidence for minimally 2 Ca sites. One site with a K_a of 1-4 μM couples Y_Z to WOC.

University of Kentucky
Lexington, KY 40546-0091

83. The Role of Purine Degradation in Methane Biosynthesis and Energy Production in Methanococcus vannielii
E. DeMoll, Department of Microbiology and Immunology

$80,000

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*. Insofar as it has been characterized, the purine degrading pathway of *M. vannielii* resembles that of 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- and 6-carbons enter the methane biosynthetic pathway at the beginning as CO_2, however the 8-carbon apparently enters the methane pathway in the middle 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. vannielli*. 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

84. Enzymatic Synthesis of Materials
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 and engineered 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 enzymatic modification of metal and semiconductor surfaces to alter adhesive and other interfacial properties.

Genes have been cloned and expressed to allow production of natural and engineered enzymes. 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 hydrolyzing (and making) glycosidic bonds have been produced. Fluorinated sugars have been synthesized and polymerized into long chain fluorinated polysaccharides. A boron-doped silicon surface has been modified to alter both its nonspecific and specific adhesion to other materials.

Lawrence Berkeley Laboratory

Berkeley, CA 94720

85. Mapping Photosynthetic Genes in Prokaryotic and Eukaryotic Cells

J. Hearst, Chemical Biodynamics Division

$258,000

Characterization of Carotenoid Synthesis Genes

Despite the crucial role of carotenoids in photooxidative protection, no carotenoid gene sequences from any organism have been previously reported, nor have the molecular mechanism regulating carotenogenesis been elucidated. We have recently determined the complete nucleotide sequence, 11039 bp, of the BamHI-J, -M, -G, and -H fragments of pRPS404 (Armstrong et al.). This region contains seven of the eight previously identified *R. capsulatus* carotenoid genes (crtA, B, C, D, E, F, and I). In addition we have identified a new gene located between crtB and crtC from the DNA sequence. This gene, designated crtK, seems to be required along with crtC for the conversion of neurosporene to hydroxyneurosporene. We have observed two regions of exceptional amino acid homology (54% over 39 residues at the C-termini and 41% over 44 residues at the N-termini) between CrtD and CrtI, both of which are dehydrogenases. We are currently studying the regulation of the crt genes by light and oxygen during the shift from respiratory to anaerobic photosynthetic growth. We are also sequencing the remaining uncharacterized gene involved in carotenoid biosynthesis, crtJ, which is separated from the other crt genes by about 12 kb. We have recently completed the high resolution mapping of Tn5.7 insertion mutations in crtA, I, C, E, and F to between 25 and 238 bp resolution. In addition we have found that one Tn5.7 insertion in the 5' flanking region between crtA and crtI has no effect on carotenoid synthesis, while another Tn5.7 insertion which eliminates bacteriochlorophyll synthesis lies within the 3' end of crtA. We are using these Tn5.7 mutants to examine the operon structure of the crt genes in more detail. In a recent collaboration we have for the first time identified the enzymatic functions of the crtB and crtE gene products, using a ^{14}C *in vitro* incorporation assay. This assay shows that crtB encodes an enzyme which condenses two molecules of geranygeranyl pyrophosphate to yield prephytoene pyrophosphate, while crtE converts prephytoene pyrophosphate to phytoene.

Measurement of DNA Supercoiling in the Photosynthesis Gene Cluster

The expression of oxygen-regulated genes in the photosynthesis cluster of *R. capsulatus* requires DNA gyrase (Zhu and Hearst, 1988). We have shown that addition of gyrase inhibitors to photosynthetic cultures results in the rapid loss of mRNA for light harvesting and reaction center proteins, and ribulose-bis-phosphate carboxylase, the key enzyme in carbon fixation. The loss of mRNA for these genes occurs on a timescale comparable to the half-lives of the individual RNAs, suggesting that the inactivation of gyrase results in the immediate cessation of transcription. The requirement for DNA gyrase raises the possibility that photosynthesis genes may be regulated by the level of superhelicity of the chromosome. To address this model, we have developed an assay for detecting *in vivo* changes in superhelicity of small regions of the bacterial chromosome (Cook et al, submitted). Our assay is based on the preferential reactivity of trimethylpsoralen (TMP) for supercoiled versus relaxed DNA. This method allows us to determine the rate of TMP crosslinking to any restriction fragment of the genome. In control experiments, we have demonstrated the utility of the assay in detecting *in vivo* changes in supercoiling of restriction fragments between 2 and 10 kb in size. The reactivity of individual restriction fragments decreases 1.8-fold after treatment with the gyrase inhibitor novibiocin. We have utilized this assay to determine whether there is a change in superhelicity in the photosynthesis gene cluster upon a shift from respiratory to anaerobic photosynthetic growth conditions. We have also examined a restriction fragment containing the fbc operon which codes for the subunits of the cytochrome bc_1 complex. During this shift in growth conditions,

the mRNAs coding for the RC and LHI complexes are induced six-to eight-fold while the amount of mRNA from the fbc locus remains constant. Neither the genes for photosynthesis nor those for the bc_1 complex undergo a change in superhelicity during this metabolic transition as measured by our assay. Our results cast doubt on a current model for the control of gene expression in facultative anaerobic bacteria which proposes that a change in DNA supercoiling mediates differential transcription of genes for aerobic and anaerobic metabolism. Our current efforts are aimed toward understanding the topological requirements for gyrase as a consequence of transcription of the photosynthesis gene cluster.

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Berkeley, CA 94720

86. Mechanism of Water Splitting and Oxygen Evolution in Photosynthesis
M.P. Klein, Chemical Biodynamics Division

$215,000

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 water molecules 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 a Mn-Mn separation of 2.7 Å. 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 essentially identical and well described as pairs of di-mu-oxo binuclear centers with Mn-Mn distances of 2.69A and 2.79A containing Mn(III) and Mn(IV). 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 PSII 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.

Lawrence Berkeley Laboratory

Berkeley, CA 94720

87. Tissue Specific Gene Regulation in Plants
F. Leach, and J.C. Bartholomew, Chemical Biodynamics Division

$156,000

Transcription studies of *Agrobacterium rhizogenes* Ri T-DNA in *Solanum tuberosum* and *Nicotiana tabacum* have led to the conclusion that expression from the Ri T-DNA genes is highly variable and tissue-specific. In particular, *Agrobacterium rhizogenes* T-DNA bears genes which are specifically expressed in leaves or in root tissues. Four loci are responsible for adventitious root-formation, however, with a different importance in the various species tested. The exact role of the proteins they encode is not known. We are studying the molecular mechanisms which are responsible for their differential expression once the bacterial T-DNA has been inserted into the plant genome. We are, therefore, working on two Ri T-DNA genes, one root-specific, the other leaf specific. We have started by mapping the promoter elements of the two genes by RNA protection experiments and deletion analysis. Fragments of various lengths from the 5' upstream regions have been subcloned. They were modified to yield overlapping subsets of fragments ending at the same 3' nucleotide just before the ATG codon, but with several combinations of 5' end deletions. The fragments obtained were ligated to the easily assayable reporter gene beta-glucuronidase. Fusions of the Ri promoter fragments and the reporter gene were checked by supercoiled DNA sequencing and introduced into tobacco using a pTi derived binary vector. Regenerants are presently being analyzed: the earliest constructs re-introduced into plants and the positive control show expression of the reporter gene. There is no background beta-glucuronidase activity in tobacco. We have identified a region of approximately 120 bp necessary for the proper transcription of the leaf-specific gene. These results were deduced from the

analysis of regenerants maintained in sterile culture conditions. We have transferred regenerants to the greenhouse to confirm the results on adult plants developing in normal physiological conditions. By selfing flowering plants we will obtain an F1 progeny which will contain a fraction of plants homozygotes for the re-introduced chimaeric constructs. We intend to use these F1 descendents to follow the pattern of activity of the fused promoters during seedling development and to understand what role these particular genes have in rooting and general plant morphogenesis. Although the two genes under study are linked to morphogenic processes, neither bears any sequence homology to the few genes yet characterized involved in phytohormone biosynthesis. A paper concerning this part of the project is in preparation. Due to the unsuitable distribution of restriction sites available, lengthier methods were necessary to generate the several root-promoter chimaeric constructs. Although all the constructs were introduced into tobacco, the analysis is not yet as advanced.

Lawrence Berkeley Laboratory

Berkeley, CA 94720

88. Photochemical Conversion of Solar Energy

L. Packer, R.J. Mehlhorn, I.V. Fry, and J. Maguire, Applied Science Division

$130,000

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

89. Chemistry of Phycobiliproteins and Phytochrome

H. Rapoport, Chemical Biodynamics Division

$36,000

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-protein 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

90. Photosynthetic Membrane Structure and Photosynthetic Light Reactions

K. Sauer, Chemical Biodynamics Division

$310,000

We are investigating the kinetics and energetics of excitation transfer and trapping in antenna complexes and reaction centers of photosynthetic membranes using wavelength-resolved fluorescence decay measurements. This process typically occurs within less than a nanosecond following the absorption of visible light photons. Studies using X-ray crystallography from several laboratories have provided structural information for several of these complexes, and this detailed information has enabled us to carry out excita-

tion transfer calculations using exciton theory and/or Forster inductive resonance transfer applied to pigment arrays of known geometry. The cyanobacterial pigment C-phycocyanin coordinates published based on revised 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 significant excited state relaxation that is faster than has been resolved in measurements to date, and we will investigate this faster time domain using improved time resolution of our kinetic spectrometer. The availability of cyanobacterial mutants that are deficient in particular components of the phycobilisome antenna complexes has allowed us to investigate the effect of these deletions on the flow of excitation energy in these architecturally complex structures. 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 extended our studies to a chlorophyll a/b protein complex associated with Photosystem I of higher plants. This complex exhibits pronounced enhancement of long-wavelength fluorescence at low temperatures even in the absence of reaction centers. We are investigating the role that these low lying excited states play in photosystem architecture and the mechanism of energy conversion.

Los Alamos National Laboratory
Los Alamos, NM 87545

91. Structural Biology of the Plant Cell Wall
L.O. Sillerud and N.H. Fink, Division of Life Sciences

$117,000

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

92. Energy Metabolism in Methylotrophic Bacteria
C.J. Unkefer, Isotope and Nuclear Chemistry Division

$118,000

Methylotrophs are aerobic microorganisms capable of growth on one-carbon (C_1) compounds more reduced than CO_2. These organisms derive their energy and reducing power from the oxidation of the reduced C_1 compounds by O_2 and must condense C_1 units to form all of the macromolecular constituents of the cell. The ability of methylotrophs to grow on simple C_1 compounds gives them considerable industrial potential based on their ability to produce a variety of useful compounds from simple precursors such as methanol or methane. In order for this potential to be realized, the fundamental biochemistry and physiology of their metabolism must be understood.

Our work is currently focused on several closely related questions about carbon metabolism in the *icl*-serine-type methylotroph *Methylobacterium* AM1. (1) How do *icl*-organisms oxidize acetate to glyoxylate, a

function required for growth on both methanol and ethanol? (2) How is the methanol dehydrogenase coenzyme PQQ biosynthesized? (3) What are the mechanistic details of the PQQ dependent methanol dehydrogenase? The first is a specific question about a long-standing problem in methylotroph metabolism. PQQ is a novel coenzyme involved in the oxidation of methanol. Examining the enzymes that generate (methanol dehydrogenase) formaldehyde will lead us to answering the question: how do methylotrophs avoid self-poisoning by formaldehyde?

Our approach to the metabolic questions will be to incubate growing cells and cell extracts with ^{13}C-labeled substrates, the products of which can be identified by NMR. Information obtained from tracing one- and two-carbon units will give insight on metabolic conversions.

University of Maryland

College Park, MD 20742

93. Active and Passive Calcium Transport Systems in Plant Cells

H. Sze, Department of Botany

$70,000 (FY89 funds/17 months)

Many cells respond to stimuli by changing their cytoplasmic calcium levels rapidly. Since this cation may be a key regulator of many biological processes, we are interested in understanding the mechanisms and regulation of active and passive Ca^{2+} fluxes across the plasma membrane and the organellar membranes. Cytoplasmic Ca^{2+} levels are kept low by several active transport systems. 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. Our attempts to find an active Ca^{2+}-pumping ATPase on the nuclear membrane were discouraging; however, such a pump exists on endoplasmic reticulum vesicles from carrot suspension cells. We are now interested in understanding how calmodulin activates the ER Ca^{2+}-ATPase.

Massachusetts Institute of Technology

Cambridge, MA 02139

94. Analysis of the Rhizobium meliloti Surface

E.R. Signer, Department of Biology

$91,000

The formation by rhizobia of nitrogen-fixing nodules on the roots of leguminous plants presumably involves communication between bacteria and plant cells, and is thus likely to depend on interactions between the surfaces of the two symbiotic partners. We are probing the surface of the alfalfa symbiont *Rhizobium meliloti* SU47 in order to identify components that are involved. Currently we are focusing on the response of rhizobial surface lipopolysaccharide (LPS) to bacteriophages, monoclonal antibodies, and/or detergents. Sensitivity to antibodies that recognize LPS is altered during differentiation to bacteroids *in planta*, which suggests that surface changes play a role in nodule development. We have identified thirteen genes involved in biosynthesis of LPS and have isolated a cosmid clone including some of these, which we are characterizing molecular-genetically. Mutants in each of these genes, in an otherwise wild-type background, fix nitrogen in alfalfa nodules, in contrast with LPS mutants of *R. phaseoli*, which are defective in fixation. Thus LPS does not appear to play a major role in fixation by *R. meliloti*. The structure of LPS from wild-type and mutants is now being determined (in collaboration with Dr. R. Hollingsworth, Michigan State University). These studies should eventually define critical components and reveal their role in nodule development, which should in turn ultimately help in the genetic manipulation of rhizobia for improved efficiency of nodulation and/or nitrogen fixation in the field.

University of Massachusetts

Amherst, MA 01003

95. Cellulose Fermentation by Nitrogen-Fixing Anaerobic Bacteria

E. Canale-Parola, Department of Microbiology

$80,000

The project objective is to conduct studies aimed at increasing understanding of i) the physiology of anaerobic cellulolytic nitrogen (N_2)-fixing bacteria, ii) the N_2-fixing system of these bacteria, iii) the effects that N_2 fixation has on cellulose fermentation, and iv) the physiological interactions between non-cellulolytic, N_2-fixing, commensal bacteria and cellulose fer-

menters 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 nitrogen fixation and the fermentation of cellulose in biological systems in which these two complex physiological processes coexist within the same cell. Furthermore, these strains may be extremely useful in the direct conversion of biomass materials to ethanol and other fuels. Another objective of the research will be to establish diazotrophic cocultures of N_2-fixing, anaerobic, non-cellulolytic commensals and cellulolytic bacteria in order to study the effects of competition for cellulose depolymerization products on cellulose degradation.

University of Massachusetts

Amherst, MA 01003

96. Genetic Analysis of a Green Bacterial Photosynthetic Membrane

T. Redlinger and S. Robinson, Departments of Biochemistry and Botany

$120,000 (FY88 funds/two years)

The relative simplicity of the bacterial photosynthetic apparatus, and the availability of genetic techniques for analysis of the prokaryotic genome make photosynthetic bacteria excellent model systems for the study of photosynthetic energy capture. We choose to study the green filamentous phototrophic bacteria, *Chloroflexus*, because of its unique structural juxtaposition of a photosystem II-like reaction center complex in the membrane with attached chlorosome which serve as light-harvesting antennae.

Our project focuses on the regulation of synthesis and assembly of the photosynthetic apparatus in *Chloroflexus* by applying recombinant DNA techniques to isolate genes encoding photosynthetic proteins. Recently, we have isolated the gene (csmA) encoding the Bchl c binding polypeptide from *Chloroflexus*. Sequence analysis of csmA indicates that the protein is synthesized with a carboxy terminal extension. This is an exciting finding which links the D1 protein in photosystem II (which also has been shown to have a carboxy terminal extension), to the more primitive antenna system of *Chloroflexus*. We are continuing to investigate the role of the carboxy terminal extension in assembly as well as cloning other genes encoding chlorosome proteins. Results from this research provide a new approach for looking at chlorosome assembly as well as photosystem II assembly in higher plants.

Meharry Medical College

Nashville, TN 37208

97. Respiratory Enzymes of Thiobacillus ferrooxidans

R.C. Blake, Biochemistry Department

$75,000

Certain chemolithotrophic bacteria inhabit ore-bearing geological formations exposed to the atmosphere and obtain all of their energy for growth from the dissolution and oxidation of minerals within the ore. The aim of this research is to continue the systematic isolation and characterization of the respiratory enzymes expressed by these chemolithotrophic bacteria when grown on either reduced metal substrates or reduced inorganic sulfur compounds. Our current focus is on the iron-oxidation systems of *Thiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*. The periplasmic space of both gram-negative organisms contains cytochrome(s) c and an acid-stable blue copper protein called rusticyanin. The rusticyanin proteins have been purified to electrophoretic homogeneity from cell-free extracts of both organisms, and their structural and functional properties are under investigation. We have identified a particular acid-stable, membrane-bound cytochrome c in each organism that serves to catalyze the transfer of electrons from ferrous ion to purified rusticyanin. This cytochrome c-catalyzed electron transfer reaction exhibits many of the qualitative (such as anion specificity) and quantitative (such as the Km for ferrous ions) properties exhibited for ferrous oxidation by whole cells. We will continue with efforts to purify these cytochromes c, as well as to identify and study other components of the iron respiratory system. It is anticipated that this project will eventually contribute to a basic understanding of biological energy transduction. It can also provide useful information toward manipulating *T. ferrooxidans* and related organisms for commercial use.

Michigan Biotechnology Institute
Lansing, MI 48909

98. One Carbon Metabolism in Anaerobic Bacteria: Regulation of Carbon and Electron Flow During Organic Acid Production
J.G. Zeikus and G.-J. Shen

$99,000

Our project deals with understanding the fundamental biochemical mechanisms that control carbon and electron flow in anaerobic bacteria that conserve energy when coupling hydrogen and/or CO_2, CH_3OH, $HCOO^-$ and CO consumption to the production of acetic, propionic, succinic or butyric acids; and, understanding the influence of pH on the dynamics of end product formation and cell physiology of acidogenic bacteria. Two acidogens, *Propionispira arboris* and *Butyribacterium methylotrophicum* were chosen as model systems to understand the function of oxido-reductases and electron carriers in the regulation of hydrogen metabolism and single carbon metabolism. Two acidogens, *Sarcina ventriculi* and *Anaerospirillum succiniciproducens*, were chosen as model systems to understand the influence of growth pH on regulation of catabolism. Our research covers purification and characterization of electron carriers and enzymes; characterization of electron flow pathways and key regulating enzymes; documentation of the physiological function of hydrogen and C1 metabolism; and, identifying the influence of pH extremes on the growth physiology of these acidogens.

Michigan State University
East Lansing, MI 48824

99. A Structural Assessment of Species
R.I. Hollingsworth, Department of Biochemistry and Chemistry

$120,000 (FY89 funds/two years)

In our lab 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 study employs affinity chromatography using a variety of lectins to isolate molecules based on lectin-binding specificity. The lectins are chosen to complement the structural diversity of sugars observed in LPS isolates. The purified 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. Once the structures of the oligosaccharides are determined, they 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 48824

100. Role of Acyl Carrier Protein Isoforms in Plant Lipid Metabolism
J.B. Ohlrogge, Department of Botany & Plant Pathology

$77,000

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

101. Cloning, Characterization and Expression of Genes/cDNA Encoding Lignin Peroxidases in Phanerochaete chrysosporium, a White-rot Basidiomycete

C.A. Reddy, Department of Microbiology and Public Health

$68,277

Phanerochaete chrysosporium, the most extensively studied lignin degrading organism, secretes a family of extracellular heme proteins called lignin peroxidases and manganese peroxidases that are known to play pivotal roles in lignin degradation. Several lines of research are being pursued by our laboratory to understand the molecular biology and physiology of lignin biodegradation by this organism. We sequenced cDNA clones and the corresponding genomic clones that encode lignin peroxidase proteins H2 and H10; additional lignin peroxidase genes are also being characterized. We have also isolated and characterized a class of mutants that are nitrogen deregulated for lignin degradation (der mutants), and another class of mutants that lack the ability to produce lignin peroxidases (lip mutants). Additional mutants that selectively lack the ability to produce manganese peroxidases (mnp mutants) are also being isolated. These mutants will be invaluable tools for further molecular genetic studies on lignin peroxidases and manganese peroxidases. We have also developed a novel shuttle vector-based gene transfer system for *P. chrysosporium*. Further studies aimed at obtaining a better understanding of this non-integrative transformation system are in progress. We are also using this transformation system for creating lignin peroxidase gene disruptions, isolation of regulatory genes, and other studies aimed at understanding the regulation and expression of the lignin peroxidase and manganese peroxidase genes at the molecular level. Studies are also in progress to develop heterologous expression systems designed for expression and secretion of lignin peroxidases.

Michigan State University DOE Plant Research Laboratory

East Lansing, MI 48824

102. Differential Gene Expression in Bradyrhizobium japonicum

Colleagues of B.K. Chelm (deceased)

$90,000

Bradyrhizobium japonicum reduces dinitrogen to ammonia in symbiotic association with soybean (*Glycine max*). The formation of the nitrogen-fixation apparatus involves a series of developmental changes in both the plant and the bacterium. These developmental changes are most easily defined, and studied, as changes in gene expression. Our approach has been to focus on the bacterial responses to two fundamental signals that trigger developmental changes: nitrogen-limitation (Ntr regulation) and oxygen-limitation (Olr regulation). In order to probe these responses and investigate their underlying mechanisms in *B. japonicum*, we have been characterizing genes, and their transcriptional regulatory regions, which are expected to be involved in these responses. Gene-replacement techniques have been particularly useful to us in the characterization of the roles of several putative regulatory genes. The proteins encoded by these regulatory genes have been found to be involved in the transcriptional activation of genes required during nitrogen- or oxygen-limited growth. In addition, these studies have revealed that, in *B. japonicum*, the capacity for assimilation of nitrogen is controlled at the level of transcription by two separate regulatory systems. Each system relies on a different regulatory protein (NtrC or NifA) to activate the transcription of genes responsive to oxygen- or nitrogen-limited stress. An understanding of how these regulatory proteins function in gene activation and how the genes encoding these regulatory proteins, or the proteins themselves, sense the change in their environment will continue to be an active area of research in this field.

Michigan State University DOE Plant Research Laboratory

East Lansing, MI 48824

103. Enzymatic Mechanisms and Regulation of Plant Cell Wall Biosynthesis
D. Delmer

$114,000

This project seeks to elucidate the mechanism and regulation of the conversion of reduced carbon into cellulose, the world's most abundant organic compound. The overall objectives are: 1) to identify specific proteins involved in the catalysis and regulation of the polymerization of activated glucose residues into cellulose, 2) to purify and characterize these proteins in terms both of physical and functional properties, 3) to identify the corresponding genes which code for these proteins, and 4) to understand short- and long-term mechanisms by which plants regulate the process with the ultimate aim of testing the capacity of plants to survive with enhanced or decreased levels of cellulose. Current approaches include: 1) study of the relationship between the synthesis of cellulose and a related-glucan, callose, and purification and characterization of the callose synthase; 2) identification and characterization of a protein receptor for a herbicide (DCB) which specifically inhibits cellulose synthesis; and 3) testing of a model which proposes that the DCB-receptor is a regulatory protein which specifies both the extent and type of glucan (cellulose or callose) synthesized by a cellulose/callose synthase.

Michigan State University DOE Plant Research Laboratory

East Lansing, MI 48824

104. Molecular Mechanisms That Regulate the Expression of Genes in Plants
P. Green

$203,000

The aim of our research is to elucidate molecular mechanisms for gene expression in higher plants with emphasis on posttranscriptional events. Although many genes are regulated after the mRNA has been synthesized, relatively little is known about how such control is exerted. In particular, we would like to learn how mRNA stability is regulated in plant cells and how translation may influence this process. To this end we are developing strategies to clone genes that encode stable and unstable mRNAs, and to localize important determinants of mRNA stability within these genes. Chimeric gene constructs in transformed tobacco cells and transgenic plants are being used for these studies. Another objective is to identify the factors, such as ribonucleases, which interact with mRNA sequence determinants to affect stability. For these experiments, we will use both biochemical and genetic approaches in *Arabidopsis*. We are also interested in the effects of hormonal and environmental stimuli on mRNA stability, and whether these effects require ongoing protein synthesis.

Michigan State University DOE Plant Research Laboratory

East Lansing, MI 48824

105. Resistance of Crop Plants to Environmental Stress
A.D. Hanson

$192,000

Plants have biochemical or metabolic adaptations to environmental stresses, as well as adaptations expressed at higher levels of organization. Were metabolic adaptations to stress better understood, they could be used in crop improvement, via conventional or recombinant DNA technologies. This project aims (1) to identify adaptive metabolic 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 metabolic 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 purified this enzyme to homogeneity, raised antibodies against it, and obtained amino acid sequence data. Several immunopositive clones have been isolated from a spinach leaf cDNA library, and are being analyzed further. We have shown using $^{18}0$ labeling techniques that the first step is most probably an oxygenase; purification of this enzyme has begun. Lactate dehydrogenase (LDH) activity is induced by hypoxia in roots and catalyzes lactate glycolysis, which is important during hypoxia. We have developed an improved purification for barley LDH. Preparatory to cDNA cloning, we have raised antibodies against highly purified protein and obtained amino acid sequences of several tryptic peptides.

Michigan State University DOE Plant Research Laboratory

East Lansing, MI 48824

106. Action and Synthesis of Plant Hormones
H. Kende

$205,000

The project objective is to gain knowledge on the regulation of 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-aminocyclo-propane-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 very important enzyme in plant development and have produced antibodies against it. Currently, we are trying to obtain amino acid sequences of ACC synthase as a first step to clone the gene for this enzyme. We are also screening expression libraries with ACC-synthase antibody in an attempt to isolate ACC-synthase clones. We are also investigating the enzyme responsible for ethylene formation from ACC. We have characterized some of its properties in isolated vacuoles and found that it requires membrane integrity and probably a transmembrane ion gradient. 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. the breakdown of internodal starch and biosynthesis of cell wall. 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.

Michigan State University DOE Plant Research Laboratory

East Lansing, MI 48824

107. Cell Wall Proteins
D.T.A. Lamport

$178,000

Recent work with tomato cell suspension cultures shows that during rapid growth there is a surprisingly large precursor pool of monomeric extensin bound ionically to pectin *in muro* but rapidly eluted from intact cells by dilute salt solutions. Facile extraction of these flexible rod-like macromolecules (visualized via EM) suggests their anticlinical (radial) orientation *in muro*. Thus the long axes of cellulose (warp) and extensin (weft) may be in perpendicular planes. The presence of the cross-linked amino acid isodityrosine in covalently bound extensin implies that cross-linkage of extensin monomers occurs *in muro*. We suggest that extensin networks of defined porosity form around microfibrils, thereby mechanically coupling the load-bearing polymers. Current work deals with the two extensin precursors P1 and P2 (i.e., their primary structure, EM visualization, and immunochemistry). P1, P2, and their HF-deglycosylated polypeptides dP1 and dP2 were antigenic, eliciting four sets of rabbit polyclonal antibodies that cross-react specifically with the glycosylated and non-glycosylated epitopes whose primary structure was further elucidated. After HF-deglycosylation, and tryptic degradation of dP1 and dP2, a relatively few major peptides dominate each peptide map; (1) p1/H5 Ser-Hyp-Hyp-Hyp-Hyp-Thr-Hyp-Val-Tyr-Lys; (2) P1/H20 Tyr-Lys, P2/H4 Ser-Hyp-Hyp-Hyp-Hyp-Val-Tyr-Lys. Tryptic degradation was essentially complete; these major peptides represent extensively repeated units, hence a highly periodic polypeptide. The hexapeptide Val-Lys-Pro-Tyr-His-Pro domain of the P1 hexadecapeptide H20 is the prime candidate for intermolecular cross-linkage. If so, the average intermolecular cross-link frequency corresponds remarkably to that predicted for a network penetrated by cellulose microfibrils.

Michigan State University DOE Plant Research Laboratory

East Lansing, MI 48824

108. Interaction of Nuclear and Organelle Genomes
L. McIntosh

$194,000

Our overall goal is to elucidate at a molecular level the regulation of energy transduction in photosynthetic organisms.

Photosynthetic organisms contain unique membrane complexes capable of light-driven charge separation. In some cases these complexes are located on thylakoid membranes which may occur in specialized organelles, chloroplasts. Charge separation and electron transport occur within "reaction centers" consisting of chromophore molecules positioned within a membrane-protein architecture. Our aim is to understand the regulation of electron flow in photosynthesis.

In order to achieve this goal, a model photosynthetic organism, the unicellular cyanobacterium *Synechocystis* 6803, is being employed. Specific genes encoding Photosystem II and Photosystem I polypeptides are isolated and the regulation of the expression of these genes is being studied. In addition, genes encoding reaction center components are modified *in vitro* and used to transform *Synechocystis* 6803. Analyses of these site-directed photosynthetic mutants are being employed to understand electron flow within Photosystem II. A similar approach is being developed for Photosystem I.

Respiration in green plants also takes place in specialized organelles, mitochondria. A majority of plants investigated differ from many eukaryotes in that they contain a branched pathway for mitochondrial electron flow: with a normal cytochrome pathway and an alternative pathway that is not coupled to production of a proton gradient. There is a fundamental lack of knowledge concerning the regulation of electron flow between these two pathways of plant respiration, the structure of the alternative pathway and the function of the alternative pathway in higher plants. Our initial goals are to determine the possible mechanisms regulating electron flow between these two pathways and to investigate the structure of the alternative oxidase at a molecular level. Polyclonal and monoclonal antibodies have been employed to characterize the alternative oxidase in several plant species and the fungus *Neurospora crassa*. These antibodies are now being used to aid in cloning components of the alternative oxidase from a number of plant species and *N. crassa*.

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

109. Sensory Transduction in Plants
K.L. Poff

$178,000

The primary objective of this project is to understand the mechanisms for the acquisition of environment 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. In addition we have identified a strain with its threshold fluence for phototropism increased by a factor of 50. This represents a probable candidate as a photoreceptor pigment mutant. We are continuing to study the pathways for photoreceptor and gravitropism through mutant isolation, and genetic, physiological and biophysical characterization. This genetic approach should permit positive identification of the photoreceptor pigment, access into the transduction sequence, and eventual understanding at the molecular level of the events from photoreception to the bending response.

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

110. Developmental Regulation of Gene Expression in Plants
N.V. Raikhel

$178,000

The first objective of our research is to understand the mechanisms that control developmentally and hormonally regulated, tissue-specific gene expression. The research therefore combines cellular and molecular approaches. Our general aims are: (1) to isolate and characterize enzymes and genes that are tissue-specific and hormonally and developmentally regulated, (2) to define the DNA sequence elements and cellular processes that control tissue-specific gene expression, and (3) to define the DNA sequences that control hormonal regulation of plant gene expression. Cereal and rice lectins are excellent systems to study these regulatory mechanisms because lectin accumulation is organ-, tissue-, and cell-specific, lectins are developmentally regulated, and lectin levels are modulated by abscisic acid. The second objective of our research is directed toward understanding molecular mechanisms of protein targeting. Proteins that enter the secretory pathway are eventually either secreted or localized in one of several distinct subcellular compartments: the endoplasmic reticulum, the Golgi complex,

the protein body/vacuoles or the plasma membrane. Protein delivery to the protein body/vacuoles has been extensively studied in higher plants. However, relatively little is known about molecular mechanisms responsible for the targeting of proteins to particular destinations. The regulatory mechanisms which control the targeting of vacuolar proteins are being investigated.

Michigan State University DOE Plant Research Laboratory

East Lansing, MI 48824

111. Physiological and Molecular Genetics of Arabidopsis
C.R. Somerville

$198,000

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 lipids has been altered by specific deficiencies in one of several fatty acid desaturases or glycerol phosphate acyltransferase. 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 partially regulated at the posttranscriptional level. 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.

Michigan State University DOE Plant Research Laboratory

East Lansing, MI 48824

112. Molecular Basis of Disease Resistance in Barley
S.C. Somerville

$178,000

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. graminis* 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. graminis*. Our conclusion is that the "gene-for-gene" hypothesis, in its simplest formulation, does not adequately describe barley *E. graminis* interactions. We will continue the mutational analysis of the barley Ml-az 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. graminis.* The specific activity of two extracellular peroxidase isozymes increased between 8 and 16 hours post-noculation. We will prepare isozyme specific antibodies so that these isozymes can be localized in the infection court relative to structural features such as papillae and other wall depositions.

Michigan State University DOE Plant Research Laboratory

East Lansing, MI 48824

113. Molecular Biology of Toxicogenic Plant Pathogens
J.D. Walton

$178,000

The objective of this project is to understand the biosynthesis and mode of action of toxins produced by plant pathogenic fungi. We are characterizing the fungal enzymes which biosynthesize toxins, with the ul-

timate goal of studying the evolution of the genes for the enzymes. This will allow a better understanding of how fungal pathogens can overcome the disease resistance genes incorporated into crop plants by plant breeders. We have discovered two enzymes involved in the biosynthesis of HC-toxin made by the pathogen *Helminthosporium carbonum.* Using antibodies to these enzymes and cloned fragments of the corresponding genes we are studying strains of the isolates which are unable to make HC-toxin, and also other species of fungi which make closely related toxins. We are also studying the mode of action of toxins from plant pathogens, to better understand both the biochemical bases of resistance and susceptibility, and also the cellular processes affected by the toxins. We are currently studying the mode of action of victorin from *Helminthosporium victoriae.* It is the most toxic compound known against plants, and has striking effects on the important process of cell wall synthesis.

Michigan State University DOE Plant Research Laboratory

East Lansing, MI 48824

114. Developmental Biology of Nitrogen-Fixing Cyanobacteria

C.P. Wolk

$194,000

Certain cyanobacteria (blue-green algae) trap solar energy via photosynthesis and use the resulting chemical-reducing power to fix atmospheric nitrogen gas (N_2). They thereby produce ammonia, which is used as the nitrogen source for cellular growth. The initial steps of N_2 assimilation take place in specialized cells called heterocysts. The project objective is to understand the supply of energy and electrons to, and the oxygen protection of, nitrogen fixation within heterocysts. Our approach makes use of conjugal transfer of cloned genes from *Escherichia coli* to nitrogen-fixing cyanobacteria. By putting the genes for *Vibrio* luciferase under the control of *Anabaena* promoters and visualizing luciferase-produced light with a photon-counting microscope, we are able to identify the cells in which strong promoters are active. We are attempting to develop a system for amplification of the activity of weaker promoters. Efforts to develop a system for efficient transposon-mutagenesis of *Anabaena* are continuing. A genetic map of *Anabaena* has been constructed based on the PHOGE system of pulsed field gel electrophoresis that uses homogeneous orthogonal fields. Highly effective selection for double recombinants and, thus, site-specific mutagenesis has been achieved using the sac (levan sucrase) gene. We are developing tools for genetic analysis of photoautotrophic, nitrogen-fixing cyanobacteria. This work will facilitate understanding of cellular differentiation and construction of modified strains particularly suitable for applied, biological conversion of solar energy.

Michigan State University DOE Plant Research Laboratory

East Lansing, MI 48824

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

J.A.D. Zeevaart

$180,000

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 *Silene armeria* photoperiodic control of stem growth is mediated by gibberellins (GAs). The following members of the early 13-hydroxylation pathway, GA53, GA44, GA19, GA20, GA17, GA1, GA29, and GA8, have been identified. GA1 accumulates to very high levels in expanding leaving and shoot tips of plants in LD. It appears that GA1 is the GA that induces stem elongation, since the level of GA1 is always related to the degree of stem growth observed.

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. The same ^{18}O labeling pattern has been observed for ABA from turgid bean leaves and from immature maize embryos. In fruits, the biosynthesis of ABA is developmentally regulated. In the case of avocado mesocarp, the pattern of ^{18}O incorporation into ABA is similar to that of ^{18}O-labeled ABA from stressed leaves. However, in apple fruit tissue, the highest ^{18}O enrichment is in the tertiary hydroxyl group of ABA. A possible explanation for this anomaly is that the initial cleavage product from a larger precursor molecule is relatively slowly converted to

ABA in apple tissue, thus allowing the ^{18}O of the aldehyde to exchange with ^{16}O of water. In leaves, further oxidation of the cleavage product would be rapid, or occur in an environment that precludes ^{18}O exchange. Thus, the ^{18}O incorporation studies indicate that there is a universal pathway for ABA biosynthesis in higher plants. However, the regulation of ABA biosynthesis is clearly different in the various tissues.

University of Minnesota
Navarre, MN 55392

116. Genetics of Bacteria that Utilize Carbon Compounds
R.S. Hanson, Gray Freshwater Biological Institute

$67,000

Bacteria that grow on methane, methanol and methylamine are a morphologically and physiologically diverse group of bacteria. Several of these bacteria have been shown to be clustered in two phylogenetically related groups by analysis of 16S RNA sequences. This information is useful for the synthesis of probes to detect methanotrophs in samples from bioreactors and in a variety of environments. We have observed considerable sequence homology in the methanol dehydrogenase genes of all gram negative methylotrophs. However, the upstream regulatory sequences of the two methylotrophic bacteria from which MDH genes have been completely sequenced have little similarity. The genetic maps of 12 genes required for MDH synthesis show several differences in two closely related methylotrophs. The MDH messenger RNA has a chemical half life of approximately one-minute although the protein represents 20% of the cellular protein.

We now intend to map and sequence cloned genes encoding five proteins of *Methylosinus trichosporium* OB3b required for methane monooxygenase activity. We will continue to characterize the twelve genes (and their gene products) required for MDH synthesis and will continue 16S rRNA sequencing in order to complete the molecular phylogeny of methanotrophic bacteria.

University of Minnesota
Minneapolis, MN 55455

117. The Biochemistry and Enzymology of Halomethylcorrinoids
H.P.C. Hogenkamp, Department of Biochemistry

$77,000

Methylcorrinoids, such as methylcobalamin and methylcobamides, participate in the biosynthesis of methane, acetate and methionine. In the methanogens, the corrinoids function as coenzymes for the methanogenesis from methanol, formaldehyde and acetate as well as for the synthesis of acetate from C-1 substrates. The synthesis of methionine is catalyzed by methionine synthase, a cobalamin dependent enzyme that transfers the methyl group of 5-methyltetrahydrofolate to homocysteine. The unique feature of the corrinoid coenzyme is the carbon-cobalt bond.

In this project we propose to synthesize and characterize several corrinoids with halomethyl groups attached to the cobalt atom. We plan to synthesize cobalamins, cobinamides and trimethylbenzimidazoylcobamides with CXH_2-, CX_2H- and CX_3-ligands (X=F, Cl, Br and I) and evaluate the effect of the electronegative and bulky halogen on the stability and reactivity of the carbon-cobalt bond. We also propose to test these halomethylcorrinoids as substrates and/or inhibitors of methanogenesis, acetogenesis and methionine formation.

We have already demonstrated that corrinoids are able to catalyze the reductive dehalogenation of chlorinated C-1 hydrocarbons in the presence of suitable reducing agents.

University of Minnesota
Minneapolis, MN 55455

118. The Mechanism of Switching from an Acidogenic to a Butanol-Acetone Fermentation by Clostridium acetobutylicum
P. Rogers, Department of Microbiology

$79,000

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, determined the kinetic constants for the other key enzymes in the two pathways branching from butyryl-CoA producing either butyric acid or butanol. We are now studying the mechanism of rapid turnover of butyric acid during the solventogenic phase of the fermentation.

Prior to solvent formation the synthesis of five or six enzymes is induced 40 to 100 fold. 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 butyraldehyde dehydrogenase, butanol 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

119. Response of Desulfovibrio Colonies to Oxygen Exposure

J.D. Wall, M.F. Brown, and B.J. Rapp, Biochemistry Department and Plant Pathology Department

$70,000

Oxygen tolerance of the strictly anaerobic sulfate-reducing bacteria is well documented and poorly understood. This capacity for surviving brief exposures to oxygen must be a major factor in the diversity of environmental niches observed for these bacteria. In pursuing genetic studies with *Desulfovibrio desulfuricans* (ATCC 27774), we had an opportunity to observe that viable cells could be found in colonies on the surface of solidified medium exposed to air for periods as long as a month. During exposure to air, the originally black colonies became greyish-white, presumably due to the air oxidation of the metal sulfide deposits. A black, brittle deposit formed at the bottom of the colony and, simultaneously, the colony descended into a dimple that developed into a well in the agar. Eventually the colony reached the bottom of the Petri dish. These changes did not take place when the colonies were maintained in an anaerobic chamber. The morphological changes took place with all strains tested; three strains of *D. desulfuricans*, *Desulfovibrio gigas*, and *Desulfovibrio multispirans*. Documentation of these morphological changes will be presented. Continued sulfate reduction appeared to be essential. Cyclic sulfate (thiosulfate or sulfite) reduction to sulfide and reoxidation of sulfide by the oxygen in air are proposed to maintain the viability of the bacteria by providing substrates for energy production and by reducing oxygen tension. Scanning and transmission electron microscopy of colony and cellular changes will be shown.

Mount Sinai School of Medicine
New York, NY 10029

120. The Respiratory Chain of Alkalophilic Bacteria

T.A. Krulwich, Department of Biochemistry

$166,000 (FY89 funds/two years)

The focus of the coming project period is the pH-regulation of the cytochrome content of the extremely alkalophilic bacilli. These bacteria, as part of their adaptation to unusual energetic problems, consistently exhibit an extraordinarily high membrane cytochrome content when grown at optimal pH values such as pH 10.5. During growth at lower pH values, a facultative alkalophile such as *Bacillus firmus* OF4 has a lower cytochrome content and different cytochrome ratios than at alkaline pH. The genes encoding the cytochrome oxidase will be cloned and characterized with a view to elucidating the basis for pH-dependent regulation of cytochrome gene expression.

National Institutes of Health
Bethesda, MD 20892

121. Identification of the Enzymatic Steps in Methanogenesis from Acetate: Studies on the Structural and Catalytic Properties of Carbon Monoxide Dehydrogenase from Methanosarcina barkeri

D.A. Grahame, National Heart, Lung, and Blood Institute

$60,500 (FY88 funds/two years)

The objectives of the proposed research are, in Part I: To identify, purify, and characterize the enzymatic components involved in methanogenesis from acetate by reconstitution of the factors required for the partial reaction in which the methyl group of acetate is converted to the methyl moiety of 2-methylthioethane-sul-

fonate (methyl-S-CoM) and to demonstrate whether CODH is essential for methyl-S-CoM formation; and in part II: To determine specific characteristics of CODH including the subunit location of the active site and the chemical properties of nickel in enzyme.

The *in vitro* enzymatic system of methane formation from acetate will be resolved of CODH using immobilized anti-CODH antibodies and then the pure enzyme will be added back to establish its importance in the conversion. Identification and purification of the other required enzymes will be carried out within the NIH Anaerobic Laboratory using an assay which has been developed for methyl-S-CoM formation from acetate. Proof for a physiological role for CODH will come from experiments in which it is recombined with the other purified components.

The subunit location of the active site of CODH will be determined using an immunological approach in which antibodies to the individual subunits are tested for inhibitory effects. This question will also be addressed by the development of conditions for subunit dissociation wherein activity is retained. Structural information about the active site will be obtained from experiments using a radiolabeled, active site-directed inhibitor. Measurement of the release of nickel and iron from ^{55}Fe and ^{63}Ni labeled preparations of CODH upon disruption of the iron-sulfur centers will provide comparative information on the nature of binding of these metals in the enzyme. In these experiments, an attempt will be made to determine whether cyanide inactivation results from binding to nickel or to iron by observing the effects of cyanide inactivation on the release of both metals. The stoichiometry of cyanide binding during inactivation will be determined using [^{14}C]NaCN.

University of Nebraska
Lincoln, NE 68583-0722

122. 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 viruses 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-methyldeoxycytosine and 0 to 37% N^6-methyldeoxyadenosine), 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 non-prokaryotic 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

123. Anaerobic O-demethylation of Phenylmethylethers
A.C. Frazer and L.Y. Young, Department of Microbiology

$73,000

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. In order to achieve this goal, studies are underway to improve the *in vitro* assay for AOD activity in cell-free extracts. In addition, some fractionation of

the AOD system from cell extracts has been obtained recently by reconstituting the AOD activity in various fractions with the addition of a small amount of extract from uninduced cells.

North Carolina State University

Raleigh, NC 27695-7612

124. Phosphoinositide Metabolism and Control of Cell Growth

W.F. Boss, Department of Botany

$64,000

Polyphosphoinositides, the negatively charged phospholipids that have been shown to play a pivotal role in signal transduction in animal cells, are found in plant cells. The question being addressed is whether or not they are involved in signal transduction in plant cells and if so what is the mechanism of their involvement. If the polyphosphoinositides play a similar role in signal transduction in plants as they do in animals, then the second messenger, inositol trisphosphate (IP_3), which is produced upon hydrolysis of phosphatidylinositol bisphosphate (PIP_2) by phospholipase C, should be present. We have not been able to detect IP_3 in the carrot cells using both ^{32}P and $[^3H]$ inositol labeling. Pulse-chase labeling experiments indicate that the cell wall metabolites which label with $[^3H]$ inositol but not the inositol phospholipids or inositol phosphates are readily chased with 10 mM inositol. The fact that we could not detect IP_3 and the fact that PIP_2 was present in very low amounts (approximately one tenth) compared to PIP in these cells suggested that the role of the phospholipids might be that of "membrane effectors", that is, they may alter membrane enzyme activity directly rather than serve as a source of the second messenger IP_3. To test this hypothesis, we are studying the regulation of the enzymes involved in the synthesis of PIP and PIP_2 to determine whether or not increased synthesis of these lipids correlates with the initial response to external stimuli such as fungal enzymes and light.

Northwestern University

Evanston, IL 60208-3500

125. Genetics of Thermophilic Bacteria

N.E. Welker, Department of Biochemistry, Molecular Biology and Cell Biology

$29,000

The goal of this project is to develop efficient and reliable genetic technology to analyze and manipulate *Bacillus stearothermophilus* NUB36. Over sixty different auxotrophic and resistance markers have been mapped by transduction and protoplast fusion. Biochemical and genetic analyses reveal that most of the markers are analagous to markers on the well-characterized *Bacillus subtilis* chromosome. An efficient protoplast transformation system was established and we have constructed several shuttle plasmid vectors that are stably maintained and express antibiotic resistance in *B. subtilis* and in *B. stearothermophilus* at temperatures above 65°C. A vector carrying transposon Tn917 MLS[1] was constructed and shown to carry out insertional mutagenesis in this strain. The differential regulation of thermophile genes by temperature was examined by two different methods. Promoter fragments of the chromosome of *B. stearothermophilus* were cloned in the shuttle plasmid pMH109 Tc^r (contains a promoter-less cat gene). Transcription of this gene at various temperatures was estimated by growth rate in media containing chloramphenicol and by the detection of cat-specific mRNA. The other method involves the detection of cellular proteins that are synthesized only at 45 or 70°C. We have identified six promoter fragments that exhibit stronger activity at 70°C than at 45°C and two promoter fragments that exhibit stronger activity at 45°C. Twenty unique proteins were detected by two-dimensional PAGE that appeared to be synthesized in cells growing either at 45°C (four proteins) or at 70°C (sixteen proteins). Studies are underway to biochemically characterize these growth-related proteins and to clone and genetically analyze the putative "thermophile" genes.

Ohio State University

Columbus, OH 43210

126. The Molecular Characteristics of the Lignin-forming Peroxidase

L.M. Lagrimini, Department of Horticulture

$60,646

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 will be assayed for peroxidase activity, isoenzyme synthesis, and tissue specific expression. In addition, these plants will be assayed for lignin content and distribution, cell wall morphology, growth and photosynthetic rate, wound-healing capability, and susceptibility. Both sense and antisense plants exhibit dramatic alterations in phenotype. The anionic peroxidase gene and promoter/regulatory sequences will be isolated, sequenced, and joined to the B-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

127. Structure and Regulation of Methanogen Genes

J.N. Reeve, Department of Microbiology

$83,500

The goals of this project are to determine the structure and mechanisms of regulation of expression of genes in the methane producing archaebacteria known as methanogens. Several genes encoding enzymes involved in amino acid and purine biosyntheses (hisA, hisI, argG, proC, purE) or encoding enzymes involved in methane biosynthesis (mcrBDCGA; methyl coenzyme M reductase; mvhDGAB, methyl viologen reducing hydrogenase) have now been cloned and sequenced from a range of very different methanogens. Promoters have been identified by correlating results obtained with RNA polymerase footprinting, S1-nuclease protection and primer extension procedures. Comparison of the mvhDGAB sequence obtained from *Methanobacterium thermoautotrophicum* strains ΔH and Winter with sequences of eubacterial hydrogenases has demonstrated a common ancestry for these archaebacterial hydrogenases and many eubacterial hydrogenases. Highly conserved cysteinyl residues have been identified which are likely to be essential for hydrogenase activity. The mvhB gene appears to encode a novel type of electron transport protein which we have termed a polyferredoxin. The product of the mvhB gene is predicted to contain six tandomly-repeated bacterial ferredoxin-domains. Each molecule could contain as many as 48 Fe atoms and may be capable of transferring or storing 12 electrons. Its potential role as an electron conduit in methanogenesis is being investigated. Methyl reductase and hydrogenases constitute large percentages of the total cellular protein of methanogens and therefore the mcr and mvh operons which encode these enzymes are likely to be transcribed from very strong promoters. These promoters have been subcloned and are being used to construct plasmids which should confer antibiotic resistance when introduced into methanogens. Development of a transformation system for methanogens remains an important feature of this project.

Oklahoma State University

Stillwater, OK 74078

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

A. Mort, Department of Biochemistry

$87,500

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. We have succeeded in obtaining fairly small (2-8 repeat units) fragments of the backbone of RGI by HF solvolysis at -40°. From these we will determine the distribution of acetate esters and perhaps of galactose sidechains. We have also isolated sections of homogalacturonans which retain their methyl esterification. Using commercial pectins from citrus and apple we have succeeded in converting all of the esterified acids to galactose. We are perfecting methods to cleave the resulting mixed galactan/galacturonan at the acid residues (with Li + ethylenediamine) or at the galactosyl residues (with HF). From the fragments so produced, we will be able to determine the local distribution of methyl esters in pectins. By treatment of walls with endopolygalacturonase followed by strong alkali, we can extract all of the RGI like material and xyloglucan from cotton walls. We will use the Li + ethylenediamine reaction to isolate the sidechains of RGI. These will then be characterized by NMR and mass spectroscopy.

University of Oklahoma

Norman, OK 73019

129. Metabolism and Bioenergetics of Syntrophomonas wolfei

M.J. McInerney, Department of Botany and Microbiology

$70,000

Anaerobic, hydrogen-producing syntrophic bacteria degrade fatty acids which are important intermediates in anaerobic degradation and methanogenesis. The physiology of one of these bacteria, *Syntrophomonas wolfei* was studied. *S. wolfei* grew in pure culture in a defined mineral medium with crotonate, lipoic acid, biotin, para-aminobenzoic acid, and cyanocobalamin. Pure cultures of *S. wolfei* used trans-2-pentenoate, tran-2-hexenoate, trans-3-hexenoate and 2,4-hexadienoate. The latter compound was fermented to acetate, propionate and butyrate. The formation of propionate suggested that another pathway in addition to β-oxidation occurs. *S. wolfei* contained formate dehydrogenase activity. However, its specific activity was 1000-fold lower than hydrogenase activity. The production of formate from radioactive bicarbonate by whole cells or crude extracts of *S. wolfei* could not be detected. These studies showed that *S. wolfei* has the enzymatic ability to metabolize formate, but at a slow rate. All the reducing equivalents generated in the oxidation of butyryl-coenzyme A and 3-hydroxybutyryl-coenzyme A by crude extracts of *S. wolfei* were detected as hydrogen.

Oregon Graduate Center

Beaverton, OR 97006-1999

130. Expansion of Bioconversion Technology at the Oregon Graduate Center

M.H. Gold, Department of Chemical & Biological Sciences

$600,000

This expansion will establish a multidisciplinary program for bioconversion research at OGC. During the initial period three 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 laccases 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 and genetic studies on laccases at OGC.

2. Molecular biology of plant cell wall proteins D.R. Corbin

We are investigating the molecular biology of plant cell wall structural proteins in several plant species. We have identified hydroxyproline-rich glycoprotein (HRGP) sequences in several woody plant species, in-

cluding alder and poplar. Our emphasis is on the isolation of HRGP genes, elucidation of their structures via gene sequencing, and subsequent analysis of gene expression during development and lignification. We are also studying the structure and expression of HRGPs in leguminous plants with respect to their potential role as barriers to infection of roots by symbiotic nitrogen-fixing bacteria.

3. Oxidative enzymes involved in cellulose degradation V. Renganathan

Phanerochaete chrysosporium produces three enzymes capable of oxidizing cellobiose. These include a heme- and flavin-containing cellobiose oxidase and two flavin-containing cellobiose dehydrogenase: quinone oxidoreductases. The object of this project is to characterize the mechanisms of these enzymes and their roles in cellulose degradation. During the past year, culture conditions and protein purification methods for the preparation of these enzymes have been developed. All three proteins bind tightly to cellulose and the significance of this interaction is also under investigation.

Oregon Graduate Center

Beaverton, OR 97006-1999

131. Biochemical Genetics of Lignin Degradation by Phanerochaete chrysosporium
M.H. Gold, Department of Chemical and Biological Sciences

$100,000

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 lignin degradation on the molecular biological and biochemical levels.

During the past year we have isolated and characterized cDNA and genomic clones for both LiP and MnP. We also reported on the first DNA transformation system for *P. chrysosporium*. This system utilizes an adenine auxotrophic strain isolated in our laboratory and the corresponding biosynthetic gene from a related fungus. Using this transformation system, we are studying the regulation of the genes and function of the enzymes involved in lignin degradation. We have also continued our spectroscopic and transient-state kinetic studies on the native enzymes and their oxidized intermediates, in order to characterize the structure and catalytic cycle of these peroxidases. Finally, we are studying the pathways by which these peroxidases degrade lignin and lignin model compounds.

Oregon Graduate Center

Beaverton, OR 97006-1999

132. Chemistry of Ultrathin Membranes and Films
J.K. Hurst Department of Chemical and Biological Sciences

$500,000

This award represents second-year support for a program which has the overall objective of establishing a focused research group to study the molecular forces controlling supramolecular assembly and dynamics of ultrathin organic membranes and films. In the first year, syntheses of novel materials patterned after archaebacterial models were initiated. Archaebacteria were chosen because their membranes: (i) contain unique chemical bonds that render them exceptionally durable, hence, attractive to technological applications; and (ii) possess unique structural elements which control their organizational and dynamic properties in as yet ill-understood ways. Synthetic strategies have been developed that are modular in concept, allowing rational and systematic exploration of relationships between molecular structure and film/membrane properties.

A second area of activity that will soon be initiated involves theoretical modeling by stochastic methods of dynamic processes including lateral diffusion and aggregation on two-dimensional surfaces, passive transport, transmembrane oxidation-reduction, domain formation and phase separation. These activities will be complemented by experimental work involved with: (i) mechanisms of transverse diffusion and charge separation across bilayer membranes, currently in progress; and (ii) other physical methods, e.g., magnetic or microscopic imaging techniques, for determining the ultrastructural organization of dopant molecules, including lateral phase separation. A faculty addition is planned to provide expertise in this area.

An additional faculty appointment is planned in the coming year in research areas dealing with interactions of biologically active materials with synthetic films and membranes and/or nonbiological surfaces.

Oregon State University

Corvallis, OR 97331

133. Analysis of Potyvirus Proteolytic Processing: A Basis for Pathogen Derived Resistance

W.G. Dougherty, Department of Microbiology

$142,000 (FY89 funds/two years)

Potyviruses express their genetic information from a genome-length RNA as a single polyprotein which is co- and post-translationally processed by viral encoded proteinases. One of the viral-encoded proteinases recognizes a consensus sequence. This 49kDa proteinase cleaves the polyprotein at five locations and is responsible for the production of six virus proteins. Biochemical inhibitor studies and a molecular genetic analysis of the proteinase gene suggests that this proteinase has characteristics of a trypsin-like serine proteinase with an active site triad comprised of a histidine, aspartic acid and cysteine residues. Cysteine has replaced the nucleophilic serine residue. The 49kDa proteinase is unique as it recognizes a consensus sequence which spans seven amino acids. Four of the amino acids are conserved between all natural cleavage sites and three of the positions are variable. The conserved positions are essential to define a functional cleavage site. This has been demonstrated by cell-free processing reactions of precursors with cleavage sites altered by site-directed mutagenesis. A number of different amino acid residues may occupy the variable positions and may affect proteinase-substrate interaction and hence the rate of cleavage. A proteinase with such a high degree of specificity is a logical target in the design of new resistance schemes.

Oregon State University

Corvallis, OR 97331

134. Genomic Variation in Maize

C. Rivin, Department of Botany and Plant Pathology

$60,000

Our longterm 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

135. The Role of Turgor Pressure and Solute Uptake in Plant Cell Growth

D.G. Cosgrove, Department of Biology

$86,000

Plant cell expansion involves simultaneous water absorption, solute uptake, and wall relaxation and synthesis. Our studies have shown that water absorption by the growing stems of pea (*Pisum sativum L.*) and other young seedlings is fast and is regulated primarily by wall yielding, which generates the lower water potential necessary for water absorption. Our current focus is on the nature of wall yielding, its dependence on cell turgor pressure, and the mechanism by which cell turgor is maintained in expanding cells. We have developed new relaxation methods to study wall yielding. With the pressure-probe technique we find that wall yielding in excised growing tissues is proportional to cell turgor pressure and exhibits a definite yield threshold. With the pressure-block method we can measure relaxation in intact growing tissues and find that such tissue show more complicated growth and relaxation responses than excised ones. Rapid feedback-regulation of growth is apparent from the pressure-block experiments. With these methods we have examined how drought-stress, light and growth retardants modulate stem elongation. The last two agents act exclusively on cell wall yielding properties. With droughted pea plants, inhibition is partly through reduced cell turgor pressure and partly through reduced wall relaxation. Drought appears to alter feedback-regulation of cell expansion. Experiments are currently underway to characterize such regulation. In

a related line of study we find close correspondence between rates of solute uptake from the phloem and rates of cell expansion. Work is underway to determine the nature of such coupling.

Pennsylvania State University

University Park, PA 16802

136. Enzymology and Molecular Biology of Lignin Degradation

M. Tien, Department of Molecular & Cell Biology

$80,000

Lignin is an aromatic polymer which accounts for a large percentage of the energy the earth captures from the sun. Approximately 25% of the carbons fixed by photosynthesis are transformed into lignin. The research focuses on degradation of lignin by the filamentous fungus *Phanerochaete chrysosporium*. We have isolated and characterized the enzymes which catalyze this degradation, in particular the lignin and Mn-dependent peroxidases. These are hemeproteins which are secreted by the fungus and utilize hydrogen peroxide as the oxidant. Our research is focused on the mechanism of these enzymes and how they are regulated by the fungus. Our methodology includes the use of electrochemistry and nmr spectroscopy to characterize the enzyme active site; kinetics using mechanism-based inhibitors to characterize the binding site for substrates; and transient-state kinetics to understand the reactivity of the heme. We have also isolated the genes which encode these enzymes. Utilizing the modern tools of DNA manipulation, we are studying how these genes are regulated. We are also attempting to express the lignin peroxidase in other organisms to aide our biochemical studies.

University of Pennsylvania

Philadelphia, PA 19104

137. DNA Sequences Encoding Chlorophyll a/b Binding Polypeptides

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

$113,000

We have been studying regulatory elements within the promoter of genes encoding polypeptide components of the light-harvesting chlorophyll a/b (CAB) protein complex. We have characterized a light regulatory element (LRE) within the promoter of the *Nicotiana plumbaginifolia* cab-E gene. We also characterized both a positive (PRE) and negative (NRE) regulatory element. We have now demonstrated that both the PRE and NRE elements, in addition to the LRE elements, mediate light-regulated expression in dark-adapted plants when the elements are fused to the constitutively expressed CaMV 35S promoter. Furthermore, truncated cab-E promoters when fused to CaMV 35S enhancer elements also promote light-regulated expression, in dark-adapted plants. We propose to re-examine these various constructs for light-regulated expression in dark-grown seedlings.

Purdue University

West Lafayette, IN 47907

138. Biosynthesis and Assembly of Cell Wall Polysaccharides in Cereal Grasses

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

$80,700

We are investigating the biosynthesis of mixed-linkage β-D-glucan and glucuronoarabinoxylans which make up the hemicellulosic matrix of the primary cell walls of maize and other cereal grasses. The Golgi apparatus was enriched from plasma membrane and other organelles by flotation density gradient centrifugation. Glucan synthase I and II, which are established markers for Golgi and plasma membrane, respectively, displayed considerable overlap in conventional separations with sucrose density gradients. Flotation gradients improved separation of the membranes substantially, but the different synthases themselves also incorporated radioactivity from either 10 μM or 1 mM UDP-[^{14}C]-glucose into polymer. Relative incorporation of radioactivity into polymers from UDP-[^{14}C]-xylose by the various membrane fractions was nearly identical to relative IDPase activities, indicating that combined xylosyl transferase-xylan synthase represents a new, unequivocal marker for the Golgi apparatus. We also have developed techniques of gas-liquid chromatography and radiogas proportional counting to achieve capillary quality separation of partially methylated alditol acetates with simultaneous determination of radioactivity in the derivatives. We determined that both terminal and 4-linked xylosyl units from UDP-[^{14}C]-xylose and 3- and 4-linked glucosyl units from UDP-[^{14}C]-glucose were formed with Golgi membranes, whereas the plasma membranes made mostly 3-linked glucosyl units. We now are using digestion of polymeric products by specific endo-glycanohydrolases to diagnostic oligosaccharides that reveal specific kinds of polysaccharides synthesized by the

Golgi membranes. We will then verify the linkage structure of these diagnostic oligomers by separation of partially methylated alditol acetates and detection of radioactivity by radiogas proportional counting. Efforts in the coming year are directed toward determining the factors required for branching of the xylan with arabinose and formation of the cellobiosyl- and cellotriosyl-(1→3)β-D-glucose units of the *bona fide* mixed-linkage β-D-glucan.

Purdue University

West Lafayette, IN 47907

139. Ca^{++} Gated Proton Fluxes in Energy Transducing Membranes

R.A. Dilley, Department of Biological Sciences

$85,000

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. The molecular mechanisms for H^+ ion movement in and through membranes are not understood, and represent an important 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. When calcium ions are tightly bound, H^+ fluxes and the H^+ gradient stay within localized domains, but when the calcium ions are displaced, the H^+ ions flow out of the postulated gate 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

140. 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 the regulation of genes that code for photosynthetic membrane proteins and the precise structure/function relationship of one of these genes. We will concentrate on photosystem II in the transformable cyanobacteria *Synechococcus* strain PCC7942 and *Synechocystis* strain PCC6803. We will analyze genes that we have already determined are regulated either by iron or by light. We have identified at least one operon that is regulated by an "iron-box" that is similar to an *E. coli* DNA sequence that is under iron-control. We will try to determine if a related sequence is involved in light-regulated gene transcription. Our ultimate goal will be to determine the mechanism by which photosynthetic organisms regulate gene expression under a variety of environmental conditions. We will pay particular attention to an analysis of the *wox*A gene which codes for MSP the manganese stabilizing protein involved in PSII O_2-evolution. We have cloned this gene and will perform random and site-directed mutagenesis to determine if specific residues involved in Ca^+ or Cl binding-sites can be identified and to determine how these mutations affect O_2-evolution. These objectives will be approached by using a combination of biochemical, genetic, and molecular biological techniques. This project is aimed at a much finer understanding of gene regulation in photosynthetic organisms. In addition, we hope to better define how Ca and Cl function in photosynthetic O_2-evolution.

The Rockefeller University

New York, NY 10021-6399

141. Asparagine Synthetase Gene Family: Differential Expression During Plant Development

G. Coruzzi, Lab of Plant Molecular Biology

$85,000

We propose to characterize the structure and expression of the family of genes for the N-metabolic enzyme asparagine synthetase (AS), and to begin to determine the molecular mechanisms involved in the regulated expression of these genes during plant

development. Using a molecular cloning approach, we will answer the following questions concerning this important, but poorly characterized enzyme: 1. What are the number and sizes of the AS subunit polypeptides? 2. What are the subcellular locations of the AS polypeptides (chloroplast, mitochondria, cytosol)? 3. What is the organ distribution of the AS polypeptides? 4. How are the genes for the AS related in structure? 5. Are different AS genes expressed differentially during plant development? 6. How do metabolites, hormones, or light influence the expression of AS genes?

We have recently identified two AS mRNA species which accumulate differentially during development, in a fashion which parallels biochemical studies on the AS enzyme. AS1 mRNA accumulates to high levels in leaves of dark-grown plants in a "photophobic" fashion, while AS2 mRNA accumulates to high levels in cotyledons during germination. We will determine how phytochrome, metabolites, or hormones affect AS1 & AS2 mRNA levels, and whether these changes reflect differential transcription. A comparative analysis of AS gene expression to our ongoing studies of GS gene family will enable us to begin to elucidate the molecular basis for coordinate or differential regulation of genes along a N-metabolic pathway in plants.

Rutgers University

New Brunswick, NJ 08903-0231

142. Cellulase - A Key Enzyme for Fermentation Feedstocks

D.E. Eveleigh and J.D. Macmillan, Department of Biochemistry and Microbiology

$119,776 (FY88 funds/ 20 and 1/2 months)

Biomass can be fermented to a range of useful products. For efficiency a well characterized and effective cellulase is necessary to gain fermentable sugars from cellulosics (forest by-products, agricultural wastes and municipal refuse). We have selected a thermally stable cellulase system from the actinomycete *Microbispora bispora* for development. The system of enzymes has been characterized using cellulases from the native strain in comparison to those produced via heterologous expression in *E. coli*. Secretion/expression of the *M. bispora* endoglucanase (mb celA gene) in *E. coli* initially resulted in low enzyme yields. Attempts to gain greater expression were made by placing the gene under control of the lac po. In a further construction the mb celA gene was placed under control of the 1ppplacpo promoter and in frame with the *E. coli* outer membrane (Omp A) signal peptide in order to facilitate both over expression and secretion. Both constructions resulted in enhanced yields and with 50% secretion. The other two cellulase components, cellobiohydrolase and beta-glucosidase, have been characterized from the native strain. These enzymes have also been expressed in *E. coli*, though so far lower with specific activities. These studies have clarified perspectives on actinomycete cellulases, their regulation, and insights on how these enzymes can be applied to gain optimal hydrolysis of cellulose.

Rutgers University

Piscataway, NJ 08855-0759

143. Corn Storage Protein: A Molecular Genetic Model

J. Messing, Waksman Institute

$100,000

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 family in contrast to the multigenic zein family that we have studies previously. We also found that a trans-acting factor that is absent in most inbreds can boost the 10kD mRNA levels resulting in higher accumulation of 10 kD protein in mature seed. It is interesting that, by overexpression of a single gene, methionine levels in the mature corn kernel can be significantly increased. We are now studying the transacting mechanism of this overexpression.

Salk Institute for Biological Studies

San Diego, CA 92138-9216

144. Genetic Analysis of Photoreceptor Action Pathways in Arabidopsis thaliana

J. Chory, Plant Biology Laboratory

$85,000

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 promoter fused to both screenable and selectable markers into *Arabidopsis* using *Agrobacterium tumefaciens* transformation. We are currently characterizing the transgenic plants for the structure of the inserted sequence and the correct expression of the promoter with respect to light and tissue specificity. Seedlings from a 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.

Salk Institute for Biological Studies

San Diego, CA 92138-9216

145. Biosynthesis of Plant Plasmamembrane Polypeptides

C.J. Lamb, Plant Biology Laboratory

$79,000

The overall objective of this research is the study of the biogenesis of plant plasmamembranes with an initial emphasis on the biosynthesis of specific plasmamembrane proteins particularly in relation to the molecular mechanisms involved in protein trafficking through the endomembrane system to the plasmamembrane. To this end we have generated a panel of monoclonal antibodies to a set of epitopes on plasmamembrane (glyco) proteins, which provide rigorous markers for fractionation of the plasmamembrane *in vitro* and allow immunoaffinity purification and biochemical characterization of specific plasmamembrane (glyco) proteins. In particular MCA 16.4B4 is reactive with a glycan epitope present on a family of plasmamembrane glycoproteins M_r 130-185 kDa elaborated from a single polypeptide species M_r 50 kDa (designated p50) which is rich in serine and threonine. Polyclonal antisera and MCA to peptide epitopes present in plasmamembrane polypeptide p50 have been generated by immunization with the corresponding mature glycoproteins purified to homogeneity by immunoaffinity chromatography on MCA 16.4B4-Sepharose columns. Differential partitioning between aqueous and Triton x 100 phases indicates that this glycoprotein family partitions into the aqueous phase and hence is unlikely to be an integral plasmamembrane protein, but rather an extrinsic protein located on the external face of the plasmamembrane. Therefore, in parallel we have started to examine the sucrose transporter protein, which is likely to be an intrinsic plasmamembrane protein. The sucrose transporter was identified by sucrose protection from inactivation by ^{3}H-N-ethylmaleimide and antiserum to the corresponding 42 kDa polypeptide was shown to specifically block the uptake of sucrose but not other sugars into isolated protoplasts.

We are now in the process of identifying molecular clones encoding polypeptide p50 sequences and sucrose transporter sequences by screening cDNA libraries cloned in λgt11 with appropriate antibodies and by protein microsequencing and synthesis of oligonucleotide probes. These molecular clones and extant antibodies are being used to study the biosynthesis and processing of the family of plasmamembrane glycoproteins based on polypeptide p50 as an ex-

ample of an extrinsic plasmamembrane glycoprotein and the sucrose transporter protein as an example of an intrinsic plasmamembrane protein. Specific emphasis is placed on analysis of the molecular basis for transport, post-translational modification and sortive trafficking through the endomembrane system to the plant plasmamembrane.

Smithsonian Institution
Washington, D.C. 20560

146. A Primary Light Harvesting System: The Relationship of Phycobilisomes to Photosystem I and II
E. Gantt, Botany Department, University of Maryland, College Park

$74,000

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 labeling. The stoichiometry of reaction centers and the antennae is being determined by photochemical techniques. Results to date indicate that the stoichiometry of PS I and PS II is little affected over a photon flux range (white light) of 5 to 280 microeinsteins per meter square per second in *Porphyridium cruentum.* Major effects however occur in the phycobiliprotein antennae. High photon flux densities can result in a reduction of phycobilisome size, or a reduction of phycobilisomes per thylakoid area depending on the growth conditions.

Solar Energy Research Institute
Golden, CO 80401

147. The Water-Splitting Apparatus of Photosynthesis
M. Seibert, Photoconversion Research Branch

$125,000

The light reactions of photosystem II (PSII) in series with the dark reactions of the water-oxidation process supply the reductant ultimately used to fix carbon during oxygenic photosynthesis. The objective of this project is to elucidate structural and functional relationships between the PSII reaction center (RC) and O_2-evolving complexes. Procedures that stabilize the isolated PSII RC (D1/D2/cytochrome b-559/4.8 kD proteins) complex at 20°C in the light were refined, and femtosecond time domain flash studies were performed with this material. The primary charge separation act (P680-Pheo $\xrightarrow{h\nu}$ $P680^{+}$–$Pheo^{-}$) occurs with a $\tau = 3.0 \pm 0.6$ ps at 4°C and 1.4 ± 0.2 ps at 15 K, and no spectral or kinetic evidence for an acceptor prior to Pheo was obtained. The results are similar to those reported for the bacterial RC. On the other hand, absorption, fluorescence, and CD studies using stabilized material suggest that the primary donor of PSII may not be a bacterial-type special pair. Biochemical approaches examining the donor side of PSII have identified high-affinity Mn-binding sites localized on the isolated PSII RC complex although photoactivation of the complex could not be demonstrated. Comparative analyses of *Scenedesmus* WT and Mn-deficient LF-1 mutant PSII membranes have identified two types of high-affinity Mn-binding sites, one associated with histidine residues on the D1 protein and the other involving carboxyl residues on RC proteins. Both types of sites are composed of two components sufficient for binding a total of four Mn. At least one of the histidine sites may be located near the carboxyl end of the D1 protein. This work indicates that although sites for binding all functional Mn may exist on RC proteins, other PSII core proteins and/or cofactors are required for successful O_2-evolution function.

Southern Illinois University
Carbondale, IL 62901

148. Regulation of Alcohol Fermentation by Escherichia coli
D.P. Clark, Department of Microbiology

$73,997

The purpose of this project is to elucidate the way in which the fermentative synthesis of ethanol is regulated in the facultative anaerobe *Escherichia coli.* We are also investigating the control of other genes required for fermentation and 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. In conse-

quence 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

149. Host Range and Other Symbiotic Genes of Rhizobium meliloti

S.R. Long, Department of Biological Sciences

$222,933 (FY88 funds/two years)

The intense use of petrochemicals for fuel in fertilizer production is one of the major energy costs of agriculture. Plants which can grow in symbiotic association with nitrogen fixing bacteria are able to flourish without input of nitrogen fertilizer, which makes possible high protein plants, and allows some such plants to be used as green manure in crop rotation. Why some plants and not others can establish nitrogen fixing symbioses with *Rhizobium* bacteria is not known. We are using genetics and molecular biology to identify the bacterial genes responsible for host range, and we are using biochemistry and cell biology to investigate the mechanism by which common and host-specific nodulation genes have their function in the plant. In the past year, we have identified two new nodulation genes, nodP and nodQ, have established their nucleotide sequence and have determined their protein products using *in vitro* expression systems. We have also achieved the partial purification of the protein products for the host specific gene nodH. Using protein purification techniques and using *in vivo* metabolism labeling, we hope to elucidate the nature of nod gene action on the recipient host plant.

Texas A&M University

College Station, TX 77843-2128

150. 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. The spatial separation of successive phases of chloroplast biogenesis in barley leaves will be used to identify nuclear genes encoding plastid proteins which are expressed early in chloroplast development. The expression of these genes will be characterized using cDNA clones and through analysis of the *cis* and *trans*-acting elements which regulate expression of genomic clones. Finally, the influence of plastid transcription on the expression of nuclear genes such as *rbcS* and *cab* will be examined.

Virginia Polytechnic Institute & State University

Blacksburg, VA 24061

151. Enzymology of Acetone-Butanol-Isopropanol Formation

J.S. Chen, Department of Anaerobic Microbiology

$72,000

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 *Clostridium butylicum*) can produce all three compounds, with some strains producing mainly butanol and isopropanol. Industrial solvent fermentation is limited by several biological factors. Efforts aimed at alleviating these limitations require an understanding of these organisms and their solvent-producing machinery. This project focuses on the elucidation of

the molecular properties of solvent-forming enzymes and mechanisms for regulating the metabolic pathway and the expression of solvent-production genes. We have shown that a primary/secondary alcohol dehydrogenase (ADH) is responsible for butanol/isopropanol formation in *C. beijerinckii* producing both alcohols, whereas a primary ADH is responsible for butanol formation in *C. beijerinckii* producing butanol but not isopropanol. We have determined the N-terminal amino acid sequence of the primary/secondary ADH from *C. beijerinckii*, and the information is used in the cloning of the ADH gene from genomic libraries. The cloned ADH gene will be used in elucidating the transcription and translation properties of solvent-production genes. A *C. beijerinckii* strain which produces butanol only appears to have two primary ADHs which differ in their coenzyme specificities and other molecular properties. We are purifying both ADHs and will compare their properties with the characterized primary/secondary ADH. In solvent production, the conversion of acetoacetyl CoA to acetoacetate is a key reaction. We have been studying all acetoacetyl-reacting enzymes of *C. beijerinckii* to understand this reaction. Our recent results show that phosphotransbutyrylase has a high acetoacetyl CoA-reacting activity. The physiological significance of this activity will be studied together with that of acetoacetyl CoA:acetate/butyrate CoA-transferase and acetoacetyl CoA hydrolase.

Virginia Polytechnic Institute and State University

Blacksburg, VA 24061

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

J.G. Ferry, Department of Anaerobic Microbiology

$87,540

The pathway of carbon flow during acetate conversion to methane in *Methanosarcina thermophila* is now generally understood. Six proteins have been purified and characterized that catalyze the following steps in the pathway. Acetate kinase and phosphotransacetylase catalyze the activation of acetate to acetyl-CoA. The activated acetate binds to a five-subunit complex that contains a dimeric carbon monoxide dehydrogenase and a dimeric corrinoid/iron-sulfur protein. The complex is proposed to cleave the carbon-carbon and carbon-sulfur bonds of acetyl-CoA and bind the resulting carbonyl group to a nickel-iron-carbon center. The methyl group is proposed to bind to the corrinoid/iron-sulfur protein of the complex. A methyltransferase catalyzes the transfer of the bound methyl to coenzyme M. Electrons for the reductive demethylation of methyl-coenzyme M to methane are derived from oxidation of the bound carbonyl to carbon dioxide. The electron acceptor for the carbon monoxide dehydrogenase is a ferredoxin. All of the above mentioned proteins have been characterized and antibodies raised in rabbits. Western blots have shown that the synthesis of acetate activating enzymes are regulated in response to the growth substrate. Putative clones containing the gene encoding phosphotransacetylase were obtained from a lambda gt11 library of *M. thermophila* DNA using a plaque-lift assay.

Virginia Polytechnic Institute and State University

Blacksburg, VA 24061

153. Unravelling Lignin Formation and Structure in Living Plants

N.G. Lewis, Departments of Wood Science, Forest Products and Biochemistry

$84,000

Vascular plants have evolved with a unique capacity to produce lignin, a complex phenylpropanoid polymer which performs essential structural and defense functions. Because of its intractable nature, little is known about lignin bonding patterns *in situ*. We have recently demonstrated that the bonding patterns of this polymer can be observed *in situ* by administering specifically-labeled (C-13 enriched) lignin precursors to *T. aestivum* and *L. leucocephala* plants, respectively. We are now developing this methodology for application to softwood plant tissue, as well as establishing changes that the lignin macromolecule undergoes during delignification.

Mechanisms controlling lignin monomer transport into the cell wall, and subsequent initiation of lignification are not understood. In this study, we are identifying the mechanisms by which a) hydroxycinnamic acids are esterified to cell walls, b) monolignols, or their conjugates, are transferred from the cytoplasm to the cell wall, and c) why only glucosylation of Z-monolignols, and not the corresponding E-isomers, occurs in beech bark.

Washington State University
Pullman, WA 99164

154. Regulation of Terpene Metabolism
R. Croteau, Institute of Biological Chemistry

$84,000

Oils and resins from plants are important renewable resources. Knowledge of the biochemistry of these terpenoid substances is needed to deduce regulatory mechanisms at the enzyme level. The objective of this project is to provide such understanding through the investigation of two models: (1) camphor metabolism in *Salvia officinalis* and (2) menthone metabolism in *Mentha piperita*. The pathways of biosynthesis have been established. As the plant matures, both terpenoids undergo catabolism by a pathway involving conversion to glycoside which is transported to the root/rhizome. Following hydrolysis, the terpenoid undergoes oxidative degradation to acetate which is metabolically recycled into acyl lipids and phytosterols of membranes. Terpene catabolism thus represents a salvage mechanism which provides a carbon source to the developing root/rhizome. The pathways of monoterpene catabolism are being deciphered. During the transition from terpene biosynthesis to catabolism, the epidermal oil glands (primary site of terpene accumulation) undergo dramatic ultrastructural modification. Cytochemical localization of the biosynthetic and catabolic enzymes is being carried out, and the appearance and disappearance of the various activities are being correlated with ultrastructural changes. Based on these studies, research on the influence of foliar applied bioregulators which alter terpene yield, and work with tissue culture systems, a model for the regulation of terpene metabolism is being constructed. Results from this project will have important consequences for the yield and composition of terpenoid oils and resins that can be made available for industrial exploitation.

Washington State University
Pullman, WA 99164-6340

155. D-Erythroascorbic Acid: Its Preparations, Chemistry, and Metabolism (Fungi and Plants)
F.A. Loewus, Institute of Biological Chemistry,

$146,820 (FY88 funds/two years)

D-erythroascorbic acid (EAA) was synthesized in four steps starting from D-glucose. Oxidation of D-glucose with oxygen in alkali followed by esterification gave 65% methyl D-arabinonate, which was oxidized to D-erythro-pent-2-ulosonate (14%) using VO_5 and $NaClO_3$ in methanol. The 2-keto methyl ester was transformed in hot methanolic Na acetate to EAA in 50% yield. Crystalline EAA was identified by elemental analysis, mass spectrometry, and ^1H- and ^{13}C-nmr spectrometry. Bakers' yeast contained 50 µg EAA/g dry yeast when analyzed by HPLC with amperometric detection. In bread-making tests, no EAA was detected in dough during 1-3h fermentation. Added EAA failed to increase the elastic nature of wheat dough as did L-ascorbic acid at 200 ppm based on flour. Conditions were developed for a kinetic study of the peroxidative cleavage of EAA to oxalic acid (OA) in the presence of Na_2O or H_2O_2. In 33 mM H_2O_2 buffered with 90 mM CAPS (pH 10.0) at 30°C, 3.4 mM EAA was cleaved to produce a stoichiometric amount of OA in 3h. OA was assayed by HPLC on a Waters IC-PAC anion column with ion conductivity detection. An unidentified intermediate, R_t relative to OA of 1.19, which accompanied OA production, slowly decayed as OA accumulated. Mycelial cultures of one edible mushroom, *Pleurotus sajor-caju*, and two phytopathogens, *Whetzelinia sclerotiorum* and *Sclerotium cepivorum*, have been established and these oxalate-producing fungi are currently being examined for their EAA-producing capacity with the further objective of testing EAA biosynthesis as a regulatory process leading to OA formation during invasive growth on the host.

*(Project involves collaboration with Dr. P.A. Seib, Department of Grain Science and Industry, Kansas State University.)

Washington State University
Pullman, WA 99164-4660

156. Isocitrate Lyase and the Glyoxylate Cycle
B.A. McFadden, Biochemistry/Biophysics Program

$72,000

Our objectives are to shed light upon the structure, regulation and catalytic function of isocitrate lyase, 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 isocitrate lyase gene of *Escherichia coli* [*J. Bacteriol.*, *170*, 4528 (1988)]. In this publication, the deduced amino acid sequence of the subunit

of isocitrate lyase from *E. coli* and castor bean were compared. We have also described considerably improved purifications of isocitrate lyase from *E. coli* and watermelon cotyledons [*Prep. Biochem.*, *18*, 431 (1988)]. In the remainder of the project period, the amino acid sequences of up to five active-site peptides from the *E. coli* enzyme will be elucidated. Mutagenesis of the *E. coli* gene will be directed towards functional residues that are conserved in the castor bean enzyme to test our postulated catalytic mechanism.

These studies will provide basic information about isocitrate lyase. 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

157. Enhancement of Photoassimilate Utilization by Manipulation of the ADPglucose Pyrophosphorylase Gene

T.W. Okita, Institute of Biological Chemistry

$64,000

Starch biosynthesis is regulated in large part by the gene activation and expression of ADPglucose pyrophosphorylase, as well as the allosteric control of ADPglucose pyrophosphorylase enzyme activity by 3-phosphoglycerate and Pi. Our research has focused on the pyrophosphorylase genes expressed specifically in the storage organs of rice endosperm and potato tubers. Recombinant DNA clones have been isolated for both the mRNA transcript and genomic sequences of rice and their DNA structures have been determined. The pyrophosphorylase subunit is initially synthesized as a larger precursor containing a putative transit sequence for amyloplast localization. No obvious homology is evident between the transit peptides of pyrophosphorylase and other nuclear encoded plastid localized proteins. Analysis of the genomic clones revealed that the pyrophosphorylase gene is a complex structure spanning 5500 bp with 9 introns varying in lengths from 84 bp to over 2400 bp. A partial cDNA clone has been isolated from a potato tuber cDNA library. Northern blot hybridization analysis indicates that the stolon and tuber pyrophosphorylase is coded by two transcripts with molecular lengths of 3.4 and 2.3 kb. Current efforts are being directed at a more definitive analysis of the subunits comprising the tuber ADPglucose pyrophosphorylase enzyme, isolation of full length cDNA clones for the stolon and tuber specific transcripts, and isolation of their respective genes. These studies will lead to the identification of cis-acting regulatory elements responsible for the expression of this gene during somatic tissue differentiation. *In vivo* starch biosynthetic studies have been conducted with potato tuber discs labeled with ^{14}C-sucrose. The results indicate that starch synthesis can be increased when the tuber discs are incubated in the presence of 10mM mannose; an inorganic phosphate sequestering agent. Overall, these studies will allow us to improve the catalytic conversion of photoassimilates into starch due to increased gene expression and/or molecular alteration of the allosteric site of ADPglucose pyrophosphorylase.

Washington University

St. Louis, MO 63130

158. Genetic Engineering with a Gene Encoding a Soybean Storage Protein

R.N. Beachy, Department of Biology

$78,000

Our work with genes encoding the α' and β-subunits of β-conglycinin demonstrated that expression of each gene is regulated in transgenic tobacco and petunia plants in like manner as in soybean. We have examined and compared the DNA sequences flanking the α' and β-subunit genes for their capacity to bind proteins that may be involved in regulating expression of these genes. Four protein binding activities were found and termed Soybean Embryo Factor (SEF) 1, 2, 3 and 4. SEF 3 binds almost exclusively to the DNA sequence previously identified as a transcriptional enhancer associated with the α'-gene. A series of *in vitro* derived mutants of this sequence are being tested for their functionality *in vivo* by using a transgenic plant assay. SEF 1, 2, and 4 to bind to DNA sequences 5' to both the α' and β-genes; DNA sequences that bind SEF1 and 4 have been characterized by footprinting techniques. In addition, DNA fragments that bind SEF 1, 2 and 4 were used to construct chimeric promoters on the β-glucuronidase gene and will be tested for function in transgenic plants. Experiments are in progress to further characterize the proteins that bind the DNA sequences so that additional studies to understand the nature of regulation of the β-conglycinin gene family can be carried out.

Washington University
St. Louis, MO 63110

159. Processing and Targeting of the Thiol Protease, Aleurain
J.C. Rogers, Division of Hematology-Oncology

$83,000

We have identified a cDNA clone from barley aleurone mRNA that encodes a protein with unusual homologies: the C-terminal portion, about 270 amino acids, is 65% identical to the mammalian 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. The N-terminal 1/3 of the protein, about 140 amino acids, has no detectable homologies to other known protein sequences; its function is unknown. In aleurone tissue, the mRNA level is increased by gibberellic acid and decreased by abscisic acid, but is expressed apparently constitutively at high levels in leaf and root tissues. The amino acid sequence and cathepsin H homology suggest that the protein will be both secreted into the endoplasmic reticulum and glycosylated. Using our cDNA clone in a bacterial expression system, we have made a fusion protein containing the protease domain of aleurain, and have used it to raise specific antisera in rabbits. These antibodies identify a 32 kd protein in extracts of aleurone layers that is induced with GA treatment but not secreted; a similarly sized protein is specifically identified in extracts of leaf tissue. Experiments are underway to characterize the pattern of expression in different tissues, to identify the subcellular locations of the protein, to characterize processing of the precursor to the 32 kd mature form, and to purify the enzyme from barley.

Washington University
St. Louis, MO 63130

160. Hydroxyproline-rich Glycoproteins of the Plant Cell Wall
J.E. Varner, Biology Department

$91,000

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 printing a freshly cut surface of the tissue at different developmental stages on nitrocellulose paper and probing the paper with antibodies against extensin. This technique has shown that 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 are using differential scanning calorimetry to examine the properties of isolated plant cell walls. Walls isolated from the growing region of soybean hypocotyls undergo a phase transition—a change in specific heat—with a midpoint at 53°. 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. Current work is centered on the possibility of studying pectin-extensin interactions *in muro* by calorimetry.

University of Washington
Seattle, WA 98195

161. Studies on the Control of Plant Cell Enlargement by Cellular Parameters
R.E. Cleland, Department of Botany

$40,000

This project is directed towards an understanding of how plant cell enlargement is controlled and regulated at the cellular level, emphasizing the mode of action of the hormone auxin. We have shown that in coleoptile and stem tissues, auxin induces cell enlargement, in part, by causing cells to excrete protons. The resulting decreased wall pH then leads to a loosening of the wall. In oat coleoptile cells, the wall loosening is apparently enzymatic, and does not involve wall-bound calcium. Studies during the past year on the mechanism of wall loosening in dicot stem cell walls has shown that the situation is quite different in this tissue. The wall extensibility is altered by either the addition or removal of wall calcium. We have proposed that part of the wall loosening is due to solubilization of wall calcium by the auxin-induced wall acidity, and have obtained preliminary evidence to support this idea. In addition, protons induce an enzymatic effect. This is indicated by the fact that long-term *in vitro* tension chances at pH 4.5, but not if the proteins are removed by trypsin. Thus the mechanism of acid-induced wall loosening in dicot walls is fundamentally

different from that of coleoptile walls. Studies are underway to complete this study on the mechanism of dicot stem cell wall loosening.

University of Wisconsin
Madison, WI 53706

162. Enzymology of Biological Nitrogen Fixation
R.H. Burris, Department of Biochemistry

$99,887 (two years)

Dinitrogenase from a nifV mutant of *Klebsiella pneumoniae* contains an altered form of FeMoco that lacks a biologically active homocitric acid molecule. Change in the composition of FeMoco leads to substantial change in the kinetics of nitrogenase action. The Km for N_2 is higher and the kM for N_2O is lower than for the wild type enzyme. Observation that different numbers of electrons are stored in CO-inhibited as compared to non-inhibited dinitrogenase before H_2 is released suggests that the mutant enzyme has more sites responsible for H_2 evolution than the wild type enzyme. Dinitrogenase reductase activating glycohydrolase activity is present in extracts both of *Azospirillium brasilense* and *A. lipoferum*; it is membrane associated. A post-translational regulatory system for nitrogenase activity is present in *A. brasilense* and *A. lipoferum*, and it operates via ADP-ribosylation of dinitrogenase reductase. The effect of oxygen, ammonium ion and amino acids on nitrogenase of *Herbaspirillum seropedicae* was compared with *Azospirillum* spp. and *Rhodospirillum rubrum*. Ammonium reversibly inhibited nitrogenase activity when added to a derepressed cell culture, but the inhibition was only partial. There is no clear evidence that ADP ribosylation of the dinitrogenase reductase is involved in the ammonium-inhibition of *H. seropedicae*.

University of Wisconsin
Madison, WI 53706

163. Molecular Genetics of Ligninase Expression
D. Cullen and T.K. Kirk, 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 lignin peroxidases (LiP's) isozymes are present, and production is derepressed under carbon, nitrogen, or sulfur limitation. Our objectives are to: 1. elucidate the organization/regulation of the genes encoding LiP's of *P. chrysosporium*, and 2. investigate the expression of LiP genes in *Aspergillus nidulans*. Toward these goals, we have cloned and sequenced three closely related LiP genes. Because of the focus on gene regulation, we have concentrated on genomic clones and substantial 5' untranslated distances are being sequenced. Linkage relationships and transcriptional regulation of these *P. chrysosporium* genes are under investigation. With respect to *A. nidulans* expression, the LiP gene encoding isozyme H8 has been translationally fused to the bacterial B-galactosidase (lacZ) gene and the resulting fusion protein detected in *A. nidulans* transformants. Such fusions provide a powerful and convenient tool for studying promoter structure/regulation in *A. nidulans*, a model expression system. In addition to yielding fundamental information concerning gene regulation in lower eukaryotes, these studies provide the necessary groundwork for expression in commercially relevant fungi, e.g. *A. niger*. The long-term goal, production of highly purified recombinant peroxidases, may aid in the development of processes such as biological bleaching of pulps, effluent treatment, and in biopulping.

University of Wisconsin
Madison, WI 53706

164. Role of Transit Peptides in the Localization of Nuclear-encoded Chloroplast 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 stromal space and the thylakoid lumen respectively. Genes for precursor proteins destined for the chloroplast envelope membranes are being isolated and 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

165. Organization of the R Chromosome Region in Maize

J.L. Kermicle, Laboratory of Genetics

$122,000 (FY88 funds/two years)

The maize R gene governs the presence, distribution and intensity of anthocyanin pigmentation, plant part by plant part. Genetic analysis reveals the number, kind and arrangement of the components involved, providing a framework for molecular-level investigations. Forty Ds insertion mutations were used to define the functional limits of an allele that confers strong seed color. We now are focusing on the segment that distinguishes this allele from one that specifies only plant color. Separate attention is being given to the pattern of recombination occurring when duplications and insertions are present.

University of Wisconsin

Madison, WI 53706

166. The Role of Proteolytic Enzymes in Degradation of Plant Tissue

J. Lewosz, A. Kelman, and L. Sequeira, Department of Plant Pathology

$56,000

Free hydroxyproline was not released from potato cell walls by partially purified proteolytic enzymes of *Erwinia carotovora* subsp. *carotovora* (Ecc) obtained from various media and from infected rotting tubers. These proteases did not degrade potato lectins or hydroxyproline-rich proteins isolated from carrots, even when these proteins were denatured by boiling or treated with reducing compounds.

The Ecc proteases did not degrade other proteins present in potato tuber extracts or proteins ionically bound to cell walls. Furthermore, deglycosylation of cell walls by boiling them at pH 1.0 did not promote solubilization of cell wall proteins by protease-containing filtrates of Ecc. Treatment with Ecc proteases did not increase electrolyte release from potato tuber slices and did not affect vital staining with neutral red. Also, neither cell wall degradation nor electrolyte release by PL was accelerated in presence of protease. However, lectin-like, hydroxyproline-containing proteins extracted from cell walls with 5 M ammonia were degraded when incubated with Ecc proteinases. Cell walls pretreated with proteinases of Ecc and subsequently extracted with 5 M ammonia also gave higher yield of ammonia soluble compounds.

The results indicate that some glycosidic alkali-labile bonds have to be broken before proteases of Ecc can degrade cell wall protein components. Thus, proteases of the soft rot bacterium may only act in concert with other enzymes that split glycosidic bonds.

University of Wisconsin

Madison, WI 53706

167. Carbon Monoxide Metabolism by Photosynthetic Bacteria

P.W. Ludden, Department of Biochemistry; G.P. Roberts, Department of Bacteriology

$70,000

Rhodospirillum rubrum is capable of carbon monoxide oxidation to CO_2 and assimilation of this carbon to cellular material. This process also causes the *in vivo* evolution of H_2 and thus is analogous to the water-gas reaction carried out industrially. The oxidation of carbon monoxide by *Rhodospirillum rubrum* and other microorganisms plays a major role in the global carbon monoxide cycle. The carbon monoxide dehydrogenase (CODH) enzyme has been isolated in its holo form containing iron, sulfur, nickel and zinc, and in an apo form lacking nickel. The apo form can be activated *in vivo* and *in vitro* by the addition of nickel. CN and COS are Ni-specific, tight-binding competitive (vs CO) inhibitors of CODH. Pretreatment of apo-CODH with CN or COS does not affect the ability of the enzyme to incorporate Ni. NO is an Fe-specific inhibitor that destroys both holo- and apo-CODH. Transfer of electrons from holo-CODH to the Fe-S center of apo-CODH can be mediated by electron carriers such as methyl viologen.

The enzyme is induced specifically by the presence of carbon monoxide in the medium and is specifically repressed by the presence of oxygen in the medium. Both oxygen and cyanide accelerate the degradation of CODH. The mechanism of these inductions and

repressions are unknown and a goal of this research is to understand how dissolved gases can effect changes in gene expression in this microorganism. Isotopically substituted CODH (containing ^{61}Ni and ^{57}Fe) have been investigated spectroscopically. These studies have led to the conclusion that there is a Ni-Fe redox center in the enzyme. Other metals such as cobalt have been substituted for Ni and these alternate forms of the enzymes will be investigated as well. The path of carbon monoxide assimilation into the cell will be studied and the gene for carbon monoxide dehydrogenase and related activities will be isolated, mutagenized *in vitro* and used to generate mutants of these gene products in *Rhodospirillum rubrum.*

University of Wisconsin

Madison, WI 53706

168. Starch Synthesis in the Maize Endosperm as Affected by Starch-Synthesizing Mutants
O.E. Nelson, Department of Genetics

$81,000

The goal of the project is to investigate the steps in starch synthesis in the developing maize endosperm using the maize mutants in which a step is disrupted as experimental probes. The dull mutant synthesizes less starch than its nonmutant counterpart, and the starch has a higher amylose content (35%) owing largely to the reduced amount of amylopectin present. We find that dull endosperms have much reduced activity for a kinase that synthesizes Glc-1, 6-bis-phosphate from Glc-1-phosphate and ATP. The kinase activities in a gene dosage series suggest that the Du allele is the structural gene for this enzyme. Transposon tagging via a defective Suppressor-mutator (dSpm) transposable element has enabled the identification of a Brittle-1-specific sequence, and we are proceeding to clone the gene. We have found that a number of inbred lines lack one of the two endosperm phosphoglucomutase (PGM) isozymes present in other inbred lines, but this lack does not appear to be affecting starch synthesis. The significance of this observation is not yet clear.

University of Wisconsin

Milwaukee, WI 53706

169. Mechanism of Formation of the Carboxyl of Acetate by Acetogenic Bacteria
S.W. Ragsdale, Department of Chemistry

$77,000

Acetogenic bacteria are anaerobic bacteria which synthesize acetate by a novel mechanism of CO_2 fixation. Acetate is formed from CO, H_2/CO_2, or organic substrates such as pyruvate or hexoses. The enzymes involved in the formation of the carboxyl group of acetate are CO dehydrogenase (CODH) and, in the synthesis from pyruvate, pyruvate-ferredoxin oxidoreductase (PFOR). An intermediate in this process appears to be an enzyme-bound complex on CODH, CODH-C_1, which we are characterizing. An extremely interesting species, consisting of Ni, ~3Fe, and CO, has been identified which could be this CODH-C_1 complex. By EPR, ENDOR, Mössbauer, resonance Raman, and IR spectroscopies, we are elucidating the structure of the Ni-Fe-C species and other unique iron-containing centers in CODH. We have cloned the genes encoding the two subunits of CODH and are determining their DNA sequences.

In the synthesis of acetate from pyruvate, one pyruvate carboxyl forms CO_2 (to become the C-2 of acetate) and another pyruvate carboxyl is converted to the C-1 of acetate without exchange with CO_2. We are using NMR spectroscopy to study several exchange reactions in order to obtain insight into how the carboxyl groups of two pyruvates are channeled in different directions and the mechanism of transfer of the carboxyl of pyruvate from PFOR to CODH to form the CODH-C_1. These exchange reactions are between CO_2 and the carboxyl of pyruvate, between CO and the carboxyl of pyruvate, and between the carbonyl of acetyl-CoA and the carboxyl of pyruvate.

University of Wisconsin

Madison, WI 53706

170. Gas Exchange Characteristics of leaves as Indicators of the Basic Limiting Factors in Photosynthesis
T.D. Sharkey, Department of Botany

$73,019

The response of photosynthesis to light, CO_2 and O_2 is studied by measuring the exchange of gases (water vapor and CO_2) between the atmosphere and plant leaves. These measurements are combined with simul-

taneous measurements of fluorescence, fractionation of stable carbon isotopes, and measurements of metabolite levels and enzyme activities on leaf sections freeze-clamped during gas analysis measurements. The purpose of this research is to identify those physical and chemical factors which limit and regulate photosynthesis.

Current emphasis is on the first enzyme in photosynthetic carbon metabolism, ribulose bisphosphate carboxylase (RuBPCase), and the last enzyme, sucrose phosphate synthase (SPS). The regulation of activity of RuBPCase by CO_2 and Mg^{2+} is studied in leaves exposed to environmental conditions which alter the capacity of the leaf for photosynthesis. The reduction in activity of this enzyme which is so often limiting photosynthesis may stimulate starch synthesis and so help balance the capacity for producing and consuming triose phosphates. The activity of SPS is also regulated in response to environmental changes that affect the rate of photosynthesis. Future work will involve studies of the molecular regulation of SPS activity. In addition, analysis of mutant plants lacking another enzyme necessary for sucrose synthesis continues.

University of Wisconsin
Madison, WI 53706

171. Molecular Mechanism of Energy Transduction by Plant Membrane Proteins
M.R. Sussman, Department of Horticulture

$75,000

Proton pumps are membrane-bound proteins that act as biological energy transducers. The plasma membrane of plants and fungi contains an ATP-coupled proton pump (H^+-ATPase) that converts chemical into electrical energy. 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. The aim of this project is to identify aspects of the enzyme's primary structure that are essential for converting chemical into electrical energy. Protein modification and sequencing techniques are used to study the structure of a hypothetical protein transport 'channel' as well as to identify essential amino acids in the enzyme's other active sites. DNA cloning and sequencing techniques are used to obtain the complete amino acid sequence for ATPase structural genes present in higher plants. Based on chemical modification and computer-assisted sequence studies, amino acids that are predicted to be important in the catalytic mechanism are tested for essentiality through site-directed mutagenesis and expression of cloned genes. These studies provide data necessary for generating and testing hypothesis concerning the molecular mechanism of protein-mediated proton conduction and energy transduction.

University of Wisconsin
Madison, WI 53706

172. Mechanism for the Selective Conjugation of Ubiquitin to Phytochrome
R.D. Vierstra, Department of Horticulture

$73,000

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 rate of Pfr is approximately 100 times faster than Pr, this system offers the unique ability to rapidly and synchronously manipulate the degradation rate of a protein *in vivo* 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.

Yale University

New Haven, CT 06511

173. 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 has been implicated in the regulation of anthocyanin pigmentation patterns in both the seed and plant. Many alleles of R are comprised of multiple tissue specific genes that are highly related at the DNA sequence level. Our objectives have been to characterize a genetically well studied R allele, standard R-r, and ultimately to understand the basis of tissue specific regulations of gene expression. The R-r allele comprises multiple genes specifying plant (P) and seed (S) pigmentation and a third gene (Q) of unidentified function. We have initiated a genomic walk from each gene to determine their relative organization. Alternating field gel electrophoresis of high MW DNA has indicated that the separation of (P) and (S) genes may be several 100kb. However the (S) and (Q) genes always map to a single fragment indicating that the genomic walking will be able to resolve the relative orientation of (Q) and (S). This will be important for understanding the mutational behavior of the R-r allele which often involves intrachromosomal recombination between genes. Molecular analysis of a wide range of R alleles from maize and related species has revealed a highly diversified gene family. Sequence analysis of selected regions will help establish a phylogeny of allele that will assist our understanding of the evolution of a multicomponent allele such as R-r.

Yale University

New Haven, CT 06520

174. Control of Genes Encoding Catabolic Enzymes in Bradyrhizobium

D. Parke and L.N. Ornston, Department of Biology

$79,000

The goal of this project is to understand energy metabolism and gene control in bacteria of the genus *Bradyrhizobium*. These bacteria are versatile purveyors of metabolic energy, being capable of fixing nitrogen in symbiosis with legumes and 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 beta-ketoadipate pathway. Inducible in all other microbes studied to date, enzymes of the beta-ketoadipate pathway are expressed constitutively in *Bradyrhizobium*. One enzyme in particular, beta-ketoadipate succinyl CoA transferase, a product of the pcaE gene, is expressed at high levels in saprophytic and symbiotic *Bradyrhizobium*. The properties of this enzyme will be studied in an effort to understand the physiological basis for its high constitutive expression. The investigation will also analyze whether unregulated enzyme synthesis is a feature of other peripheral catabolic pathways in *Bradyrhizobium*. The selective basis for the observed lack of regulation will be examined by a number of approaches, including isolation of mutant strains blocked in the catabolism of plant phenolics. The mutant strains will be 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

175. Mechanisms and Control of K^+ Transport in Plants and Fungi

C.L. Slayman, Department of Cellular and Molecular Physiology

$212,000 (FY88 funds/two years)

The continuing purpose of this proposal is a comprehensive study of potassium transport mechanisms in plants and fungi, with several component objectives:

I) Definition of passive transport mechanisms in model fungal and plant protoplasts. Overall transport characteristics of non-animal cells are governed by a balance between a) active transport of H^+ and H^+-coupled substrates, and b) dissipative ion flow, usually via discrete channels which can be examined with the patch electrode (Hedrich & Schroeder, Ann. Rev. Pt. Physiol. 40:539). Work in this laboratory is extending the channel studies to protoplasts of the model plant *Arabidopsis* and the mycelial fungus *Neurospora*.

II) Cloning and sequencing of genes for potassium transport systems in *Neurospora.* A DNA probe from the structural gene for a high-affinity K^+ transporter in *Saccharomyces* (Gaber et al., Mol. Cell. Biol. 8:2848) has been used to clone a related *Neurospora* gene, thought to encode the H^+-K^+ symporter of that organism. Functional characterization of the gene product should begin soon. Also, complementation has been used to identify the genes for two established transport mutants of *Neurospora*: trk1 and trk2, of which the first affects of the ion-K_m in a low-affinity uptake system, and the second enhances K^+ leakage. Cloning of these two genes is continuing.

III) Modeling analysis of the high-affinity K^+ transporter in *Neurospora* is continuing on the basis of steady-state current-voltage data previously obtained. In addition, a full description is in preparation on temperature modulation of both proton pumping and related channel-like conductance switching in *Neurospora.* From this analysis tests are emerging for specific transport control processes which can be activated by temperature stress.

Yale University

New Haven, CT 06511

176. Transfer RNA Involvement in Chlorophyll Biosynthesis

D. Soll, Department of Molecular Biophysics & Biochemistry

$89,000

Chlorophyll is the major pigment in photosynthesis. One molecule of chlorophyll is synthesized from eight molecules of delta-aminolevulinic acid, the universal precursor of porphyrins. Chlorophyll synthesis is regulated at the synthesis of delta-aminolevulinic acid, which is formed in the stroma of greening plastids from glutamate. Another pathway of delta-aminolevulinic acid biosynthesis operates in fungi or mammals, where glycine and succinyl-coenzyme A are the precursors. The mechanism of delta-aminolevulinic acid synthesis from glutamate is still poorly 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 probably 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 delta-aminolevulinate. Studies on the *in vitro* synthesis of delta-aminolevulinate in extracts from barley chloroplasts, *Chlamydomonas* and *Synechocystis 6803* showed the involvement of $tRNA^{Glu}$. Three enzymes and a tRNA are involved in this process. In the first step glutamate is attached to tRNA via an aminoacyl bond by glutamyl-tRNA synthetase. The subsequent reduction of glutamate to glutamate semialdehyde is carried out by Glu-tRNA reductase which requires tRNA as a specific "cofactor". The barley chloroplast Glu-tRNA reductase shows high specificity among different glutamate tRNA species. In the final step an aminotransferase converts glutamate-1-semialdehyde to delta-aminolevulinic acid. The goals of our studies are the biochemical characterization of the enzymes and tRNAs involved in this process, the detailed analysis of the genes encoding these macromolecules, and an understanding of the regulation of this biosynthetic pathway. As experimental systems we shall use the cyanobacterium *Synechocystis 6803* and barley. These studies should uncover novel principles regarding the role of tRNA as a cofactor in metabolic conversions.

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