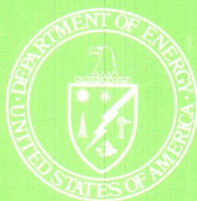
DOE/ER-0346

Annual Report and Summaries of FY 1987 Activities Supported by the Division of Biological Energy Research

September 1987



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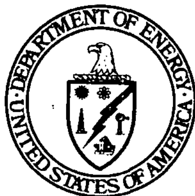

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PROGRAM OVERVIEW OF THE

DIVISION OF BIOLOGICAL ENERGY SCIENCES

(Budgetary title: Division of **Energy Biosciences**)

The Biological Energy Research program has the mission to generate fundamental data for the development of biosystems to provide enhanced renewable resources and the means of converting them biologically into fuels and other materials now derived from fossil fuels. Additionally, the program has the objective of defining, in biological terms, potential systems for the conservation of energy. The program aims at understanding principles and mechanisms rather than seeking optimization of processes.

At a time when petroleum prices are relatively low and agricultural commodities are in considerable surplus it is easy to be complacent about the need for basic research on renewable resources and their conversion. Suffice to say the current circumstances are certain to be only temporary and this period should be viewed as a valuable opportunity to establish a strong data base in preparation for the future when renewable resources will assume greater importance as other reserves diminish and energy prices escalate again.

The scope of the Biological Energy Research program has not changed appreciably over the past few years, aside from the expected evolution resulting from new scientific insights and technology. The broad basic problem areas remain targets of the program in respect to achieving greater understanding as to how biological processes work. These include in the **Plant Sciences:**

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 that 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.

The above topic areas reflect some of the principal areas of research supported by the Biological Energy Research (BER) program, however, the abstracts contained within these pages offer the best representation of the scope and flavor of the BER program. The program composition evolves to some degree each year as might be anticipated, but the objectives remain the same, namely the generation of a fund of basic information about key biological processes in plants and microorganisms that in some cases have been largely over-looked. The program remains one where research submissions are welcome on significant topics which may not fall within the most active areas. The philosophy is that information about orphan organisms and less popular research areas sometimes will be most needed for advances both from a scientific as well as from an applied viewpoint. An inspection of the program should reveal the balances within it with respect to topics, size of projects and other factors.

In FY 1987 the Biological Energy Research program grew modestly. As the program has become known to more investigators and institutions the number of proposals submitted for consideration has also grown. In the FY 1987 consideration, 116 new proposals were received and of these 21 were funded, (18%). The number of new proposals received for consideration for FY 1988 funds, received in the summer of 1987, exceeded all previous levels by a wide margin (an increase of 55% over the previous year). As can be seen in the following table, the majority of projects support research in university laboratories, however, support of research at other types of institutions is also provided.

	Number of projects	FY 87 funding (in thousands of $)	Percent of total funds
University Grants & Contracts	111	9795	59%
Michigan State University Plant Research Laboratory	14	2173	13%
National Laboratories Brookhaven National Lab Lawrence Berkeley Lab Oak Ridge National Lab Los Alamos National Lab	15	2295	14%
Solar Energy Research Institute	1	120	1%
Oregon Graduate Center	1	1200	7%
Other research institutions (federal, state, industrial, nonprofit)	6	474	3%
Small Business Innovation Research (SBIR) contribution, Conferences & miscellaneous	6	443	3%
	154	16500	

To provide some of the flavor of the BER program several representative activities are noted below:

The small cruciferous plant, Arabidopsis thaliana, has been given special attention recently at the Michigan State University/DOE Plant Research Laboratory. This plant is proving to be a powerful experimental system for basic plant research. An international symposium was convened this year at Michigan State University to discuss extensively the opportunities, advantages and techniques for utilizing this as a model system in higher plant research. Among the benefits offered by this organism are the small size of both the plants and the seeds which circumvent the need for large greenhouse or field space, the short life cycle of the plant, its small chromosome number and its small genome size. Arabidopsis has proven an excellent organism in which to find mutations, a fact that is being extensively exploited at the Plant Research Laboratory to investigate such physiological phenomena as phototropism, lipid metabolism, herbicide resistance and others. The role of the laboratory and of Dr. Chris Somerville in particular, as proponents of this experimental system made it a logical site for the Arabidopsis symposium.

Another example involves a multiple investigator project on the microbiology and physiology of anaerobic bacteria at the University of Georgia which has provided insight into the relationship between the numerous forms of hydrogenase found in many anaerobic bacteria. Molecular hydrogen is believed to be the main "currency" involved in transfer of energy in anaerobic bacterial consortia. The important role of hydrogen metabolism in many anaerobic bacteria has prompted a detailed study of the hydrogenase enzymes from sulfate-reducing bacteria, methanogenic bacteria and clostridia. Three forms of hydrogenase may be categorized based upon the metal cofactors present in the enzyme. These are iron-containing (Fe form), nickel-iron-containing (NiFe form), and selenium-nickel-iron-containing (SeNiFe form). The genes encoding the NiFe form and SeNiFe form have been isolated and sequenced from a sulfate-reducing bacteria. Analysis of the new sequences show that while there are similarities in a few small domains, the two forms are very dissimilar. Comparison of the NiFe and SeNiFe hydrogenase genes with a Fe hydrogenase gene sequence shows little if any similarity. Differences in the biophysical and catalytic properties of the various hydrogenase forms are presently being extended to determine the precise physiological role of each form in the anaerobic metabolism of bacteria.

In FY 1987 the area of carbohydrate structural analysis received special attention. A Federal Register Notice was issued in January 1987 inviting submissions of proposals dealing with a program in plant and microbial

carbohydrate structural analysis encompassing, research, training and service to the wider research community. The point of reference used was a 1984 workshop report in which the structural analysis needs for carbohydrates were documented. In the workshop, the participants devised and suggested the concept of a network of analytical programs as being the potential solution to the problem of how to deal with the increasing needs for carbohydrate structural data. Six proposals were received in response to the Federal Register Notice. From among them, the proposal from the University of Georgia was selected competitively as the one best able to fulfill the criteria established in a timely and effective manner. Accordingly, in FY 1987 a program at the University of Georgia is being established as a start to the network concept. As an adjunct activity the first stages of the development of an international carbohydrate structural data base "Carbank" was accomplished at the University of Georgia with DOE support in part.

Important activities occur outside of the scientific laboratory that are sponsored by the BER program, wholly or in part. Meetings, workshops and symposia provide a forum for the necessary intellectual exchange of ideas and scientific data. The related activities supported in FY 1987 are listed below:

1. Partial support for the 10th Annual University of California-Riverside Symposium in Plant Physiology, January 1987, Riverside, California. (Proceedings published.)

2. Partial support for "Symposium on Current Topics Biochemistry and Physiology," April 1987, University of Missouri, Columbia, Missouri. (Proceedings to be published.)

3. Partial support for Workshop entitled Ribulose 1,5-Bisphosphate Carboxylase/Oxygenase - Genes, Proteins and the Regulation of Activity, April 20-25, 1987, University of Arizona, Tucson, Arizona. (Proceedings to be published.)

4. Partial support of the second, third and fourth Annual Penn State Symposium in Plant Physiology, May 21-23, 1987, Pennsylvania State University, University Park, Pennsylvania. (Proceedings to be published.)

5. Partial support of International Symposium on Plant Transposable Elements, August 23-27, 1987, University of Wisconsin, Madison, Wisconsin. (Proceedings to be published.)

6. Partial support of a conference on Molecular Biology of Mitochondria and Chloroplasts, August 25-30, 1987, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. (Proceedings to be published.)

No high quality scientific program can retain that quality without exposure to critical review. The various components of the BER program are reviewed continuously with the help and cooperation of a large number of scientists, in this country and abroad. These individuals provide time and their expertise most generously in participating in reviews of proposals and on-going programs, as well as in workshops and panels. These efforts are genuinely appreciated. Only time constraints preclude separate expressions of gratitude to the individuals involved. This annual report is used to convey our appreciation to those persons.

Any questions the reader may have concerning the technical aspects of any of the projects included herein can be addressed to the principal investigator. Questions about the overall program of the Biological Energy Research Division should be addressed to:

Dr. Robert Rabson, Director
or
Dr. Gregory L. Dilworth, Microbiologist
Division of Biological Energy Research
Office of Basic Energy Sciences, ER-17, GTN
U.S. Department of Energy
Washington, D. C. 20545

Phone: (301)353-2873

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SUMMARIES OF FY 1987 ACTIVITIES
SUPPORTED BY THE
DIVISION OF BIOLOGICAL ENERGY RESEARCH

1. **ARIZONA STATE UNIVERSITY** - Tempe, AZ 85287

Antenna Organization in Green Photosynthetic Bacteria
R.E. Blankenship, Department of Chemistry $67,000

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 1500 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 absorption and fluorescence spectroscopies. Results have identified a sequence of at least four distinct pigment species that transfer energy from the chlorosome into the membrane and eventually to the reaction center. The 740 nm-absorbing bacteriochlorophyll c that is the main pigment in the chlorosome has an extremely short 15 ps fluorescence lifetime. Spectral evidence indicates that the chlorosome pigments are probably organized by pigment-protein and direct pigment-pigment interactions into what are essentially pigment oligomers. Future work includes time-resolved fluorescence spectroscopy on whole cells, membranes, chlorosomes and pigment aggregates using both the streak camera and single photon counting techniques. Theoretical studies on the spectroscopic properties of pigment aggregates will also be carried out.

2. **BRANDEIS UNIVERSITY** - Waltham, MA 02254

Carbon and Hydrogen Metabolism of Green Algae in Light and Dark
M. Gibbs, Institute for Photobiology of Cells and Organelles
$62,950

The primary focus of this project is an understanding of anaerobic metabolism in the eukaryotic green algae. Recently developed methods for isolating photosynthetically competent chloroplasts from Chlamydomonas reinhardii capable of being adapted to a hydrogen metabolism makes it possible to study the metabolism of the organelles directly. Photosynthesis, photoreduction (assimilation of CO_2 with H_2) but not the oxyhydrogen reaction (assimilation of CO_2 in the dark with energy provided by H_2 and O_2) have been observed in the isolated chloroplast. Under N_2, Chlamydomonas cells evolve H_2 and CO_2 per mole of acetate consumed to support the occurrence of anaerobic and light-dependent citric acid and glyoxylate cycles. Enzymic distribution indicates that the bulk of C. reinhardii succinic acid dehydrogenase and the NADP-linked glyceraldehyde 3-phosphosphate dehydrogenase resides in the chloroplast. About half of the NAD-glyceraldehyde 3-phosphate dehydrogenase is located in the chloroplast. Anaerobically adapted chloroplasts evolve in the light H_2 from malate. The reductive carboxylation by ferredoxin of acetyl-coenzyme A to pyruvate has been characterized in extracts of C. reinhardii wild type and mutant F-60.

3. **BROOKHAVEN NATIONAL LABORATORY** - Upton, NY 11973

Chlorophyll-Protein Complexes of Photosystem II: Structure, Biosynthesis and Phosphorylation
J. Bennett, Biology Department $245,000

The project studies the structure, function and formation of chlorophyll-protein 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-translational 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.

4. **BROOKHAVEN NATIONAL LABORATORY** - Upton, NY 11973

Plant Molecular Genetics
B. Burr and F.A. Burr, Biology Department $275,000

Molecular genetics has an impact on plant improvement in at least two areas: the development of analytical techniques and the study of basic genetic mechanisms that influence crop productivity. In the former area we have developed a rapid method of gene mapping that is based on the use of recombinant inbreds. This method constitutes a permanent segregating population and, since all mapping information obtained for a family is cumulative, may be employed by any investigator interested in mapping a novel trait for which differences can be found between the original parents. This, of course, includes quantitative traits such as response to photoperiod, plant morphology, combining ability, and multigenic resistance to pathogens. Presently, we have mapped 130 markers in two recombinant inbred families and estimate that we have better than a 90% chance of detecting linkage with any new marker we wish to map. The result is a rapid means of mapping recombinant DNA clones and replaces the need for *in situ* hybridization to chromosomes. The second area of study in this laboratory concerns regulatory genes. We have developed a technique for gene isolation by transposon tagging that has permitted us to clone *o2*, a regulator of storage protein biosynthesis, and *c1* and *p1*, regulator of anthocyanin biosynthesis. Mutants of both these systems show how changes in the genes modulate their action, and defeat auto-regulation. We are attempting to identify and express the products of the regulatory genes. Since target genes for these regulatory genes have previously been isolated, we anticipate being able to study the interaction of the regulatory proteins with their target sequences.

5. **BROOKHAVEN NATIONAL LABORATORY** - Upton, NY 11973

Mechanisms of Energy Conversion in Photosynthesis
G. Hind, Biology Department $355,000

The project goal is to discover how energy is transformed in photosynthetic membranes and how the end-product varies in response to metabolic need. Cyclic electron transport is studied in intact chloroplasts of the C-3 plant, Spinacia oleracea and the C-4 plant, Zea mays, and in heterocysts of the filamentous bacterium, Anabaena 7120. It is mediated by combined activities of the cytochrome b/f and photosystem 1 complexes. The generation of reduced $NADP^+$ competes with cyclic electron flow at a branch point, probably the enzyme ferredoxin:$NADP^+$ reductase, whose flexible regulation and attachment to the membrane are under study.

Relative electron fluxes through the cyclic and linear pathways are explored using flash, steady-state and photoacoustic 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 studied to elucidate the coupling mechanism.

State transitions 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. Isolation and characterization of these enzymes is in progress. The mechanism through which kinase activity is controlled by ambient redox poise is unknown and will be studied toward a goal of reconstituting State transitions in an artificial membrane.

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.

6. **BROOKHAVEN NATIONAL LABORATORY** - Upton, NY 11973

The Physiology and Biochemistry of Cyanobacteria
H.W. Siegelman, Biology Department $190,000

Cyanobacteria are cosmopolitan organisms frequently responsible for water blooms, and they may cause serious water management problems. The molecular structure and composition of their photosynthetic energy collection system, which consists of an assembly of biliproteins called phycobilisomes, are being characterized. A hydrophobic-interaction

chromatographic procedure was devised to isolate large amounts of phycobilisomes. The constituent biliproteins are then purified, and their aggregation state, mass, and molecular morphology are being determined by combining high performance gel permeation and neutron scattering analyses. At low concentration, phycocyanin and phycoerythrin exist in monomer, trimer, and hexamer states which are pH and protein concentration dependent. At high concentration, the hexamer is dominant at pH 6 and the trimer at pH 5 or 7. These aggregation states are readily reversible by variation of pH and protein concentration. Crystallization of phycocyanin and phycoerythrin is providing useful material for the determination of the three-dimensional structure of the biliproteins using synchrotron radiation. Mouse antibody screening of a λGT11 genomic library of *Synechocystis 6701* DNA provided several clones encoding sequences of phycoerythrin, phycocyanin, and allophycocyanin. Several heptapeptide toxins from *Microcystis aeruginosa* are being purified by hollow fiber ultrafiltration, and Fractogel HW-40 F and octadecyl silica chromatography. Pathophysiological studies show that the toxin containing leucine and arginine (LR) can be blocked by trypan red for up to three months, about 15% of the lifespan of the mouse. The lethality of the toxin containing leucine and alanine (LA) is not blocked by trypan red. Newborn mice are resistant to the LR toxin but are not protected from the lethality of LA toxin.

7. **BROOKHAVEN NATIONAL LABORATORY** - Upton, NY 11973

Molecular Bases and Photobiological Consequences of Light Intensity Adaptation in *Dunaliella tertiolecta*
P. G. Falkowski, Department of Applied Science $60,000

To some degree, all photosynthetic organisms adapt to variations in growth irradiance levels, however, compared with higher plants, physiological adaptation to variations in light intensity is especially pronounced in algae. For example, changes in cellular pigmentation of over five-fold occur in algae strictly in response to changes in light levels. These responses reflect fundamental changes in the synthesis and organization of pigment protein complexes which comprise the photosynthetic apparatus. The mechanisms responsible for these responses are not known, however they do not appear to be mediated by phytochrome. 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 in a unicellular marine chlorophyte, *Dunaliella tertiolecta*, which has a similar photosynthetic apparatus to that found in higher plants. The project aims at understanding how pigment synthesis and protein synthesis are coordinated and regulated so that functional pigment protein complexes are formed or degraded in response to light intensity. Attention is focused

on the level of control of protein synthesis by irradiance, namely at differentiating between transcriptional, translational and post-translational regulation of the apoproteins. Simultaneously the effect of irradiance on pigment synthesis are assessed using radioactive 13N to trace precursors. In collaboration with Dr. John Bennett in the Biology Department, attempts are being made to transform D. tertiolecta in order to genetically engineer strains with altered light harvesting properties. Such transformations will allow us to experimentally examine the relationship between light harvesting capability and photosynthetic electron transport. This relationship determines the maximum quantum yield of photosynthesis.

8. **CALIFORNIA INSTITUTE OF TECHNOLOGY** - Pasadena, CA 91125

Genetics in Methylotrophic Bacteria
M.E. Lidstrom, Environmental Engineering Science $82,958

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 used mutants and gene probes generated from our studies of Methylobacterium AM1 to isolate C-1 specific genes from methane-utilizers. These genes have been mapped and characterized by standard techniques. We are now cloning promoter regions from these genes using a broad host-range promoter cloning vehicle we have constructed. The promoter-lacz fusions generated by this cloning will be used to study transcriptional regulation of key C-1 genes in these methane-utilizers. Our studies so far suggest that the structural genes for central C-1 functions are highly conserved in methylotrophs in general, and future work will be directed towards comparative structure-function studies.

9. **UNIVERSITY OF CALIFORNIA** - Berkeley, CA 94720

Transcription Control Elements and Manipulation of Chloroplast Genes
W. Gruissem, Department of Botany $90,000

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 elements that are involved in the expression of genes. The proposed research project focuses on the molecular mechanisms of chloroplast gene expression. We have investigated DNA sequences in the

5' flanking regions of several spinach chloroplast genes for their promoter function _in vitro_. While the promoter regions for most of these genes have considerable structural and DNA sequence homology with the prokaryotic consensus promoter, some of the tRNA genes do not contain 5' upstream promoter elements. We will continue and extend our structural and functional analysis of chloroplast promoter regions. Besides the transcriptional regulation exerted at the promoter level, we have evidence that post-transcriptional mechanism(s) play a significant role in the control of chloroplast gene expression. Experimental analysis and combined evaluations of changes in RNA polymerase levels, relative promoter strengths, and the post-transcriptional stability of mRNAs at different developmental stages, will enable us to correlate the relative contribution of regulatory mechanisms with observed changes in chloroplast gene activity. These studies will be based on _in vitro_ and run-on transcription system from spinach chloroplasts. The putative chloroplast genes for RNA polymerase subunit proteins will be instrumental in extending the characterization of the transcription apparatus. To support the _in vitro_ analysis of regulatory elements, we have initiated work with characterized chloroplast promoter regions and selectable marker genes in DNA constructs designed for transformation experiments and integration into selected sites of the chloroplast genome. These experiments will be carried out with tobacco using _Agrobacterium_ vector systems and direct DNA transfer techniques. The development of a reliable and reproducible chloroplast transformation system will be a significant progress in our attempts to manipulate chloroplast genes.

10. **UNIVERSITY OF CALIFORNIA** - Berkeley, CA 94720

The Regulation of Enzyme Synthesis and Secretion in Plant Cells
R.L. Jones, Department of Botany $90,000

The cereal aleurone is a digestive gland that secretes hydrolytic enzymes to the cell exterior via the constitutive pathway. In barley the production of hydrolases is controlled by gibberellic acid (GA) and the calcium ion (Ca^{2+}). We have shown that, whereas GA controls the production of α-amylase by regulating the synthesis of this enzyme, Ca^{2+} regulates the process of intracellular transport or secretion of the protein. Our research is focused on the role of Ca^{2+} in the aleurone of barley. Wall-less protoplasts prepared from aleurone layers respond to both GA and Ca^{2+} as does intact aleurone tissue. Using these protoplasts we are measuring the levels of cytoplasmic free Ca^{2+} using Ca^{2+}-sensitive dyes, and the sites of Ca^{2+} sequestration in isolated membrane fractions using ^{45}Ca uptake. We have examined the utility of the esters of Quin, Fura and Indo dyes for the measurement of Ca^{2+} in aleurone protoplasts. Quin is poorly taken up by cells; Fura-2 is taken

up but accumulates in vacuoles as well as cytoplasm; and Indo-1 is taken up, but uptake rates are variable. We have shown that the acid form of Indo-1 is rapidly taken up by aleurone protoplasts and accumulates in the cytoplasm as the anion. The fluorescence of Indo anion is used as a measure of cytoplasmic free Ca^{2+}. The sequestration of Ca^{2+} in microsomal membranes is also being studied in membrane fractions isolated from protoplasts. We have identified an ATPase activity in membranes of the endoplasmic reticulum responsible for Ca^{2+} uptake and accumulation.

11. **UNIVERSITY OF CALIFORNIA** - Berkeley, CA 94720

Determination of Genes and Phenotypes of Bacteria Necessary for Epiphytic Colonization and Survival on Plants
S.E. Lindow, Department of Plant Pathology $63,933

Bacteria that live as epiphytes on the surface of healthy plants are important as plant pathogens, in causing plant frost injury by catalyzing ice formation, and in other processes. The objectives of this study are to determine the traits of these epiphytic bacteria which allow them to grow and/or survive in the hostile leaf surface environment. The genes and/or phenotypes of strains of *Pseudomonas syringae* and *Erwinia herbicola* that are necessary for epiphytic fitness on bean leaves will be determined by an evaluation of the fitness of 10,000 individual Tn*5* induced insertion mutants in both species and subsequent determination of phenotypes associated with these insertional mutations. Parameters optimizing insertional mutagenesis in this strain have been determined. Auxotrophs represented 1.02% of the Tn*5* mutants recovered. Tn*5* was shown to insert randomly into the chromosome of strain B728a as tested by the normality of sizes 8 *Eco*RI, *Not*I and *Sfi*I restriction fragments which contained Tn*5*. The epiphytic fitness of Tn*5* induced mutations *P. syringae* are being determined indirectly on leaves, after subjecting them to fluctuating moist and dry conditions, by a leaf freezing assay. Population size of ice nucleation bacteria on leaves and the mean freezing temperature of leaves is directly related, and differences in leaf surface population sizes of from 3 - 4 fold or more can be detected. Preliminary results indicate that approximately 0.8% of Tn*5* induced mutants of *P. syringae* are deficient in epiphytic fitness as determined by this assay. The phenotype of at least some Tn*5* induced mutants will be determined *in vitro* and will be associated with alterations in growth and survival on leaf surfaces and in other environments.

12. **UNIVERSITY OF CALIFORNIA** - Berkeley, CA 94720

The Bioenergetics of Salt Tolerance in Cyanobacteria
L. Packer, Department of Physiology/Anatomy $75,000

This project seeks to understand mechanisms that enable photosynthetic cells to survive under conditions of high salinity. Cyanobacteria are used as models of how cells are affected by hypersaline shock and how they adapt to altered ionic environments. It is hypothesized that exposure of cells to a sodium chloride shock elicits altered gene products effecting the redirection of energy-flow from photosynthesis and respiration towards salt extrusion, and the synthesis of osmoregulatory substances. Biophysical and biochemical assays being applied to the study of salt-stressed cells include: 1) magnetic resonance techniques to determine the impact of salt, both on the initial intrusion of NaCl and the subsequent osmoregulatory changes, in intact cells; 2) ^{31}P- ^{13}C-NMR (using $H^{13}CO_3^-$ enriched cells) to determine energy status and carbohydrate turn-over during the initial exposure to salt and after salt adaption; 3) ESR spin label techniques to monitor energetic parameters (internal volume, pH, and trans-membrane 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; 5) and determination of ultrastructural changes, in conjunction with changes in glycogen and lipid composition to assess physiological responses to salt stress. We also are investigating salt stress effects on the energetic parameters responsible for the exchange of essential ions, both during the initial inhibition of photosynthesis and the adaptive increase in respiration accompanying development of salt tolerance.

13. **UNIVERSITY OF CALIFORNIA** - Berkeley, CA 94720

Phytochrome from Green Plants: Assay, Purification and Characterization
P.H. Quail, Molecular Plant Biology, U.C. Berkeley and USDA Plant Gene Expression Center, Albany, CA $80,000

This project is designed to investigate the molecular properties and biogenesis of phytochrome in light-grown tissue. We have confirmed that the predominant species in green Avena tissue behaves as a Mr-118,000 molecule under appropriate electrophoretic conditions. Apparent inability to separate this molecule from the Mr-124,000 etiolated-tissue species under some electrophoretic conditions has led to some confusion in the literature. The suggestion that the Mr-118,000 species is a degradation

product appears incorrect as it is extractable from fresh green tissue into hot SDS. Proteolytic degradation of the Mr-118,000 molecule can occur *in vitro* but is prevented by thiol-protease inhibitors. We have developed a partial purification protocol that yields preparations with unaltered spectral properties, indicating maintenance of structural integrity during preparation. A zinc-induced fluorescence assay for tetrapyrroles shows that the Mr-118,000 species is the predominant chromophore-bearing polypeptide in the preparations. This species comigrates with dimeric, Mr-124,000 phytochrome from etiolated tissue on size exclusion chromatography under nondenaturing conditions suggestive of similar quaternary structure. The Mr-118,000 molecule is present in the dry embryo of *Avena* seeds and remains at a constant level during germination irrespective of light or darkness. This result contrasts with the behavior of the Mr-124,000 species which accumulates to high levels in the dark but not in the light. The Mr-118,000 species, therefore, appears to be constitutively expressed and to have the characteristics of the minor phytochrome pool referred to in the literature as "seed phytochrome" and "light-stable" phytochrome.

14. **UNIVERSITY OF CALIFORNIA** - Berkeley, CA 94720

Characterization of Embryo-Specific Genes
Z.R. Sung, Department of Genetics & Plant Pathology $61,462

Many plant cells are totipotent, that is, once differentiated, they can be stimulated to dedifferentiate and divide. In some tissues, the proliferating cells can acquire embryogenic potential, and be triggered to undergo embryogenesis. This process is referred to as somatic or *in vitro* embryogenesis. The embryos deriving from somatic cells undergo morphogenetic transitions similar to those developing from a fertilized egg, i.e., *in vivo* or zygotic embryogenesis.

The phenomenon of somatic embryogenesis offers a unique opportunity to the study of experimental embryology, which has been hindered due to the inaccessibility of zygotic embryos. It may also contribute to the understanding of developmental processes in the adult plant. The research described in this proposal is aimed at understanding the genetic program underlying somatic embryogenesis. Are they similar or are there alternative pathways leading to the same morphogenetic events?

To address this question, we plan to compare gene expression in somatic and zygotic embryogenesis. Using cDNA and antibody probes, we have identified a set of genes whose expression is associated with somatic embryogenesis, that is, their gene products are found in embryogenic, but not in differentiated adult tissues. We will find out if they are also expressed during zygotic embryogenesis, and if their temporal and spatial pattern of expression is similar in the two embryogenic processes. Finally, we hope to assess the functional role of these genes in embryogenesis, *in vitro* and *in vivo*.

15. **UNIVERSITY OF CALIFORNIA** - Davis, CA 95616

Restriction of Virus Infections by Plants
G. Bruening, Department of Plant Pathology $85,000

The productivity of a particular cultivar, in terms of biomass, food and/or fiber, often is limited by the action of plant pathogens. A direct and generally ecologically sound approach to limiting the deleterious effects of a pathogen is to develop a cultivar that is resistant or immune to it. Unfortunately, naturally-occurring plant genetic sources of resistance to economically important viruses often are not available. The objective of this research is to identify the mechanisms by which certain plants resist specific viruses. We hope to develop the necessary information to make the engineering of resistance to plant viruses possible. Previous work identified a cowpea line, Arlington, from which cowpea mosaic virus (CPMV)-resistant protoplasts were recovered. Analyses showed that Arlington protoplasts, unlike protoplasts from susceptible Blackeye 5 cowpeas, interfere with the production of CPMV proteins. Extracts of Arlington cowpea protoplasts, but not Blackeye 5 cowpea protoplasts, interfered with an *in vitro* assay of a CPMV-specified proteinase that cleaves a CPMV polyprotein and is essential to CPMV replication. The proteinase inhibitor now has been demonstrated in Arlington cowpea plants. It was shown to be specific for the CPMV proteinase and to be co-inherited, with resistance to CPMV, in crosses of Arlington and Blackeye 5 cowpeas. Our evidence shows that the proteinase inhibitor, and not other potentially virus-inhibiting activities, is the mediator of the resistance of Arlington cowpeas against CPMV. The purification of this inhibitor is in progress.

16. **UNIVERSITY OF CALIFORNIA** - Davis, CA 95616

Characterization and Expression of *Clostridium* Cellulase Genes
R.H. Doi, Dept. of Biochemistry & Biophysics $91,000

Relatively little is known about the genetic properties of anaerobic strains of bacteria. We have recently isolated a novel mesophilic *Clostridium* sp. that is capable of degrading cellulose, rice straw, and sawdust. Our long term plans are to use this anaerobic organism for basic studies on gene expression and protein secretion. As a model genetic system we will initially study the genes for the three major extracellular cellulases with MWs of 90,000, 75,000, and 50,000. We have recently developed methods for isolating DNA from the organism and constructed a plasmid gene bank. We have cloned and expressed the gene for the 50,000 dalton cellulase enzyme in *E. coli*. Interestingly the enzyme was secreted into the growth medium. Our plan is to clone all three cellulase genes and determine their base sequences, their regulatory signals for transcription,

translation, and secretion processes, and their evolutionary relationships. We will isolate and characterize high and low copy number Clostridium plasmids in order to develop specific expression and cloning vectors for the analysis of genetic regulatory signals under homologous environments. We will develop efficient methods for transforming recombinant DNA and plasmids into Clostridium cells and to test for recombination, gene conversion, and other genetic processes. We will test the ability of other gram positive plasmids (e.g. from Bacilli) to replicate and express heterologous genes in Clostridium and determine the feasibility of expressing heterologous genes in this species. This Clostridium sp. should be a good model system for studying gene expression in an anaerobic organism and it has the potential capability for efficiently converting cellulosic biomass into useful energy sources.

17. **UNIVERSITY OF CALIFORNIA** - Davis, CA 95616

Physiological Genetics of Denitrification: A Route to Conserving Fixed Nitrogen
J.L. Ingraham, Department of Bacteriology $62,000

Denitrification is the biological process by which certain bacteria reduce fixed nitrogen in the form of nitrate or nitrite to the gaseous products N_2O and/or N_2 and thereby deplete the available nitrogen in terrestrial and aquatic environments. Many aspects of this process including the role of some of the proteins that participate in it and the mechanism by which denitrification genes are expressed remain to be elucidated. We will continue our studies on these aspects using physiological genetics as an approach and Pseudomonas stutzeri as a test organism. P. stutzeri is an active denitrifier capable of growing rapidly with N_2O and of being naturally transformed by DNA in solution. Analysis of a set of frame-shift mutations that block the last step of the denitrification pathway (the reduction of N_2O to N_2) revealed a new protein (protein a) which, along with the copper-containing N_2O reductase, is essential for this step to occur. Experiments will be done to determine whether this membrane-bound protein is a copper permease or an accessory protein that functions in conjunction with N_2O possibly as an electron transporter or an anchor to attach the reductase to the cytoplasmic membrane. The protein will be purified. By placing a Tn10 element close to the gene encoding protein a attempts will be made to clone this gene into E. coli. Experiments will be done to determine the kinetics of synthesis protein a and other essential denitrification proteins following a shift of a culture from aerobiosis to anaerobiosis.

18. **UNIVERSITY OF CALIFORNIA** - Irvine, CA 92717

Membrane Bioenergetics of Salt Tolerant Organisms
J.K. Lanyi, Department of Physiology and Biophysics $75,000

Aspects of membrane bioenergetics important for salt tolerance are studied in microorganisms which have adapted to high salt concentrations. The goal is to gain insights into the functioning of ionic pumps. Three systems of particular interest to us are examined for similarities and differences in their molecular mechanisms of ion translocation: a) halorhodopsin, a light-activated chloride pump in halobacteria, b) halobacterial ion (proton) transport ATPase(s), and c) the quinone reduction-linked sodium transport system of the halotolerant pseudomonad, Bal. We will continue our structural and functional studies with halorhodopsin, which is already available as pure, solubilized protein in large quantities. We will develop purification and reconstitution schemes for the other two systems, and begin to identify the components of these oligomeric proteins, as well as elucidate their individual catalytic, transport and regulatory functions in the complexes they form.

19. **UNIVERSITY OF CALIFORNIA** - La Jolla, CA 92093

Characterization and Biosynthesis of Complex Protein-Bound Carbohydrates
M.J. Chrispeels, Department of Biology $73,000

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 great variety of complex glycans. We have studied 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. We are now applying this general knowledge to the specific protein invertase. Invertase is an important enzyme in carbohydrate metabolism which occurs both in the cell wall and in the vacuole of plant cells. How does the same enzyme arrive at 2 different locations? We are presently studying the possibility that the glycans of cell wall and vacuolar invertase are different, and that these differences are important for the transport and targeting of this enzyme.

20. **UNIVERSITY OF CALIFORNIA** - La Jolla, CA 92093

Identification and Manipulation of Rhizobium Phytohormone Genes
G. Ditta, Department of Biology $76,000

Nodule development during Rhizobium-legume symbiosis involves specific changes in the growth pattern of root cortical cells. It has been known for many years that Rhizobium species can produce both auxin (indoleacetic acid; IAA) and cytokinin during vegetative (asymbiotic growth) but it is not known whether phytohormones produced by the invading bacteria play a role in this process. The enzymatic functions of primary importance for auxin and cytokinin biosynthesis by Rhizobium are also unknown. We are attempting to answer such questions by obtaining Rhizobium mutants defective in auxin production. Physiological and biochemical studies indicate that production of indolepyruvate from tryptophan by aminotransferase activity is likely to be the primary route for IAA biosynthesis in R. meliloti. Four distinct aromatic aminotransferases have been identified in R. meliloti. Genes for two of these have been mutated, leading in one case to a reduction in the ability of cells to convert exogenously added tryptophan to IAA. The symbiotic phenotype of the mutants is normal. We are currently attempting to isolate mutations in genes for the remaining two enzymes.

An alternative experimental strategy has been to create strains of R. meliloti that overproduce auxin from constructed plasmids and to evaluate the symbiotic competence of such strains. Despite a 150 fold increase in IAA production and production of a tsr (thick short root) phenotype on alfalfa, such strains have been found to be capable of forming a normal symbiosis.

21. **UNIVERSITY OF CALIFORNIA** - Los Angeles, CA 90024

Energy Capture and Use in Plants and Bacteria
P.D. Boyer, Molecular Biology Institute $94,000

This project concerns how plants use energy from light, and bacteria energy from oxidations, to make ATP, the "currency" of the cell. Present studies focus on the function of the subunits of the complex membrane-spanning ATP synthase. Emphasis is on unraveling the catalytic and control functions of the up to six adenine nucleotide bindings sites on the enzyme. Present approaches include the labeling and identification of catalytic and noncatalytic sites through use of 2-azido ATP and ADP. These are excellent substrate analogs and photolabeling in high yield is obtained. Preliminary results indicate two binding loci on beta subunits although one may be interfacial with the alpha subunit. Identification of specific sites of labeling is being correlated with kinetic and ^{18}O exchange studies that

have promise of clarifying control functions of nucleotide binding and providing further assessment of the binding change mechanism with sequential participation of three catalytic sites. Identification of binding loci will guide site-directed mutagenesis studies now underway with the E. coli enzyme. Other studies with the E. coli enzyme are assessing occurrence of catalytic subunit positional interchange as catalysis proceeds.

22. **UNIVERSITY OF CALIFORNIA** - Los Angeles, CA 90024

Molecular Biology and Genetics of the Acetate-Utilizing Methanogenic Bacteria
R.P. Gunsalus, Department of Microbiology - 5304 LS $70,042

Methanogenic acetate degradation is a primary rate limiting step in anaerobic degradative processes yet gene regulation of this pathway has not been studied because representative species have not been available that are practical for genetic study. A recently described acetate-utilizing species, Methanosarcina acetivorans, is uniquely suited for genetic studies because unlike previously described acetotrophic methanogens that have a thick heteropolysaccharide cell wall, this species has a protein cell wall that can be gently disrupted to obtain protoplasts or lysed to yield intact DNA. Our laboratory is conducting basic experiments to develop a gene transfer system in this bacterium. Methods are being developed for the efficient plating of M. acetivorans on defined media, chemical mutagenesis, and the isolation of mutants. M. acetivorans and a number of related isolates have been screened for the presence of plasmid DNA that may serve as a gene shuttle vector. A plasmid has been isolated from M. acetivorans. This plasmid has characteristics that could make it useful for construction of a shuttle vector. Once a plasmid shuttle vector is made, genetic markers will he created using Escherichia coli gene libraries and mutants of M. acetivorans. Carbon monoxide dehydrogenase appears to be associated with the acetotrophic pathway and is regulated in response to the availability of alternative substrates for methanogenesis. Chromosomal libraries have been constructed from M. acetivorans which will be used to select for and clone carbon monoxide dehydrogenase. Once cloned, we will begin studies on the molecular mechanisms that control this gene. These studies should aid our understanding of the mechanisms of acetate regulation by M. acetivorans and serve as a model for gene regulation by other acetotrophic methanogens.

23. **UNIVERSITY OF CALIFORNIA** - Riverside, CA 92521

Catalytic Mechanism of Hydrogenase from Aerobic N_2-Fixing Microorganisms
D.J. Arp, Department of Biochemistry $47,000

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, hydrogen 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. We are interested in determining the role of the metals and each of the subunits in catalysis. The experimental approaches we are using include determination of the effect of subunit dissociation, metal extraction, or protein modification (e.g. modification of sulfhydryls) on the various activities of these enzymes (H_2 oxidation, isotope exchange, H_2 evolution). The results of this project will provide insight into the catalytic mechanism of H_2 oxidation by Rhizobium and Azotobacter hydrogenases that, in turn, will lead to a better understanding of H_2 cycling in these organisms. This information is important to genetic engineering of new strains and may lead to improved techniques for selecting the best strains of Rhizobium for inoculation of legumes.

24. **UNIVERSITY OF CALIFORNIA** - Riverside, CA 92521

Mechanisms of Flooding Tolerance in Roots and Leaves of Maize, Studied using in vivo NMR Spectroscopy
J.K.M. Roberts, Department of Biochemistry $100,000 (two years)

Ethanol is the major fermentation end-product in hypoxic corn roots. If ethanolic fermentation is blocked, by mutation in the Adhl locus so that ADH activity is greatly reduced, roots exhibit reduced tolerance of hypoxia. Our previous studies have implicated cytoplasmic acidosis, due to fermentation to lactic acid instead of ethanol, as the factor which reduces viability in these mutant roots under hypoxia. Leaves of wild-type corn are much less tolerant of hypoxia than are roots. And like the Adhl mutant above, leaves contain much less alcohol dehydrogenase (ADH) than normal corn roots. We have examined metabolism in corn leaves using in vivo ^{31}P nuclear magnetic resonance (NMR). In the dark, and under

oxygenated conditions, cytoplasmic pH in leaf cells is alkaline. However, under hypoxia cytoplasmic pH quickly decreases by more than 1 pH unit. Thus, the relative intolerance of leaves to hypoxia can be attributed to cell damage resulting from severe cytoplasmic acidosis. We are currently investigating the effects of nitrogen nutrition on this acidification reaction in corn leaves. We are also modifying our spectrometer to permit extension of *in vivo* NMR methods to the study of the photosynthetic reactions in leaves.

25. **UNIVERSITY OF CALIFORNIA** - Santa Cruz, CA 95064

Tonoplast Transport and Salt Tolerance in Plants
L. Taiz, Division of Natural Sciences $64,000

The vacuole, by sequestering excess ions, plays a critical role in plant salt tolerance. Halophytes have been shown to have an enhanced capacity for vacuolar NaCl accumulation compared with glycophytes. The differences in vacuolar transport capacities could be due to differences in the primary proton pumps and/or carrier proteins. We are characterizing the tonoplast H^+-ATPase of the glycophyte, *Zea mays*, for future comparison with the tonoplast H^+-ATPase of a halophyte. We have shown that the maize enzyme is large, M_r ~400 kDA, consisting of three major subunits: 72 kDa, 62 kDa and 16 kDa. On the basis of immunological and inhibitor-binding studies we have concluded that the 72 kDa subunit is the catalytic subunit, while the 16 kDa subunit makes up the proton channel. Antibodies to the 72 kDa and 62 kDa subunits are currently being used to screen a lambda gt11 cDNA library. The cloned cDNA's will be sequenced and the amino acid sequences derived. Radioactively labeled cDNA probes will be used to study gene expression during salt treatment. In addition, the antibodies are being used to localize the enzyme within cells and in different tissues by EM immunocytochemistry as a function of salt treatment. A putative Na^+/H^+ antiporter has also been detected in tonoplast vesicles derived from maize mesocotyls. Attempts to purify this antiporter are underway.

26. **UNIVERSITY OF CALIFORNIA** - Santa Cruz, CA 95064

Carbon Dioxide and the Stomatal Control of Water Balance and Photosynthesis in Higher Plants
E. Zeiger, Division of Natural Sciences $75,000

This research project studies the stomatal responses to carbon dioxide and their interaction with other environmental signals modulating stomatal movements. Work with suspensions of *Vicia* guard cell protoplasts has characterized a red light-induced medium alkalinization that, in photosynthetically competent preparations, is diagnostic for photosynthetic

carbon fixation. The response was coupled to oxygen evolution and it was blocked by DCMU. These results suggested to us that the current concept of a lack of the photosynthetic carbon reduction pathway (PCRP) in guard cells needs reexamination. We analysed the fate of radiolabelled carbon dioxide supplied to Vicia guard cell protoplasts irradiated with red light, with paper and thin layer chromatography. Rates of carbon fixation were 5- to 8-fold higher in the light than in the dark. Malate and aspartate had up to 90% of the total radioactivity in the dark; in the light, 3-PGA, sugar monophosphates and sugar diphosphates had up to 60% of the label. Phosphatase treatment and rechromatography of labelled sugar diphosphates showed the presence of ribulose, a specific PCRP metabolite. In time-course experiments, labelled 3-PGA was detected within 5 s. These results provide strong evidence for the operation of the PCRP in guard cells. The modulation of the PCRP by light quality and its role in the sensing of carbon dioxide by guard cells is being currently investigated.

27. **CARNEGIE INSTITUTION OF WASHINGTON** - Washington, DC 20008

Isotope Fractionation During Oxygen Production and Consumption by Plants

M.L. Fogel, Geophysical Laboratory, Washington, D. C.
J.A. Berry, Department of Plant Biology, Stanford, CA

$56,000-two years (FY86 funds)

The $^{18}O/^{16}O$ of atmospheric oxygen is 1.023 times that of seawater (i.e. the atmosphere is enriched in ^{18}O by +23 ‰). Known as the Dole effect, this difference is thought to result from isotopic discrimination during photosynthetic oxygen production and respiratory oxygen consumption. A few experiments conducted with microorganisms seem to confirm this explanation. Knowledge of the fractionation of oxygen isotopes in the important reactions of the oxygen cycle, however, is incomplete. Studies of isotopic fractionation by plants in reactions which produce or consume oxygen are being conducted under this project. Our studies show that illuminated spinach thylakoids supplied with $K_3(FeCN)_6$ or Anacystis nidulans cells provided with CO_2 produce oxygen that is very similar to that of the source water ($\Delta = 0.13 \pm 0.27$ ‰), when rigorous precautions are taken to avoid simultaneous oxygen uptake reactions. In contrast when Asparagus sprengeri mesophyll cells are illuminated in a closed vessel at their O_2 and CO_2 compensation point, the oxygen in the vessel comes to a steady-state composition of about +22 ‰ relative to the source water. Substantial fractionation occurs in the reactions of photorespiration. The glycolate oxidase reaction (conducted with or without catalase) had a fractionation value (α) of 21.9 ‰. Further experiments are in progress to measure simultaneously the oxygen and carbon isotope fractionation during O_2 uptake and CO_2 fixation, respectively, by Rubisco. Corresponding studies of

isotope fractionation by intact leaves in a gas-exchange system will also be conducted under different CO_2 and O_2 concentrations and at different temperatures to extend the understanding of the environmental regulation of these processes. Studies with respiration indicate a large variation among organisms. Yeast has a fractionation factor of $\alpha = 16.0$, whereas whole alfalfa sprouts had an α of 21.5 ‰ and Asparagus cells had an α of 20.8 ‰. Preliminary results show that respiration in the presence of cyanide results in greater fractionation indicating a possible difference in the mechanism of oxygen uptake. These studies have significance in an improved understanding of the oxygen cycle and may lead to further possible applications of stable isotope methods to plant biology.

28. **UNIVERSITY OF CHICAGO** - Chicago, IL 60637

Organization and regulation of the genes for nitrogen fixation in Rhodobacter capsulatus
R. Haselkorn, Dept. of Molecular Genetics & Cell Biology $77,085

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. We propose to determine the physical linkage of these fragments, to determine their relationship, if any, with the corresponding nif genes of Klebsiella, and to determine the nucleotide sequence of some of the fragments. So far we have identified five regulatory genes among these, using a nifH::lac fusion. Lac fusions to the regulatory genes themselves have been used to define the regulatory circuit in R. capsulatus. The products of two genes nifR1 and nifR2, are required to turn on a third nifR4. The latter is formally analogous to the nifA gene of Klebsiella but its sequence is different. The nifR1 and nifR2 genes are homologous in sequence with the ntrC and ntrB genes of E. coli and Klebsiella. Current efforts are aimed at constitutive expression of nifR4 in R. capsulatus and overproduction of its product in E. coli. Assays for DNA gyrase activity in crude extracts of R. capsulatus have been developed and are being applied to the study of the relation between DNA supercoiling and nif gene expression. The gyrB gene of R. capsulatus has been cloned. Both random and site specific mutagenesis of that gene are being attempted.

29. **COLUMBIA UNIVERSITY** - New York, NY 10032

Regulation and Genetic Organization of Hydrogenase
A.I. Krasna, Department of Biochemistry & Molecular Biophysics, College of Physicians and Surgeons $72,000

Hydrogenase is an enzyme of unique biochemical interest because of the nature of its substrates, H^+ and H_2, which are the simplest stable molecules. The enzyme plays an important role in the anaerobic metabolism of many bacteria and algae; in fermentative reactions, photosynthesis, and nitrogen fixation. The objectives of the research are to elucidate the regulation and genetic organization of hydrogenase. This laboratory has isolated a number of mutant strains of _E. coli_ with altered hydrogenase activity. A 0.9 kb DNA fragment has recently been cloned from an _E. coli_ genomic DNA library which restores hydrogenase activity in a hydrogenase-negative mutant strain of _E. coli_. This gene codes for a polypeptide of subunit mw 36 kd and is required for hydrogenase synthesis but is not a structural gene. It is involved in nickel metabolism. Using a number of different hydrogenase-negative strains of _E. coli_, the hydrogenase structural and regulatory genes of _Chromatium_ and _Proteus vulgaris_ will be cloned and characterized. A gene required for growth of an _E. coli_ mutant on H_2 and fumarate has also been cloned as a 1.4 kb DNA fragment. This gene codes for a polypeptide of subunit mw 30 kd. This mutant strain may synthesize some hydrogenase isoenzymes and not others and the nature of these enzymes and the regulatory mechanisms responsible for their synthesis will be studied.

30. **CORNELL UNIVERSITY** - Ithaca, NY 14853

Anaerobic Metabolism of Aromatic Compounds by Phototrophic Bacteria
C.S. Harwood, Department of Microbiology
J. Gibson, Section of Biochemistry, Molecular and Cell Biology
$49,478

Vast quantities of aromatic compounds in the form of lignin, lignin derivatives, and aromatic pollutants are continually being introduced into the biosphere and much of this material accumulates in anaerobic environments. The objectives of this project are to elucidate anaerobic routes of benzoate and 4-hydroxybenzoate metabolism by the phototrophic bacterium, _Rhodopseudomonas palustris_. Recent evidence suggests that diverse aromatics must first be metabolized to form one or the other of these compounds prior to cleavage of the aromatic ring and so these pathways probably play a general role as major degradative routes. _R. palustris_ is particularly well suited for these studies because its ability to separate carbon metabolism from energy generating mechanisms frees it

from the thermodynamic constraints that restrict the anaerobic metabolism of aromatics by pure cultures of fermentative bacteria. Information obtained in these studies can then be used to develop models which can be tested with nonphototrophic anaerobic cultures and consortia. Studies include identification of the number and specificity of enzymes involved in benzoate and 4-hydroxybenzoate metabolism, identification of cofactors and electron carriers involved in each pathway, and a determination of the precise nature of the products formed. Mutants that are blocked in aromatic metabolism have been isolated and will be used to corroborate the biochemical data. These mutants will also be used, together with physiological approaches, to identify compounds (inducers and repressors) that regulate the expression of genes for aromatic degradation. This work will provide basic information about the biochemistry, regulation, and genetics of anaerobic metabolism of aromatics. At present very little is known about mechanisms responsible for the degradation of this large and quantitatively important group of compounds in anaerobic environments.

31. CORNELL UNIVERSITY - Ithaca, NY 14853

Mechanisms of Inhibition of Viral Replication in Plants
P. Palukaitis, Department of Plant Pathology $72,000

Measures designed to increase plant cultivation or biomass, for the purpose of conversion to fuels and chemicals, must take into account the problems of crop losses caused by pathogens such as viruses, and incorporate control measures for such pathogens into the program. This project is concerned with analyzing the molecular mechanisms of inhibition of viral replication and movement in plants. The RNAs of a broad host-range virus, cucumber mosaic virus (CMV), have been cloned and clones are being characterized. The gene 3a will be inserted into plant genomes via a Ti-plasmid vector, and the transgenic plants will be tested for the ability to potentiate the cell-to-cell movement of unrelated viruses, as well as to interfere or interact with plant genes involved in restricting viral movement. Protoplasts from such plants will be analyzed for the molecular interactions that occur when such viral movement genes interface with plant genes involved in inhibiting the cell-to-cell movement of plant viruses. Preliminary work on viroid movement within plants indicates a mechanism similar to that used by plant viruses. Minus-sense RNA transcripts of cloned segments of the CMV genome will be tested for the ability to block the replication of CMV after co-electroporation into tobacco protoplasts. Segments that are effective in inhibiting CMV replication in the above test, will be inserted into plant genomes and will be assessed for the ability to block the replication of different strains of CMV and of other related viruses; i.e., a test of the anti-sense RNA inhibition mechanism for cross-protection.

32. **CORNELL UNIVERSITY** - Ithaca, NY 14853

Effects of Freezing and Cold Acclimation on the Plasma Membrane of Isolated Cereal Protoplasts
P.L. Steponkus, Department of Agronomy $94,000

The principal 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. Recent studies have focused on i) the role of freeze-induced electrical transients in cryoinjury, ii) dehydration-induced lamellar to $hexagonal_{II}$ phase transitions in the plasma membrane, and iii) the effect of cold acclimation on the lipid composition of the plasma membrane. Currently, the emphasis is directed to establishing a causal relationship between changes in the lipid composition and cryobehavior of the plasma membrane following cold acclimation. Our previous studies demonstrated that the plasma membrane of winter rye has a lipid composition that is quite distinct in that glucocerebrosides are the principal glycolipid (16 mol% of the total lipid) and that sterols and sterol derivatives (steryl glucosides and acylated steryl glucosides) comprise >50 mol% of the total lipids. Following cold acclimation there are numerous changes in the lipid composition of the plasma membrane. One such change is a large proportional decrease in the glucocerebroside content. Calorimetric studies indicate that the phase behavior of the plasma membrane glucocerebrosides is quite different from that of cerebrosides isolated from mammalian tissues and that glucocerebrosides may play an important role in determining the cryobehavior of the plasma membrane. Therefore, we are determining the phase behavior of glucocerebrosides and mixtures of glucocerebrosides and phospholipids (molecular species present in the plasma membrane) as a function of temperature and hydration.

33. **CORNELL UNIVERSITY** - Ithaca, NY 14853

Phytoalexin Detoxifying Enzymes in the Plant Pathogenic fungus *Nectria haematococca*
H.D. VanEtten, D.E. Matthews, Department of Plant Pathology $69,000

The ability of plants to synthesize phytoalexins in response to microbial infection is believed to provide a defense against some potential pathogens. Successful pathogens may overcome this defense by detoxifying the phytoalexins. Genetic studies indicate that the *Pisum sativum* phytoalexin pisatin is effective only against isolates of *Nectria haematococca* which cannot metabolize pisatin, or cannot do so rapidly. This fungus possesses at least six genes for pisatin demethylation, some of

which are distinguishable by the rate of demethylation they confer. Only the more rapid of these phenotypes are associated with virulence towards pea. We are examining the relationship between these genes and the enzyme pisatin demethylase, a substrate-inducible microsomal monooxygenase composed of cytochrome P-450 and the flavoprotein NADPH-cytochrome P-450 reductase. Current genetic and biochemical evidence suggests that the genes for pisatin demethylation are structural genes for cytochrome P-450 isozymes, which may differ quantitatively in their activity towards pisatin as well as in their regulation. Some isolates of N. haematococca can also detoxify maackiain, which is a major phytoalexin produced by chickpea, Cicer arietinum. Maackiain detoxification occurs via three alternative initial reactions, all hydroxylations such as might be catalyzed by cytochrome P-450. Recent work has shown that maackiain hydroxylation by N. haematococca is controlled by multiple independent genes, each of which confers ability to attack a specific carbon atom of the substrate. Most of these genes are independent of those controlling pisatin demethylation, but one of them is so tightly linked to a demethylase gene that they might prove to be a single bifunctional gene. Preliminary results of our genetic studies also indicate that the ability to metabolize maackiain is important for the virulence of this fungus towards chickpea. One of the genes for pisatin demethylase has been cloned, and is being exploited to help characterize the demethylase it encodes as well as other phytoalexin detoxifying enzymes that may be homologous to it.

34. **CORNELL UNIVERSITY** - Ithaca, NY 14853

Studies of the Genetic Regulation of the Thermomonospora Cellulase Complex

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

$72,000

Thermomonospora fusca is a thermophilic soil bacterium that produces large amounts of thermostable cellulase and xylanase activities. The objectives of this project are to purify and characterize any T. fusca cellulases that are present in addition to the five known endoglucanases; to clone and sequence the structural genes encoding each of the major T. fusca cellulases; to crystallize endoglucanase E_5 and if suitable crystals are obtained to arrange to determine the three-dimensional structure of the enzyme by x-ray crystallography. Four different T. fusca cellulase genes have been isolated by cosmid cloning into E. coli and four by plasmid cloning into Streptomyces lividans. These genes include the gene encoding cellulase E_5 and this gene has been partially sequenced. In addition, preliminary evidence indicates we probably have cloned the genes encoding E_1 and E_2. Another gene encodes a protein that is inhibited by E_3 antiserum but differs from E_3 in its other properties. The other two genes

code for enzymes that are clearly distinct from E_1-E_5 and from each other. S. lividans transformants which contain the E_5 gene excrete about 50 mg liter of the enzyme into the culture medium and the excreted enzyme is about 30% pure. Each cloned cellulase gene is being introduced into S. lividans to see if high levels of the enzyme it encodes is produced and excreted. If high levels of any other active cellulase are produced in large amounts, attempts will be made to crystallize those enzymes.

35. **CORNELL UNIVERSITY** - Ithaca, NY 14853

Microbial Ecology of Thermophilic Anaerobic Digestion
S.H. Zinder, Department of Microbiology $83,000

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 ethane. Special attention is focused on formation and breakdown of acetic acid, the precursor of two-thirds of the methane produced by the bioreactor. Among the methods used to study these organisms are: 1) viable counts and culture studies using habitat and niche-simulating media; 2) direct microscopic observation of populations using phase-contrast, epifluorescence, and electron microscopy; 3) $_{14}$C-radiotracer methods to study carbon flow to methane. Recent results include: 1) the isolation of a thermophilic Methanothrix which grows much more rapidly (Td = 24 h) than do mesophilic cultures 2) the demonstration that this culture has high levels of carbon monoxide dehydrogenase, an enzyme implicated in methanogenesis from acetate but has little or no hydrogenase; 3) the isolation in axenic culture of the acetate-oxidizing member of a thermophilic two-membered cuculture which converts acetate to methane using interspecies hydrgeon transfer; 4) the demonstration that the acetate-oxidizer can grow axenically on a mixture of acetate and betaine, and that such cultures oxidize acetate to CO_2 and contain high levels of CO dehydrogenase; 5) the demonstration that the acectate oxidizing organism can also grow on H_2-CO_2 and produce acetate as well as oxidize it; 6) the isolation of a thermophilic sulfate reducer which can apparently grow on benzoate in coculture with a methanogen; 7) the demonstration, using HPLC techniques, that $_{14}$C-labeled glucose was metabolized directly to acetate and CO_2 by populations in the bioreactor with no significant formation of intermediate products. Current research centers on further characterization of the thermophilic Methanothrix and on the role of interspecies hydrogen transfer in biodegradation of acetate and benzoate.

36. **UNIVERSITY OF DELAWARE** - Lewes, DL 19958

Metabolic Mechanisms of Plant Growth at Low Water Potentials
J.S. Boyer, College of Marine Studies $73,000

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 the limitations by focusing on the processes of photosynthesis and cell enlargement. The inhibition of photosynthesis by low leaf water potential was caused primarily by losses in chloroplast activity in sunflower (Helianthus) and could be simulated by preincubating chloroplasts at Mg^{2+} concentrations expected to be present at low water potentials. Further investigations showed that high leaf contents of magnesium caused earlier inhibition than in leaves having low magnesium contents. This suggests that losses in tissue water content could cause high enough concentrations of ions to be inhibitory to chloroplast activity in intact leaves. Our studies of cell enlargement showed that, in localized growing regions, photosynthate accumulated and maintained turgor completely at moderately low water potentials. These regions contained gradients in water potential because of a large frictional resistance to water movement from the veins to the enlarging cells. The primary signal causing decreased growth was a decrease in the gradient. This was followed by a loss in cell wall extensibility and conductance of the tissue for water, which eventually contributed to the growth limitation. This work shows that, although growth may be inhibited in some tissues by turgor loss, growth also can be inhibited by factors that affect water delivery to the cells. The metabolic changes that follow provide an opportunity to alter the growth response to these conditions.

37. **DUKE UNIVERSITY** - Durham, NC 27706

Molecular Studies of Functional Aspects of Higher Plant Mitochondria
J.N. Siedow, Department of Botany $63,311

Both cytoplasmic male sterility and sensitivity to a toxin (T-toxin) derived from the fungus, Bipolaris maydis, race T, in cms-T lines of maize appear to be associated with a unique mitochondrially-encoded 13 kDa protein. A major goal of this research is to ascertain the mechanism by which the 13 kDa protein promotes male sterility and sensitizes the inner mitochondrial membrane to T-toxin. To this end, the topographic orientation of the 13 kDa protein will be ascertained using protease treatments and membrane impermeant labeling reagents on right side- and inside-out mitochondrial membrane vesicles. Additional studies will attempt to determine which, if any, mitochondrial membrane protein complex the 13 kDa protein specifically associates with, and the extent to which

that complex's functional properties are affected by the presence of the protein. Experiments performed to date indicate that there is an association of the 13 kDa protein with purified maize cytochrome c oxidase. Future studies will consider the extent to which this association 1) affects the function of isolated cytochrome oxidase and 2) is specific for cytochrome oxidase and not simply the result of a tendency for the 13 kDa protein to nonspecifically bind to mitochondrial protein complexes. Additional studies of the mode of action of the 13 kDa protein involve the development of a suitable vector for the expression of the protein in _E. coli_ (carried out in C.S. Levings' laboratory, N.C. State University). Initial experiments indicate that both respiration and growth are totally inhibited by low levels of T-toxin (or the toxin analogue, methomyl) when the 13 kDa protein is expressed in _E. coli_. Future work will be oriented toward establishing the exact site and mechanism of action of this respiratory inhibition. A second line of experimentation is oriented toward characterizing the nature of the cyanide-resistant respiratory pathway in higher plant mitochondria. Initially, a series of studies will be undertaken to attempt to determine the molecular basis for the maternally inherited loss of cyanide-resistant respiration in the pea variety, Progress No. 9. These initial studies will include comparison of the restriction endonuclease patterns obtained with mitochondrial DNA from Progress No. 9 and a second variety, Alaska, that displays the cyanide-resistant pathway. Attempts will also be made to identify products of mitochondrial transcription that are specifically associated with either the presence (or absence) of the cyanide-resistant pathway.

38. **FLORIDA STATE UNIVERSITY** - Tallahassee, FL 32306

Guard Cell Biochemistry: Response to Environmental Stimuli Causing Changes in Gas Exchange
W.H. Outlaw, Jr., Department of Biological Sciences $95,000

The aperture size of stomatal guard cells in leaves 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, resulting from accumulation of solutes (K^+ and, to a lesser extent, Cl^-) from the apoplast and synthesis of low MW substances (e.g., malate) from osmotically inert substances (e.g., starch). The specialized metabolism that these cells have evolved to fulfill their crucial role in the plant's physiology extends to more subtle aspects, which this project studies. Despite the presence of green plastids, which conduct linear electron transport, guard cells lack the ability to photosynthetically reduce significant quantities of CO_2. To clarify its role in guard cells, the organization of PS II has been investigated by studying the kinetics of the Chl a fluorescence rise in DCMU-poisoned

single cell pairs upon illumination. (These results have been correlated with ultrastructure of the guard cell plastids determined in other studies.) Presumably, ABA is an endogenous signal that mediates stomatal closure, but little was known regarding the presence of ABA or of any enzyme specific for its metabolism in guard cells *in situ*. Using a sensitive immunological assay, we have measured ABA in various types of cells from control and water-stressed leaves. On another DOE-support project, we have assayed Rubisco by microgel electrophoresis, an approach that required instrumentation development. Our ongoing projects are: (1) kinetic analysis ABA accumulation in various leaf compartments, and (2) (micro) characterization of PEPC in guard cells.

39. **UNIVERSITY OF FLORIDA** - Gainesville, FL 32611

Regulation of Glycolysis in *Zymomonas mobilis*: The alcohologenic enzymes

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

$92,000 (FY86 funds, two years)

Two enzymes, pyruvate decarboxylase (E.C. 4.1.1.1) and alcohol dehydrogenase (E.C. 1.1.1.1), function as the primary NAD^+ regeneration system for the homofermentative production of ethanol by *Zymomonas mobilis* and *Saccharomyces cerevisiae*. A similar NAD^+ regeneration system is active in many plant roots and is essential for plant survival under anoxic conditions. The non-oxidative decarboxylation of pyruvate represents the first committed step in this process. The enzyme responsible, pyruvate decarboxylase, is present in large amounts and represents approximately 4% of the total soluble protein of *Z. mobilis* and *S. cerevisiae* during fermentative growth. This enzyme is rare among the genera of bacteria. The evolution of replacement enzymes has led to the production of a variety of mixed fermentation products in other microorganisms. *Z. mobilis* contains two alcohol dehydrogenase isozymes, both of which are expressed at high levels and appear to function together during the reduction of acetaldehyde to ethanol.

In the current study we are investigating the regulatory mechanisms which permit high levels of expression of the ethanologenic enzymes (PDC, ADH I and ADH II) in *Z. mobilis*. These studies involve enzyme purification and the production of antibodies, investigations on the influence of growth conditions on the levels of expression, cloning and characterization of structural genes, construction of alcohol dehydrogenase hybrid genes, mutation of alcohol dehydrogenases, and investigations of transcriptional and translational regulation. In addition, we are investigating the feasibility of replacing the NAD^+ regeneration systems of other bacteria with an artificial operon containing *Z. mobilis* genes for ethanol production (pyruvate decarboxylase and alcohol dehydrogenase II), the "PET" operon.

40. **UNIVERSITY OF FLORIDA** - Gainesville, FL 32611

Gene-Enzyme Relationships in Somatic Cells and Their Organismal Derivatives in Higher Plants
R.A. Jensen, Department of Microbiology and Cell Science $97,000

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 level. We have shown that an intact aromatic pathway which proceeds to L-phenylalanine and to L-tyrosine via L-arogenate exists in the plastid 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. 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.

41. **UNIVERSITY OF GEORGIA** - Athens, GA 30613

Studies on Oligosaccharins: Carbohydrates Possessing Biological Regulatory Activities
P. Albersheim, Complex Carbohydrate Research Center $165,000

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 plant cell walls, regulate various biological functions within the plant. We are studying the following oligosaccharins: [1] Oligosaccharins isolated from plant cell walls that elicit phytoalexin (antibiotic) accumulation in plant tissues. Elicitors of phytoalexins from dicotyledon and monocotyledon cell walls are being studied: bioassay systems using both monocotyledon and dicotyledon plant tissues are being used in the studies. [2] An oligosaccharin that may be a trigger for the hypersensitive-resistance response in plants. We are purifying enzyme(s) secreted by Pyricularia oryzae that release this oligosaccharin from plant cell walls. [3] Oligosaccharins that can induce flowers, roots, vegetative buds, and callus 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 bud development, and callus development in these tissues. [4] Oligosaccharins capable of determining the sex of flowers in the dioecious plant Mercurialis annua.

The above examples are the major areas of study in our laboratory that concern the identification and characterization of oligosaccharins. We are also collaborating with many laboratories around the world to identify oligosaccharin activities in other biological systems.

42. **UNIVERSITY OF GEORGIA** - Athens, GA 30613

Development of Methods to Structurally Characterize Complex Carbohydrates
P. Albersheim, A. Darvill, Complex Carbohydrate Research Center
$145,000

This research focuses on the development of methods to structurally characterize complex carbohydrates isolated from microorganisms, plants, and animals. We are using a high-field FAB mass spectrometer and a 500-MHz NMR spectrometer, among other equipment, to develop new methods to study complex carbohydrates from a variety of perspectives: this approach is necessary in order to obtain eventually a complete three-dimensional picture of the molecules. We are also attempting to develop methods 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 our structural investigations include the development of chemical reactions for specifically cleaving complex carbohydrates and techniques for labeling oligosaccharides to allow rapid and highly sensitive detection during purification. For example, we are investigating methods for attaching fluorescent and UV-absorbing tags to the reducing end of oligosaccharides, while attempting to retain any biological activity of the complex carbohydrate being studied.

Included in this project has been the formulation of a computer program designed to serve the needs for an international carbohydrate structure data base given the name "Carbank". With the recent endorsement of the computer program by the board of overseers of the data bank the inputting of structures is to begin in the coming year. Carbank is to be based at this institution at least for the next two years.

43. **UNIVERSITY OF GEORGIA** - Athens, GA 30613

Structural Studies of Complex Carbohydrates in Plant Cell Walls
A. Darvill, Complex Carbohydrate Research Center $ 200,000

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 cell-wall polysaccharides, rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II). 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 3-deoxy-lyxo-2-heptulosaric acid. Although RG-I contains only five glycosyl residues, it still 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 discreet families of side chains are attached to the RG-I backbone. We are also studying the cell walls of monocotyledons and gymnosperms. So far, our studies of the polysaccharides in these cell walls show that, although the quantities of the polysaccharides in these cell walls vary greatly, every cell wall contains the same array of polysaccharides.

44. **UNIVERSITY OF GEORGIA** - Athens, GA 30613

Establishment of the University of Georgia/Department of Energy Complex Carbohydrate Research Center
P. Albersheim, Complex Carbohydrate Research Center

$699,550

This program is aimed at meeting at least a part of the need for work on defining the structures of plant and microbial carbohydrates. The program is intended to serve as a national resource for basic research in complex carbohydrate science with its multidisciplinary faculty and staff. There are research, training, and service activities planned as components of this program. The research will focus 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. In time, training courses and workshops will be offered to scientists from other institutions who are interested in learning more about carbohydrate structure analyses. The service activity will involve conducting routine analyses of carbohydrate samples provided by other scientists as well as collaborations with other scientists that may involve visiting the Center for short or longer periods depending on the complexity of the problems. These collaborative investigations will be on non-proprietary research. Prior correspondence is requisite to these service activities.

45. **UNIVERSITY OF GEORGIA** - Athens, GA 30602

Genetics and Biochemistry of Surfactant Synthesis in *Arthrobacter* species H13-A
W.R. Finnerty, Department of Microbiology $82,500

The biosynthesis of extracellular biosurfactant by *Arthrobacter* species H-13A is under study with respect to the biochemistry and mechanisms of genetic transfer in this genus. The biosurfactant is a glycolipid consisting of glycerol, trehalose, glucose plus normal and 2-hydroxy fatty acids. A fatty acylase activity has been detected using deacylated glycolipid as acceptor. An efficient plasmid transformation system has been developed in *Arthrobacter* H-13A using the recombinant shuttle vector pMVS301. Transformation frequencies average greater than 10^5 transformants per μg DNA. Heterologous antibiotic resistance markers encoded on pMVS301 are expressed in H-13A. pMVS301 is stable in H-13A and *E. coli* exhibiting less than 1% loss per generation under non-selective conditions. A restriction map of pMVS301 demonstrated 14 unique restriction sites, 8 of which are present in the 3.8 Kb Hind III fragment containing the *Arthrobacter* origin of replication. pMVS301 is readily transformed into *E. coli*, *Arthrobacter* H-13A and a number of coryneform and nocardioform bacteria, including various species of *Nocardia* and *Rhodococcus*. Transpositional mutagenesis with Tn917 (an erythromycin resistance transposon) has been developed in H-13A for the purpose of isolation of mutants in the glycolipid biosynthetic pathway. Antibody to cellular and extracellular glycolipid and an ELISA assay has been developed to detect and quantify antiglycolipid antibodies. The immune sera exhibits specificity to the deacylated glycolipid backbone, indicating a common antigenic determinant. The antibody reacts with glycolipid-positive cells but not with glycolipid-negative cells. This is the first report of plasmid transformation and of foreign gene expression in *Arthrobacter* species.

46. **UNIVERSITY OF GEORGIA** - Athens, GA 30602

Environmental Stress-Mediated Changes in Transcriptional and Translational Regulation of Protein Synthesis in Crop Plants
J.L. Key, Department of Botany $91,000

The role of high temperature stress (high shock, HS) and other environmental stress agents in regulating protein synthesis in a range of crop plants is under investigation. Several aspects of the HS response are being analyzed in detail: 1) complexity of HS gene families and nucleotide sequence analysis of a significant number of the HS genes of the various families, 2) regulatory events associated with the transcriptional

activation of the HS genes and with the cessation or autoregulation of HS mRNA production under various HS regimes, 3) the mechanism(s) of the acquisition of thermotolerance during a permissive HS to a subsequent otherwise lethal HS, emphasizing analyses of the role of HS proteins in this phenomenon, and 4) determination of the cis and trans regulatory elements involved in transcriptional regulation of HS genes. Attempts will be made to isolate regulatory mutants which are altered in transcription activation,

To date about 10 HS genes and a number of cDNAs have been sequenced. Considerable progress has been made in gaining some insights into thermotolerance, but the complexity of this phenomenon is only now being understood. Functions of HS proteins, structural and/or enzymatic, will be sought, including cellular localization with antibodies to HS proteins etc. Transformation (using Agrobacterium vectors) analyses using designed HS cassettes and various sense/antisense constructs of HS genes will be used as a part of these studies.

47. **UNIVERSITY OF GEORGIA** - Athens, GA 30602

Soybean Ribulose Bisphosphate Carboxylase Small Subunit Gene Family: Gene Structure and Regulation of Gene Expression
R.B. Meagher, Department of Genetics $75,000

The effects of white light and far-red light on transcription rates and steady state mRNA levels of two closely related soybean RuBPCss genes, SRS1 and SRS4, have revealed a system of light regulated RNA turnover. Transcription of these genes is 30 to 50 fold higher in fully light grown seedlings than in etiolated seedlings. In contrast, steady state mRNA levels are only 8 to 10 fold higher in light than in darkness. Furthermore, when etiolated seedlings are placed in light, RuBPCss transcription increases to the level found in light grown seedlings within 12 hours, but the steady state mRNA levels of these genes do not begin to increase for at least 24 hours. We therefore hypothesize that light induction of RuBPCss transcription in soybean is paralleled by a significant increase in the mRNA turnover rate, perhaps related to the concomitant increase in the rate of translation. Interestingly, the high turnover rate appears to be maintained for some time after light grown plants are treated with far-red light, even though this treatment rapidly shuts down RuBPCss transcription. We plan to identify the mechanism(s) controlling mRNA stability of these soybean genes. This is important because the correct expression of foreign genes in higher plants will require not only sufficient and controlled transcription, but also appropriate patterns of mRNA stability.

48. **UNIVERSITY OF GEORGIA** - Athens, GA 30602

Microbiology and Physiology of Anaerobic Fermentations of Cellulose
H.D. Peck, Jr., L.G. Ljungdahl, L.E. Mortenson, J.K.W. Wiegel,
Departments of Biochemistry and Microbiology
$455,000 (17 months)

This project involves the biochemistry and physiology of four major groups (primary, secondary, ancillary and methane bacteria) of anaerobic bacteria, that are involved in the conversion of cellulose to methane or chemical feedstocks. The primary bacterium, Clostridium thermocellum, has a cellulolytic enzyme system capable of hydrolyzing crystalline cellulose and consists of polypeptide complexes ranging in M_r from 5 to 100 million. The complexes attached to the substrate cellulose with the aid of a low molecular (about 1000 daltons) yellow affinity substance (YAS) produced by the bacterium in the presence of cellulose. Properties of the complexes and YAS are studied. Research on the secondary and ancillary bacteria includes acetogens, clostridia, and sulfate reducing bacteria (SRB). Aspects of metabolism are being studied which appear to be relevant for the interactions on consortia and their bioenergetics, particularly related to hydrogen, formate, CO, and CO_2. The molecular basis of interspecies H_2-transfer and H_2-cycling, electron-transfer proteins, ATPase system and enzymes of one-carbon metabolism will receive special focus. Most bacteria appear to produce two or more different proteins with hydrogenase activity which are presumed to be regulated by conditions of growth. Five different hydrogenases have been characterized in detail from the bacteria of interest: The O_2-labile 12Fe bidirectional hydrogenases from C. pasteurianium and A. woodii; the O_2-labile 8Fe uptake hydrogenase from C. pasteurianium; 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. The structure of the metal clusters and their roles in the activation of H_2 are being investigated, and genes for the hydrogenases are cloned to obtain structural information, and structural relationships among the hydrogenases. The goal being the understanding of the roles and regulations of hydrogenases in interspecies H_2 transfer, H_2 cycling and the generation of a proton gradient. The formate dehydrogenases have characteristics in common with the hydrogenase system: multiple enzyme species with different metal redox centers, MoSeFe, MoFe, WSeFe and pterin; cytoplasmic and periplasmic localizations and involvement in the generation of a proton gradient by vectorial electron transfer. The structures of the metal clusters and their role in the metabolism of formate will be investigated with the goal of understanding the function of formate in the total synthesis of acetate from CO_2 and its role in the bioenergetics of these microorganisms. CO dehydrogenase, a key enzyme in the new anaerobic autotrophic CO_2 fixation pathway contains Ni plus non-heme iron and the structure of its metal redox centers will continue to be investigated. Additionally, the enzyme studies will be performed using thermophiles

also the isolation of some new pertinent species. The project will also include research on the mechanism of extreme thermophily (growth over 70°C) in bacteria that grow over a temperature span of 40°C or more. These bacteria exhibit a biphasic growth response to temperature and preliminary evidence suggests that the phenomenon is due to the expression of a new set of enzymes. These initial observations will be extended employing techniques of molecular biology.

49. **UNIVERSITY OF GEORGIA** - Athens, GA 30602

Molecular Characterization of Phytochrome from Green *Avena*
L.H. Pratt, Department of Botany $58,500

Plants create new biomass via the photosynthetic conversion of solar to chemical energy. They also sense both the quantity and the wavelength distribution of incident radiant energy and modify their growth and development in ways that increase the efficiency with which they utilize it in photosynthesis. Phytochrome is the pigment that performs this sensory function. Even though this chromoprotein thereby plays a central role in biomass production, little is known about it as it exists in photosynthetically competent plants. Not only is it present in exceedingly low quantity, but research supported by the DOE has led recently to the discovery that it is quite different from the more abundant phytochrome that is found in dark-grown plants and that is already well characterized. We have recently improved methods for purification of phytochrome from green *Avena* and have produced six monoclonal antibodies that are directed to and relatively specific for this chromoprotein. With these antibodies, we are beginning to answer the following questions. Are phytochromes from dark- and light-grown *Avena* products of the same or different genes? Are there in *Avena* even more than these two kinds of phytochrome that have so far been identified? How do these different phytochromes differ from one another? What is their temporal and spatial distribution throughout the life cycle of a plant grown in e natural environment? Collectively, these investigations will contribute to an increased awareness of how, in photosynthetically competent plants, plant growth and development is

50. **UNIVERSITY OF GEORGIA** - Athens, GA 30602

Nitrogen Control of Chloroplast Development
G.W. Schmidt, Department of Botany, University of Georgia $91,844

Nitrogen deficiency characteristically results in chlorosis, indicating that, directly or indirectly, chloroplast biogenesis is strongly influenced by the presence of nitrogen assimilates. Our goal is to define the molecular basis of this effect and to characterize the physiology of cells adapted to deficiency conditions. By growing the green alga *Chlamydomonas reinhardtii* in continuous culture systems, nitrogen availability can be precisely manipulated. At steady-state deficiency conditions, the cellular levels of chlorophylls *a* and *b* and xanthophylls are severely depressed while starch and lipid triglycerides are remarkably high. Also, thylakoid membranes from nitrogen-limited cells are substantially altered, particularly with regard to the levels of apoproteins for antenna complexes. In contrast, nitrogen limitation has rather minor effects on the levels of electron transport and carbon assimilation proteins. Measurements of protein accumulation indicate that synthesis of light-harvesting polypeptides may be altered at the levels of chloroplast import and/or maturation. Although the posttranslational effects might be modulated by chlorophyll and/or xanthophyll there also are profound effects of nitrogen limitation on the nuclear expression of some, but not all, of the genes encoding chlorophyll *a*/*b*-binding proteins. As determined by hybridization analyses, provision of reduced nitrogen to deficient cultures causes prodigious accumulation of mRNAs encoding antenna polypeptides. Investigations are in progress to further define the molecular basis for nitrogen-regulated synthesis of the proteins involved in lipid, starch and antenna complexes.

51. **UNIVERSITY OF GEORGIA** - Athens, GA 30602

Transcriptional Analysis of the *R* Locus of Maize
S.R. Wessler, Department of Botany $100,000 (FY86 funds 18 months)

The *R* locus controls where, when and how much anthocyanins are expressed in the corn plant and seed. Enormous natural variation has been seen when different *R* alleles are compared in a common genetic background. Some alleles have been shown to have a compound structure resulting from gene duplication and divergence. In these complex alleles, each member of the duplication (called *R* genic elements) has a unique pattern of expression. The function of the *R* locus is not known; genetic and biochemical analyses suggest that it may encode a protein that regulates other genes in the anthocyanin pathway.

The goals of our lab and our collaborators at the University of Wisconsin (Kermicle) and Yale University (Dellaporta) are to understand what the locus encodes, what the structure of the genic elements are and what is responsible for the different tissue specificities displayed by each genic element. Our contribution to this collaboration is to characterize the transcription unit(s) of the three genic elements (P), (S) and (Lc) and to quantify their tissue specific pattern of expression. To this end, element specific cDNAs will be isolated and sequenced. A comparison with the genomic sequences as determined by Dellaporta will help define each element. In collaboration with Dellaporta, the R protein(s) will be overproduced in E. coli and we will isolate specific antiserum to be used in conjunction with a Northern blot analysis to help elucidate tissue specific expression.

52. **UNIVERSITY OF GEORGIA** - Tifton, GA 31793

Development of Innovative Techniques That May be Used as Models to Improve Plant Performance
W.W. Hanna, G.W. Burton, Department of Agronomy $39,115

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 cytoplasmic backgrounds, ploidy levels, and chromosome numbers crossed and backcrossed with different genotypes of diploid and tetraploid pearl millet, P. americanum, to produce partially fertile interspecific hybrids and derivatives. We have shown how valuable germplasm can be masked on certain genomes by other genomes and stored in a perennial or vegetatively propagated wild species such as P. purpureum. We have been able to transfer this hidden or stored germplasm to cultivated pearl millet and are in the process of developing high-yielding grain types from it. Partially fertile and obligate apomictic derivatives have been produced between pearl millet and P. squamulatum and show potential for transferring apomixis from the wild to cultivated species to fix hybrid vigor. Both cytogenetic and cell culture techniques are being tested to transfer the genes controlling apomixis. Diverse cytoplasms from the wild species have been transferred to pearl millet and are in the initial stages of evaluation. The overall impact is on increased, more efficient, and more reliable production of food, fiber, and forage.

53. **HARVARD UNIVERSITY** - Cambridge, MA 02138

Unravelling Photosystems
L. Bogorad, Dept. of Cellular & Developmental Biology $96,000

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 in the membrane. This information is essential for understanding how the photosynthetic apparatus converts light energy into electrical potential energy and chemical bond energy. Cyanobacteria are relatively simple organisms that carry out the same type of oxygen-evolving photosynthesis as chloroplasts of higher green plants but are more convenient for certain experimental procedures. Unlike chloroplasts at the present time, cyanobacteria can be used readily for genetic engineering experiments through introduction of new DNA. One barrier to studying the molecular nature of mutations in the photosynthetic apparatus in cyanobacteria has been the lack of a convenient DNA complementation system. Synechocystis PCC 6803 is a single-celled cyanobacterium that readily takes up DNA of the same strain and recombines it homologously with its own endogenous DNA; also it can grow not only autotrophically in the light but also photo-heterotrophically on sugar in dim light. We have worked out a procedure for conveniently and easily identifying fragments of DNA that complement photosynthesis-deficient mutants to photosynthetic competence and used it to identify the site of a genetic lesion in photosystem II of one of our mutant strains. Two other mutant strains functionally deficient in photosystem II activity have also been partially characterized. This procedure is promising for further easy and prompt analysis of photosynthetic mutants and thus of the photosynthetic apparatus.

54. **HARVARD UNIVERSITY** - Petersham, MA 01366

Structure and Function of Frankia Vesicles in Dinitrogen Fixing Actinorhizal Plants
J.G. Torrey, Cabot Foundation, Harvard Forest $76,480

The filamentous bacterium Frankia of the Actinomycetales is one of few dinitrogen-fixing organisms capable of growth in nitrogen-free medium under highly aerobic conditions. This capacity is associated with the formation of swollen hyphal tips called vesicles that produce a

multilaminate envelope that provides O_2-protection for the nitrogenase that is synthesized within. Our research on vesicle initiation, development and function, both in free-living cultures of Frankia and in root nodules of Frankia-infected roots of actinorhizal plants, has been pursued with a variety of Frankia strains from different host-endophyte associations. Current research continues with Frankia strain HFPArI3 from Alnus rubra, HFPCcI3 from Casuarina cunninghamiana, HFPGpI1 from Gymnostoma papuanum and an undescribed isolate from Myrica gale. Of particular interest is the phenomenon of adaptation, either in free-living cultures or in symbioses, of the dinitrogen-fixing system to changes in PO_2 levels. Development of a better understanding of these adaptations is under continuing investigation.

55. **UNIVERSITY OF IDAHO** - Moscow, ID 83843

Genetics and Chemistry of Lignin Degradation by Streptomyces
D.L. Crawford, Department of Bacteriology and Biochemistry $77,000

Current project objectives are (1) to isolate and characterize extracellular peroxidase negative, peroxidase enhanced, and cellulase negative mutants of the ligninolytic actinomycete Streptomyces viridosporus in order to examine the role of each of these enzymes in lignin solubilization by this bacterium; (2) move recently cloned cellulase and peroxidase genes of S. viridosporus from Escherichia coli into Streptomyces lividans, a non-lignocellulose-degrading Streptomyces, using a new shuttle-vector, in order to characterize the lignin solubilizing and cellulose degrading activities of selected clones; (3) amplify peroxidase and cellulase in S. viridosporus by cloning of the genes back into this organism from E. coli on a multi-copy shuttle vector; and (4) subclone the two genes in order to determine their minimum sequences in studies where the possible clustering of the catabolic genes will also be evaluated. Current evidence indicates that cellulase is involved in lignin solubilization by S. viridosporus, and an extracellular peroxidase has been discovered which is thought to be a lignin depolymerizing enzyme. Both genes have been cloned in E. coli. By use of a shuttle vector, they can be moved into S. lividans and amplified in S. viridosporus. The research summarized above will, therefore, use a combination of mutational and gene cloning approaches to elucidate more exactly the role that each enzyme plays in lignin degradation by S. viridosporus.

56. UNIVERSITY OF ILLINOIS - Urbana, IL 61801

Fatty and Aromatic Acid Catabolizing Bacteria
in Methanogenic Ecosystems
M.P. Bryant, Department of Animal Sciences $68,055

The objectives are to isolate and to determine the systematics, physiology, and catabolic biochemistry of syntrophic obligate acetate- and H_2-forming anaerobic bacteria that require coculture with a H_2-using methanogen or other hydrogenotroph to grow and catabolize saturated fatty acids and mono-aromatics. Anaerobic bacteria not requiring syntrophy are also of interest. We previously described syntrophic species (1) β-oxidizing butyrate and longer chain fatty acids, (2) decarboxylating propionate, (3) Syntrophus that produces acetate, CO_2 and H^2 from benzoate, (4) Syntrophococcus that uses sugars as electron donors and H_2-using methanogens or benzenoids as electrons acceptor systems and (5) Eubacterium oxidoreducens (EO) that catabolizes gallate, pyrogallol and phloroglucinol to acetate and butyrate with H_2 or formate required as electron donors. In recent studies almost all of the enzymes necessary for catabolism by EO have been documented with cell-free extracts. The pyrogallol-phloroglucinol isomerase has been purified and shown to require dimethylsulfoxide or 1,2,3,5-benzenetetrol (activator or intermediate?) in phloroglucinol production. The growth factor required by Syntrophococcus is present in Selenmonas and crude egg yolk phosphatidyl-choline and is probably a phospholipid(s). Benzoate catabolism in Syntrophus proceeds with the carboxyl carbon being recovered in the carboxyl carbon of acetate rather than CO_2. This and other results suggest catabolism via either heptanoyl-CoA or pimelyl-CoA and hexanoyl-CoA.

57. UNIVERSITY OF ILLINOIS - Urbana, IL 61801

Photosynthesis in Intact Plants
A.R. Crofts, Department of Physiology & Biophysics
$202,000 - two years

The objective of the project is to study photosynthesis in intact plants, either in the laboratory or the field, with a view to determining if the limitations on growth under normal or stressed conditions arise from limitations in photosynthesis. This work will be complemented by studies on in vitro systems using biophysical methods, and by studies on genetically modified strains. We have constructed several laboratory based instruments, and have also been developing portable instrumentation for use in field studies; a field flash-fluorescence photometer, a fluorescence induction photometer, and a field flash-spectrophotometer have been built, but are under further development. The fluorescence machines have been used in the

study of the two-electron gate *in vivo*, and its modification in herbicide resistant strains. In the laboratory, we are extending these studies to look at the binding and unbinding of herbicides using a rapid mixing apparatus linked to a flash-fluorescence photometer. Several collaborative projects using the field instruments are expected to get under way during the current funding period, investigating electron and proton transfer in drought and chill stressed plants, and photosynthesis in canopies. The modified field spectrophotometer is expected to have a sensitivity in the range 1×10^{-5} absorbance units, similar to that of the laboratory machine. Other laboratory based research will be concerned with the further characterization of the two-electron gate, and the kinetic and thermodynamic parameters controlling its function and sensitivity to inhibitors, with the development of methodologies for optical resolution of the components of the water-splitting reaction, and with studies of the two-electron gate in *Anycystis* strains showing natural herbicide resistance, or with the D_1 protein modified by site-directed mutagenesis.

58. **UNIVERSITY OF ILLINOIS** - Urbana, IL 61801

Mechanism of Proton Pumping in Bacteriorhodopsin
T.G. Ebrey $57,000

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 concentrations including extreme saline environments. This project investigates the relationship between the transport of protons across the membrane and structure and conformation of bacteriorhodopsin. We have made an especially intriguing discovery, that removal of the cations drastically alters both the color and the photochemistry of bacteriorhodopsin. The color can be returned to purple by cation additions but many aspects of the photochemistry depend on the type of cation. We have also found a new intermediate in the bacteriorhodopsin photocycle.

59. **UNIVERSITY OF ILLINOIS** - Urbana, IL 61801

Studies on the *Escherichia coli* Respiratory Chain
R.B. Gennis, Dept. Chemistry & Dept. Biochemistry $96,000

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 or 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 gene 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 gene will be sequenced and the polypeptide sequence of each of the four subunits will be deduced from the DNA sequence. 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.

60. **UNIVERSITY OF ILLINOIS** - Urbana, IL 61801

Cellular Energy Metabolism
M. Glaser, Department of Biochemistry $90,000

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 aand 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.

61. **UNIVERSITY OF ILLINOIS** - Urbana, IL 61801

Genetics of the Methanogenic Bacterium, Methanococcus voltae with Attention to Genetic Expression Mechanisms and the Development of Genetic Transformation Techniques.
J. Konisky, Dept. 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 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.

To determine the molecular mechanisms of archaebacterial gene expression, studies focus on the methanogen histidine biosynthetic genes. Experiments are being carried out to elucidate genetic regulatory components and regulatory mutants have been isolated. Their characterization will lead to information on possible molecular mechanisms of gene control. The elucidation of regulatory mechanisms in archaebacteria is of considerable interest in view of their phylogenetic relationship to the eubacteria and eukaryotes.

62. **UNIVERSITY OF ILLINOIS** - Urbana, IL 61801

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 $97,760

The project deals with underlying mechanisms for cytoplasmic male sterility (CMS) in maize, and with the molecular-genetic bases for both cytoplasmic and nuclear reversions to male fertility in the cms-S sterility system. Studies involve the genetic and molecular characterizations of the organization of the mitochondrial DNA (mtDNA) genome, the reorganization that occurs upon substitution of the nuclear genotype and upon spontaneous reversions to male fertility. Cytoplasmic reversion to fertility has been characterized at the mtDNA level in the inbred line backgrounds M825, WF9 and 38-11 and has been correlated with mtDNA reorganization. Even in the absence of reversion, substitution of one nuclear genotype by another leads to reorganization of the mtDNA genome. These studies are being extended. In experiments under separate auspices we are attempting to isolate and characterize nuclear restorer genes (Rf) and to develop Rf probes that can be used to characterize the spontaneous nuclear revertants. We are also (DOE) analyzing strains that carry newly-arisen Rf elements with respect to their chromosomal locations, their transposition to both linked and unlinked sites, and whether transposition is replicative or non-replicative or both. The standard Rf3 in chromosome 2L is under study to determine if it undergoes transposition as well.

63. **UNIVERSITY OF ILLINOIS** - Urbana, IL 61801

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

The microbial formation of methane is carried out by a unique group of bacteria known as methanogens. These strict anaerobes are widespread in nature, and are found in diverse habitats wherever active anaerobic 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, and purifying the 2-propanol dehydrogenase. We are defining the electron acceptors and are using this enzyme with our enzymes from Methanobacterium thermoautotrophicum. We also propose to use 2-propanol as a tool to obtain mutants in the hydrogenase

system. We are continuing our studies with another hydrogen-independent system, aceticlastic methanogenesis. We are elucidating the role of the cofactors methanofuran and tetrahydro-methanopterin 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 aceticlastic methanogenesis, and we propose to define the role of component B in this process.

64. **THE INSTITUTE OF PAPER CHEMISTRY** - Appleton, WI 54912

Raman Microprobe Investigation of Molecular Structure and Organization in the Native State of Woody Tissue
R.H. Atalla - Chemical Sciences Division $70,000

The Raman Microprobe has revealed evidence of variability of molecular structure and organization within different domains of the cell walls of tissue from loblolly pine (*Pinus taeda L*) and from black spruce (*Picea mariana*). The objective of this project is to investigate the range of variation in composition and molecular orientation within individual cells, between adjacent cells, between cells from different annual rings, and between cells from different types of tissue. Results obtained so far indicate that the lignin in the cell walls is more highly organized than had heretofore been recognized, and that the carbohydrate components of the walls are at least as highly organized in wood as in seed hairs and bast fibers. Thus, a high level of architectural complexity at the molecular level prevails in woody tissue. A significant effort during the most recent program year has been devoted to assembly of a Raman microprobe system, with multichannel detection, optimized for our application. The system, which was jointly funded by the DOE University Research Instrumentation Program and The Institution of Paper Chemistry, will make possible more comprehensive mappings of molecular organization and compositional variation in cells from a wide range of morphological features.

The results will further fundamental understanding of the architecture of cell walls, and will provide a better foundation for analysis and design of industrial processes which use biomass as a primary resource.

65. **UNIVERSITY OF KENTUCKY** - Lexington, KY 40546-0091

Photoinhibition of PSII Reaction Centers; Photoactivation, Reconstitution and Resynthesis of PSII Reaction Center/Water Oxidizing Complex Components
G.M. Cheniae, Department of Agronomy $86,000

Disassembly of the tetra-Mn-complex of the water oxidizing complex invokes high sensitivity of the PSII Reaction Centers to photoinhibition processes even at weak light intensities. Dependent on experimental conditions, such photoinhibition inactivates the secondary donor, Z, to $P680^+$ or both Z and P680. The secondary PSII electron acceptor, Q_B, is not affected during this photoinhibition. Inactivation of Z mandates light dependent resynthesis/reassembly of the chloroplast encoded 34 kD (D_2) polypeptide. We attempt to unravel the chemistry of the photoinhibition process and the biochemistry leading to recovery from photoinhibition. Loss of susceptibility to photoinhibition is dependent on reassembly of the tetra-Mn-water oxidizing complex via a multiquantum photoactivation process. Triton-derived PSII membranes display kinetics of photoactivation entirely similar to the kinetics observed with *in vivo* systems. Using improved methodologies and PSII membranes, near complete reassembly of the catalytically active tetra-Mn-water oxidizing complex is obtainable (t1/2 $\leq$ 5 min.). We ask if photooxidation of Mn^{2+} to higher valency states is required in photoactivation and if high potential cytochrome b_{559} is specifically involved. Similarly, we ask if photoactivation specifically promotes religation of some fraction of the ~3 Ca/PSII which is ligated heterogeneously and is essential for overall water oxidation reactions. Additionally. explanations are sought for the dependence of reassembly of the 17,23 kD PSII extrinsic polypeptides with thylakoids on photoactivation and/or a functional tetra-Mn water oxidizing catalyst.

66. **LAWRENCE BERKELEY LABORATORY** - Berkeley, CA 94720

Light-Regulated Expression of Nuclear and Chloroplast Gene Expression
J.C. Bartholomew $53,000

Light controls both the replication and the expression of genes in *Euglena gracilis*. We are testing the hypothesis that the organization of gene replication is linked to the transcriptional activity of genes. In general, it has been found that genes expressed actively in particular cell types are replicated early during the period of genome replication, and silent copies of the same gene are replicated late. It is not known whether the transcriptional activity of the genes drive the replication order, or vice versa. The photosynthetic genes of *Euglena gracilis* are a very good system for this study since both the replication of the cells and

the photosynthetic activity are highly regulated by light. We have studied the growth of wild-type and bleached mutants of Euglena in the dark and light to compare their cell cycle properties. We are cloning the genes for light-harvesting chlorophyll proteins (LHCP) and for the small subunit of ribulose bisphosphate carboxylase to determine their relative positions in the genome and to have probes for studying the expression and replication of the various members of this presumed multi-gene family. Once the cloning is complete, we will synchronize the cells at the beginning of their DNA synthesis period in the cell cycle by light-dark training and measure the time in this period that each member of the LHCP gene family is replicated. We want to determine if the order of replication and transcription is altered in dark grown cells relative to light grown cells. If expression drives the time in the DNA synthetic period that a gene is replicate, then mutants or dark grown cells not expressing LHCP should replicate these genes late in S; whereas wild-type Euglena may even replicate the expressed copies of LHCP early and silent copies late.

67. **LAWRENCE BERKELEY LABORATORY** - Berkeley, CA 94720

Plant Hydrocarbon Biosynthesis
M. Calvin, J.W. Otvos, Chemical Biodynamics Division $67,000

Hydrocarbon-producing plants are a potential alternative to fossil fuels as a source of both energy and chemical feedstocks. Terpenoid compounds (isoprenoids) are one major class of hydrocarbon compounds found in many plant species. This diverse class of compounds include monoterpenes, sesquiterpenes, carotenoids, triterpenes (including sterols), polyisoprenes (rubber), and others. These compounds share a common biosynthetic pathway through the synthesis of isopentenyl pyrophosphate (IPP), the structural backbone of isoprenes. Biosynthetic steps to sterols were earlier elucidated from work with animals and yeast, and while studies with plants show many common features with the animal pathway, there still remain unanswered questions. Of particular importance to understanding the control of photosynthate allocation to hydrocarbon production are questions of intracellular compartmentation of enzymic steps in the pathway, identification of rate-limiting step(s) in IPP synthesis and the purification and characterization of the enzyme(s) involved in such steps.

Our current studies are with Euphorbia lathyris. In the last year we have used these plants to complete our investigation into the cyclization of squalene to form the sterols. We have also isolated vacuoles from the latex of these plants and identified these organelles as the site of conversion of mevalonic acid into the triterpenoids. Our present work focuses on the purification and characterization of hydroxymethyl-glutaryl

CoA reductase (HMGR), which we have found to catalyze a rate-limiting step between acetyl-CoA and the triterpenoids. We will isolate and purify this enzyme, so that we can better characterize its kinetics and regulation. We will also determine its subcellular location, which may also play a key role in the overall control of the isoprenoid biosynthetic pathway. We will use the information obtained in these studies to assess the possibility of manipulating the isoprenoid pathway to increase hydrocarbon yield in plants.

68. **LAWRENCE BERKELEY LABORATORY** - Berkeley, CA 94720

Mapping Photosynthetic Genes in Prokaryotic and Eukaryotic Cells
J. Hearst, Chemical Biodynamics Division $249,000

The purple nonsulfur photosynthetic bacterium Rhodobacter capsulatus provides an attractive model for studying the regulation of photosynthetic genes by light, O_2 and other environmental factors. When the concentration of oxygen is lowered, the cell develops an extensive intracytoplasmic membrane (ICM) and induces the biosynthesis of light-harvesting LH-I (B870), LH-II (B800-B850), reaction center (RC) complexes, as well as bacteriochlorophyll (Bchl) and carotenoids (Crt). Once assembled, the photochemically active complexes harvest and convert light energy into chemical energy. The size and structure of the photosynthetic apparatus is also influenced by light intensity.

We are studying the coordinate and differential expression of genes for LH-I and LH-II, RC polypeptides L, M, and H, Bchl and Crt biosynthesis in response to light and O_2 in Rhodobacter capsulatus. For the LH-I genes there exists two transcripts (0.5 and 2.6 kb), while the LH-II genes only have one transcript (0.5 kb). The level of RNA specifying the LH-II is more abundant, and more sensitive to change in O_2 concentration and shows a variation over a wider range than that of the LH-I, indicating that the LH-II and LH-I/RC genes are independently regulated. The RC-L and RC-M genes as well as an unknown gene C2397 hybridize to the same mRNA (2.6 kb) as the LH-I genes (beta and alpha). These five genes thus comprise a single operon (designated as puf operon). The RC-H gene has at least two transcripts (1.2 and 1.4 kb) which initiate within ORF 1696 and respond differentially to light intensity. The expression of the genes coding for RC-L, M and H are coordinately regulated by light intensity and O_2 concentration. The genes coding for the enzymes in Bchl and Crt biosynthetic pathways are also regulated by O_2. We have shown that an increase in light intensity causes a decrease in the expression of the genes for LH-I, LH-II, RC complexes and Bchl biosynthetic enzymes. However, high light or oxygen results in increased expression of the genes for Crt biosynthesis. We have particularly found that a crt A gene responsible for oxidation of

spheroidene to spheroidenone is activated by oxygen. These results are interpreted based on the protection function of Crt under high light and oxygen.

In addition, we have shown that the photosynthetic genes for LH, RC and pigment biosynthesis are interdependent. Mutation in each of the genes for RC-H (UAR87), Crt (9F4) or Bchl (MB1007) biosynthesis results in significantly reduced levels of mRNA from the rest of the genes. A close link exists between the genes for LH-II and Crt biosynthesis.

More recently, we have shown that the DNA supercoiling may play an important role in the oxygen regulation of photosynthetic genes in R. capsulatus. The formation of supercoiling of DNA is catalysed by gyrase. We have found that the biosynthesis of Bchl and the LH(I,II) bchl complexes was significantly inhibited by the gyrase inhibitors: Novobiocin, coumermycin, nalidixic acid and oxolinic acid. The mRNA levels for LH(I,II), RC(L,M,H), Bchl biosynthetic enzymes, ribulose-bis-phosphate carboxylase and an ORF Q upstream of puf operon were also immediately and markedly reduced by these drugs at various concentrations. These effects are comparable with that of proflavin, an inhibitor of transcript. In contrast, the level of mRNA for Crt biosynthetic enzymes was less sensitive to the drugs. We suggest that DNA supercoiling is involved in the differential expression of photosynthetic genes in response to oxygen in R. capsulatus.

69. **LAWRENCE BERKELEY LABORATORY** - Berkeley, CA 94720

Mechanism of Water Splitting and Oxygen Evolution in Photosynthesis
M.P. Klein, Chemical Biodynamics Division $165,000

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 H_2O and donated to the oxidized $P680^+$ reaction center. The most successful interpretation of the data regarding this cycle is Kok's S-state scheme, which postulates a series of five (S_0-S_4) states through which electrons are cycled during oxygen evolution. Manganese is thought to play a central role in these reactions. X-ray absorption spectroscopy using synchrotron radiation is used to determine the structural and electronic state(s) of the manganese sites. In Photosystem II particles of both spinach and the cyanobacterium Synechococcus sp., we have determined that the manganese occur minimally as a binuclear complex with Mn-Mn separation of 2.7 . Additional Mn occurs at 3.3 . We have observed a K-edge energy shift of ca 1 eV upon the transition from the S_1 to S_2, implying an oxidation state change 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. Removal of the 16, 23 and 33 kDa extrinsic peptides by $CaCl_2$ washing leaves the Mn cluster essentially intact. Electron Spin Echo spectroscopy on the S_2 multiline EPR signal provides the first direct evidence that the Mn centers are accessible to solvent water. Illumination at 190K of PS II particles prepared with NH_3 produces an altered EPR signal whose ESE signals show nitrogen modulation providing the first direct evidence that nitrogen becomes a ligand of Mn.

70. LAWRENCE BERKELEY LABORATORY - Berkeley, CA 94720

Tissue Specific Gene Regulation

F. Leach, J.C. Bartholomew, J.E. Hearst, Chemical Biodynamics Div.

$103,000

Our goal in this research is to identify some cellular factors which regulate the tissue specific expression of plant genes. There is evidence that the expression efficiency of genes on the T-DNA of Ri plasmids is dependent upon the plant tissue in which the DNA resides. It is our hypothesis that it is factors within the different tissues which regulate the efficiency of expression of different genes on the T-DNA. Our approach to testing this hypothesis is to use a short term transfection system to introduce into the protoplasts from the roots and leaves of *Nicotiana tobacum* the Ri T-DNA, and then to measure the transcription efficiency of the different open reading frames. From the known DNA sequence of the Ri T-DNA we are identifying promoters which control the transcription of the tissue specific regulated genes. Constructs have been made containing these plant promoters linked to the bacterial gene coding for the chloramphenicol acetyltransferase (CAT) enzyme. The activity of this enzyme can easily be measured from cell extracts. By transfecting these tissue specific CAT plasmids into protoplasts in which the promoter is normally inactive and harvesting the transcription products from these cells, and adding extracts from tissues in which the promoters are active, we expect to develop an assay for tissue specific promoter activators. Using this assay we will begin purifying the various factors from cells that confer tissue specificity on the promoters. These studies will give a better understanding of the mechanism of tissue specific regulation of gene activity, and may allow the genetic modification of plants to result in tissue specific expression.

71. **LAWRENCE BERKELEY LABORATORY** - Berkeley, CA 94720

Chemistry of Phycobiliproteins and Phytochrome
H. Rapoport, Chemical Biodynamics Division $54,000

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.

72. **LAWRENCE BERKELEY LABORATORY** - Berkeley, CA 94720

Photosynthetic Membrane Structure and Photosynthetic Light Reactions
K. Sauer, Chemical Biodynamics Division $249,000

Excitation transfer and trapping in reaction centers of photosynthetic membranes occurs in less than a nanosecond following the absorption of visible light photons. We are investigating the kinetics and energetics of this process using wavelength-resolved fluorescence decay measurements applied to well defined preparations of antenna pigment proteins or reaction center complexes. Recent studies using X-ray crystallography from several laboratories have provided detailed structural information for several of these proteins, which have enabled us to carry out excitation transfer calculations using exciton theory and/or Förster inductive resonance transfer applied to pigment arrays of known geometry. For the cyanobacterial pigment C-phycocyanin the Förster mechanism predicts transfer kinetics that are in good agreement with the best available experimental values, indicating that this mechanism is sufficient to understand excitation transfer steps as short as 10-20 psec. We are now attempting to improve the sensitivity of the kinetic measurements in the faster time domain. 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 found a simple relation between the temperature dependence of time-resolved fluorescence decay kinetics and the energy separation between the antenna excited states and the trap, and we are investigating the role that these low lying excited states play in photosystem architecture and the mechanism of energy conversion.

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

73. **LAWRENCE BERKELEY LABORATORY** - Berkeley, CA 94720

Photochemical Conversion of Solar Energy
L. Packer, R. Mehlhorn, I. Fry and J. Maguire
Applied Science Division $125,000

This project seeks to understand the mechanisms of biological energy conversion in bacterial systems. Using cyanobacteria, halobacteria and *B. subtilis*, the role of energy, both photosynthetic and respiratory, in the maintenance of cell function under normal and stress conditions is investigated.

Research on cyanobacteria investigates the response of photosynthesis and respiration to stress, and the roles played by the primary energy systems (trans-membrane pH and electrochemical gradients) in tolerance and adaptation to the stress condition. The function of trans-membrane pH gradient for ion transport, and the subsequent depolarization under stress, are being studied to determine if the observed inhibition of cellular processes are a direct result of energy depletion or a perturbation of structural components. The effect of pollutants (selenium) on ion transport and bioenergetics are being studied to determine the role of energy status on the accumulation or exclusion of this toxic element.

The molecular mechanism of the proton pump in bacteriorhodopsin is being investigated. Functional and structural comparisons, after chemical modifications of amino acid residues, are made to determine which residues are involved in the different aspects of energy conversion.

The function and assembly of the iron-sulfur clusters in the subunits of the respiratory electron transport component, succinate dehydrogenase are being studied. Using bacterial mutants deficient in particular iron-sulfur clusters, the sequence of synthesis, and the role of particular iron-sulfur clusters in catalysis and membrane attachment is being sought.

74. **LEHIGH UNIVERSITY** - Bethlehem, PA 18015

A Genetic Approach to Secretion and Hyperproduction of Cellulase by Trichoderma
B.S. Montenecourt, J. Phillips, Department of Biology/Biotechnology
$80,000 two years

Microbial cellulases are important enzymes of potential industrial application in the conversion of cellulosic biomass to glucose syrups and of current application in the food processing industry. The multienzyme cellulase complex of the mesophilic fungus *Trichoderma reesei* has been studied in the greatest depth. Our laboratory has focused on the

production of high yielding mutants and the analysis of these mutants with respect to the events involved in secretion of the enzymes. The effect of glycosylation inhibitors, tunicamycin and 2-deoxyglucose and membrane perturbing agents (ethanol and phenylethanol) have been studied at both the biochemical and morphopogical levels. Tunicamycin blocks the addition of N-linked carbohydrate to the cellulase protein chains but the enzymes seem to be secreted normally and retain their activity. They are, however, more thermolabile, more susceptible to protease inactivation and exhibit shifts in IEF and SDS-PAGE patterns. 2-Deoxyglucose blocks all secretion by the wild type, QM6a, but the hypersecretory mutant, RL-P37 is able to overcome this inhibition. Ethanol, at concentrations which do not inhibit growth, causes inhibition of secretion and the effect is more pronounced in the mutant RL-P37 whose cellulase production is reduced to that of the wild type. The cellulases which are secreted contain normal amounts of carbohydrate. Antibodies conjugated to gold-protein A particles have localized cellobiohydrolase in the endoplasmic reticulum, Golgi and secretory vesicle regions, confirming that this general pathway, which has been firmly identified in yeast and mammalian cells, also exists in *Trichoderma*. Temperature sensitive secretion mutants have been isolated and one mutant, LU-ts 1, was shown to be ts for for cellulase and not for protease, amylase and xylanase. At the non-permissive temperature heterogeneous protein bands are exhibited by this mutant which are inactive. This characteristic of ts for secretion is reversible. At the permissive temperature normal cellulase enzymes are secreted.

75. **LOS ALAMOS NATIONAL LABORATORY** - Los Alamos, NM 87545

Energy Metabolism in Methylotrophic Bacteria
J.A. Fee and C.J. Unkefer, Division of Isotope & Nuclear Chemistry
$110,000

Methylotrophic bacteria derive their energy from the oxidation of reduced one-carbon compounds and synthesize all their cellular material from compounds having no C-C bonds, for example, CH_3OH. Since methylotrophs grow on inexpensive one-carbon compounds, they are used for the large-scale production of single cell protein and are potential sources of other industrially useful metabolites. An important class of methylotrophic bacteria assimilate carbon by condensing formaldehyde and glycine to form serine. These organisms lack the enzyme isocitrate lyase, and the metabolic pathway that regenerates the glycine require for carbon assimilation is unknown. The main thrust of this new project is to combine ^{13}C labeling and NMR spectroscopy in an attempt to sort out the metabolic events of the so-called icl^- pathway for carbon assimilation. Secondary thrusts of our work are to elucidate the mechanism(s) by which bacteria, actively producing formaldehyde

through oxidation of CH_3OH, avoid "formaldehyde-poisoning" while maintaining a steady flow of electrons to dioxygen. Results from our experiments will lead to a greater understanding of the biochemistry and physiology of methylotrophic bacteria and will provide a firmer base for industrial application of these organisms.

76. **LOS ALAMOS NATIONAL LABORATORY** - Los Alamos, NM 87545

Structure of Cell Walls from Monocotyledenous Plants: ^{13}C N.M.R. Analysis of Cell Wall Polymers Isolated from Proso Millet (*Panicum miliaceum* L. cv. Abarr) Grown in Tissue Culture
L.O. Sillerud, N.H. Fink, Division of Life Sciences $110,000

Cell wall polysaccharides are so complex that elucidation of their primary structures requires the use of the most sophisticated technologies. High resolution, proton-decoupled ^{13}C N.M.R. (nuclear magnetic resonance spectroscopy) offers a nondestructive method that gives insight into not only the structure, but also the dynamics and metabolism of these carbohydrates without modification of the sample. Dr. N. C. Carpita at Purdue University has supplied us with suspension-cultured millet (*Panicum miliaceum*) cells labeled with such cell wall precursors as natural abundance glucose or [1-^{13}C]-glucose or [2-^{13}C]-glucose. We have also synthesized [1-^{13}C, 5-^{2}H]-arabinose and [2-^{13}C, 5-^{2}H]-arabinose for use in the plant cell wall metabolism study. Dr. Carpita characterized some of the wall components by means of gas-liquid chromatography and GC-MS. Sugar residues, such as the uronic acids, that were not resolved by these methods were easily identified and quantified from ^{13}C N.M.R. spectra. N.M.R. also allowed us to confirm the conformations and glycosidic linkages of each residue present in the polymer. Relative abundances of the residues and linkages determined from ^{13}C N.M.R. spectra agreed with those determined from the GC-MS experiments, where the data overlapped. We are using the information obtained from N.M.R. data to build a model of the cell wall that considers not only the structure but also the dynamics and metabolism of the polymers. Carbon-13 spin-lattice relaxation data and nuclear Overhauser enhancement measurements allowed us to observe segmental motion on the nanosecond timescale for the hemicelluloses. Since the next direction that we are pursuing is to perform *in situ* studies on the cell wall in living systems, we have obtained solid state ^{13}C N.M.R. on total wall, pectins and celluloses. The solid-state data indicated that the hemicelluloses are more disordered in the intact wall than are the celluloses.

77. **UNIVERSITY OF MARYLAND** - College Park, MD 20742

Active and Passive Calcium Transport Systems in Plant Cells
H. Sze, Department of Botany $66,000

Changes in cytoplasmic calcium levels ([Ca^{2+}]) in plant cells modulate several functions important for growth and development. Cytoplasmic [Ca^{2+}] is regulated by the balance of active and passive Ca^{2+} fluxes across the plasma membrane and organellar membranes. Yet the mechanism and regulation of Ca^{2+} fluxes are poorly understood in plant cells. We are using isolated membrane vesicles to study Ca^{2+} transport systems in oat roots and carrot suspension cells. Ca^{2+} accumulation into the vacuole depends on a proton motive force generated by the tonoplast H^+-ATPase. Using an artificially imposed pH gradient, we find the Ca^{2+}/H^+ antiport is reversible, electrogenic and sensitive to N,N-dicyclohexylcarbodiimide and ruthenium red. These properties will be useful for identifying the antiporter during purification and reconstitution. Ca^{2+} accumulated in the tonoplast vesicles can be released transiently by inositol 1,4,5-trisphosphate (IP3) which is produced in response to environmental or chemical stimulus. These results demonstrate that the vacuole plays an important role in the regulation of cytoplasmic Ca^{2+} in plant cells. A Ca^{2+}-pumping ATPase on the endoplasmic reticulum has high affinity for Ca^{2+} (Km = 1 uM) and is important for removing excess cytosolic Ca^{2+}. The role of the ER, plasma membrane and the nuclear membrane in regulating cytoplasmic Ca^{2+} levels are being investigated.

78. **MASSACHUSETTS INSTITUTE OF TECHNOLOGY** - Cambridge, MA 02139

Analysis of the Rhizobium meliloti Surface
E.R. Signer, Department of Biology $87,000

The formation by rhizobia of nitrogen-fixing nodules on the roots of leguminous plants presumably involves communication between bacterial plant cells, and is thus likely to depend on interactions between the surfaces of the two symbiotic partners. We are using a variety of techniques to probe the surface of the alfalfa symbiont Rhizobium meliloti SU4 in order to identify components that are involved. Currently we are focusing on the response of the rhizobial surface to bacteriophages, monoclonal antibodies, and/or detergents. Antibody sensitivity is altered during differentiation to bacteroids in planta, which suggests that surface changes play a role in nodule development. We have identified several genes involved in biosynthesis of lipopolysaccharide and have isolated a cosmid clone including some of these, which we are characterizing molecular-genetically. We are also determining the structure of LPS from wild-type and mutants

using High Performance Liquid Chromatography. At least some of the mutants in these genes are defective in symbiosis, but only in certain genetic backgrounds, which suggests that bacterial surface components interact during symbiosis. 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.

79. **MEHARRY MEDICAL COLLEGE** - Nashville, TN 37208

Respiratory Enzymes of *Thiobacillus ferrooxidans*
R.C. Blake, II, Biochemistry Department $74,584

Certain chemolithotropic 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. Despite the environmental and economic importance of these organisms, very little basic information is available concerning the identity and disposition of the respiratory enzymes responsible for these activities. The aim of this research is to initiate the systematic isolation and characterization of the respiratory enzymes expressed by these chemolithotropic bacteria when grown on both reduced metal substrates and reduced inorganic sulfur compounds. Our current focus is on the iron-oxidation system of *Thiobacillus ferrooxidans*. Rusticyanin, a soluble blue copper protein alleged to be the initial electron acceptor from ferrous ion, has been purified to electrophoretic homogeneity. Stopped-flow spectrophotometric studies indicate that a soluble, uncharged $Fe\text{-}SO_4$ complex is the actual reductant of purified rusticyanin, but that the rate constant for reduction is several orders of magnitude too low to explain the facile reduction of cytochromes by ferrous ion in the intact organism. We have identified and are currently purifying an acid-stable cytochrome *c* which appears to facilitate the interaction between rusticyanin and ferrous ion. We are also investigating the reduction of rusticyanin by various iron-oxyacid chelates. The 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.

80. **MICHIGAN BIOTECHNOLOGY INSTITUTE** - Lansing, MI 48909

One Carbon Metabolism in Anaerobic Bacteria: Regulation of Carbon and Electron Flow During Organic Acid Production
J.G. Zeikus, G.-J. Shen $95,000

Our project deals with understanding the fundamental mechanisms that control carbon and electron flow in anaerobic bacteria that conserve energy when coupling hydrogen consumption to the production of acetic, propionic or butyric acids. We compare the regulation of C_1-biotransformation to C_2, C_3, and C_4 compounds when *Butyribacterium methylotrophicum* and *Propionispira arboris* are grown on multiple carbon compounds by fermentation, enzyme and electron carrier analysis. To elucidate the electron transfer pathway in acidogens, the electron carriers are purified and characterized by spectrophotometric techniques. The physiological function of hydrogen metabolism in acidogens (influence on electron and carbon flow, relation to ETP, proton motive force, ATP synthesis, and energy conservation, etc.) are also investigated. The membrane associated redox centers are also identified and characterized by EPR studies under various growth conditions. To understand the relationship and regulation between catabolism and anabolism for C_1-biotransformation, single carbon metabolism mutants and either TCA cycle amino acid or vitamin B_{12} excreting mutants are proposed to be isolated and compared to elucidate the biochemical basis of alterated regulation in these mutants. The rate and yield limiting enzymatic steps associated with single carbon biotransformation are examined by enzyme and intermediary metabolic analysis. Some emphasis is placed on determination of vitamin B_{12} function during single carbon transformation by these bacteria. It is hoped that these studies may yield strategies to develop anaerobic fermentations based on H_2 and single carbon substrates for production of organic acids or expensive amino acids and vitamins.

81. **MICHIGAN STATE UNIVERSITY** - East Lansing, MI 48824

Role of Acyl Carrier Protein Isoforms in Plant Lipid Metabolism
J.B. Ohlrogge, Department of Botany & Plant Pathology $75,000

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. Fatty acids in plants are required for two major functions; membrane structure and energy (or carbon) storage. Acyl chains for both of these functions are supplied by the *de novo* FAS pathway which is localized in the plastid. 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 the research is to further examine the biochemical significance of ACP isoforms to plant lipid metabolism. Specifically, we will a) examine the distribution of ACP forms in a variety of photosynthetic species, in various tissues and under different environmental influence; b) prepare monospecific antibodies to each spinach leaf isoform to use as probes of their individual function; c) modify the structure of ACP I by in vitro mutagenesis to determine which structural features of ACP are responsible for its activity in thioesterase and acyl transferase reactions.

82. **MICHIGAN STATE UNIVERSITY** - East Lansing, MI 48824-1101

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

C.A. Reddy, Department of Microbiology and Public Health $64,332

P. chrysosporium, a lignin-degrading filamentous fungus, elaborates during its secondary metabolism several extracellular, H_2O_2-dependent, glycosylated heme proteins referred to as ligninases or lignin peroxidases. There has been much recent interest in these enzymes because these proteins, in addition to playing a key role in lignin biodegradation, are also believed to be involved in the detoxification of recalcitrant environmental pollutants such as dioxins, DDT, lindane and benzopyrenes. We have isolated and characterized two ligninase cDNA clones from P. chrysosporium. One of our objectives this coming year is to isolate and characterize the cDNA clones for the other ligninases. Our immediate objective is to develop expression/secretion system(s) for ligninase cDNA. To this end, ligninase cDNA encoding ligninase H8 will be cloned into yeast expression/secretion vectors as heterologous eukaryotic proteins are known to be expressed, glycosylated and expressed in yeast. In parallel we will also investigate the suitability of producing functional ligninases using fungal expression systems. The recombinant ligninase will be purified and characterized with regard to catalytic activity, glycosylation and enzyme yield. The expression system developed will then be used to determine the functional domains of the ligninase cDNA.

83. **MICHIGAN STATE UNIVERSITY DOE PLANT RESEARCH LABORATORY**
East Lansing, MI 48824

Differential Gene Expression in Rhizobium
B.K. Chelm $185,000

The symbiotic association between the bacterium Bradyrhizobium japonicum and the legume soybean requires a complex series of steps resulting in the development of the highly differentiated plant organs termed root nodules. Our approach to studying this interaction has been to analyze the importance of basic physiological response mechanisms for proper symbiotic development. These mechanisms can most readily be addressed from the bacterial side of the symbiosis where genetic and molecular genetic tools can be applied to the study of physiological responses. Since the movement of a bacterium from the soil to the intracellular localization found within nodules, albeit by a developmental process, is most certainly a shift in medium conditions, it seems reasonable to expect that the physiological adaptation mechanisms will be activated. We have shown that this is, at least in part, true, and is an important aspect of the proper interaction between the two organisms. We have focused on the characterization of the B. japonicum mechanisms for the regulation of carbon source utilization (catabolite repression), nitrogen source utilization (Ntr system), and the response to growth rate limitation by decreased oxygen availability (Olr system). In order to probe these responses and investigate their mechanisms in B. japonicum, we have been characterizing genes, and their transcriptional regulatory regions, which are expected to be regulated by these systems. These probes have allowed us to better characterize the physiological regulation of transcription in B. japonicum and to initiate the process of isolating mutant strains which are defective in these response mechanisms. These mutant strains can then be utilized for the characterization of the importance of the particular regulatory system in the plant-bacterial symbiotic interaction. We have shown that the Olr system regulates the transcription of nif genes in response to oxygen limited growth conditions irrespective of the nitrogen source availability in the medium. In addition, nodulation studies with B. japonicum Olr-mutant strains indicate that the oxygen limitation response is of major importance for the progress through the later stages of nodule development. An understanding of the molecular basis for this defective interaction will be a major goal for the near future.

84. **MICHIGAN STATE UNIVERSITY DOE PLANT RESEARCH LABORATORY**
East Lansing, MI 48824

Enzymatic Mechanisms and Regulation of Plant Cell Wall Biosynthesis
D. Delmer $50,000

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 β-glucan, callose; purification and characterization of the callose synthase; 2) identification and characterization of a protein receptor for an herbicide (DCB), which specifically inhibits cellulose synthesis, 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.

85. **MICHIGAN STATE UNIVERSITY DOE PLANT RESEARCH LABORATORY**
East Lansing, MI 48824

Resistance of Crop Plants to Environmental Stress
A.D. Hanson $179,000

Plants have biochemical or metabolic adaptations to environmental stresses, as well as adaptations expressed at higher levels of organization. Biochemical and genetic understanding of metabolic adaptations to stress would allow them to 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 investigating two metabolic responses to stress: 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 osmoticum during stress. Betaine is synthesized in the chloroplast by a two-step oxidation: choline $\rightarrow$ betaine aldehyde $\rightarrow$ betaine. The second step is catalyzed by a stromal, NAD-linked dehydrogenase. We have purified this enzyme to homogeneity and are now investigating its regulation by salinity, and

proceeding to cDNA cloning. We have demonstrated that the first step is light-promoted, and that light acts by reducing electron transfer chain components on the reducing side of PSI. Lactate dehydrogenase (LDH) activity is induced by hypoxia in roots and catalyzes lactate glycolysis which plays a key role in the regulation of cytoplasmic pH during hypoxia. We have purified barley LDH and have used antibodies against it to demonstrate that enzyme induction involves a 10-fold increase in the level of translatable LDH mRNA. We are now beginning cDNA cloning work.

86. **MICHIGAN STATE UNIVERSITY DOE PLANT RESEARCH LABORATORY**
East Lansing, MI 48824

Action and Synthesis of Plant Hormones
H. Kende $222,000

The project objective is to gain knowledge on the regulation of synthesis and mode of action of the plant hormones ethylene and cytokinin. These hormones regulate plant senescence and responses of the plant to stress. We have studied the enzymes that mediate the synthesis of ethylene from S-adenosyl-methionine. The first enzyme in this pathway, 1-aminocyclopropane-1-carboxylate (ACC) synthase, is usually the limiting enzyme in ethylene synthesis. It can be induced by a variety of chemicals and conditions, including stress. We have purified this very important enzyme in plant development and have produced antibodies against it, enabling us to study its regulation at the molecular level. 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. We are attempting to reconstitute it after breakage of membranes. The role of stress ethylene is being investigated in deepwater rice where low-oxygen stress occurring 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. We are attempting to localize the site of action of cytokinins using a cytokinin photoaffinity probe. We have developed several methods to synthesize such a probe, namely 8-azido-benzyladenine ($8N_3BA$). Identification of cytokinin receptors will be attempted in cultured tobacco cells and in protonemata of mosses, both of which respond to cytokinins.

87. MICHIGAN STATE UNIVERSITY DOE PLANT RESEARCH LABORATORY
East Lansing, MI 48824

Cell Wall Proteins
D.T.A. Lamport $139,000

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 Pl and P2 (i.e., their primary structure, EM visualization, and immunochemistry). Pl, P2, and their HF-deglycosylated polypeptides dPl 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 dPl and dP2, a relatively few major peptides dominate each peptide map: (1) Pl/H5 Ser-Hyp-Hyp-Hyp-Hyp-Thr-Hyp-Val-Tyr-Lys; (2) Pl/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 Pl hexadecapeptide H20 is the prime candidate for intermolecular cross-linkage. If so the average intermolecular cross-link frequency corresponds remarkably so that predicted for a network penetrated by cellulose microfibrils.

88. MICHIGAN STATE UNIVERSITY DOE PLANT RESEARCH LABORATORY
East Lansing, MI 48824

Regulation of Flowering
A. Lang $87,000

This project studies promotive and inhibitory factors in flower formation, particularly graft-transmissible, hormone-like materials (florigen and antiflorigen). The evidence for florigen and antiflorigen is so far physiological, based primarily on grafting experiments between plants maintained in flowering and nonflowering conditions. The next obvious step would be to isolate the responsible chemical compounds and determine their

structures. For florigen, this has been repeatedly attempted but so far without success. Our major effort is directed at isolation and identification of antiflorigen; solution of this problem may also aid in the search for florigen. The work is being done using extraction and diffusion techniques, and tissue explants capable of regeneration of flowers as bioassay. In a related, physiological project it has been established that a short-day plant, the Maryland mammoth cultivar of tobacco, does not seem to form antiflorigen when grown on long days in contrast to two related long-day plants, *Nicotiana silvestris* and *Hyoscyamus niger*, which under short days produce potent antiflorigen. In a third project it has been found that recently synthesized powerful inhibitors of gibberellin synthesis (A-Rest, Tetcyclacis) inhibit stem growth, but have no effect on flower formation in a long-day, a short-day and a day-neutral tobacco. In previous work with the long-short-day plant *Bryophyllum* it had been found that gibberellin does participate in the endogenous regulation of flower formation. The new results indicate that this does not hold for plants in general.

89. **MICHIGAN STATE UNIVERSITY DOE PLANT RESEARCH LABORATORY**
East Lansing, MI 48824

Interaction of Nuclear and Organelle Genomes
L. McIntosh — $185,000

Energy transducing membranes are the matrix for many biophysical and biochemical reactions supporting plant growth and development. Respiratory enzymes such as terminal oxidases and photosynthetic reaction center polypeptides are associated with mitochondrial and chloroplast membranes. The genes encoding these polypeptides are located within three different compartments: nuclei, chloroplasts, and mitochondria. Our purpose is to understand, through molecular/genetic manipulation, how limiting or "key" components of photosynthesis and respiration function.

Gene-directed modification of photosynthesis is being pursued in the transformable unicellular cyanobacterium *Synechocystis* 6803. Selected "photosynthetic" genes are isolated and modified *in vitro*. These altered genes are used to replace the wild-type genes in *Synechocystis*. In this manner we have inactivated the *psbA* gene family in *Synechocystis*. Recently, this *psbA*-mutant was complemented with a higher plant chloroplast gene.

In the mitochondria of most plant species there is an alternative pathway of electron flow that is not linked to ATP production. This pathway consists, in part, of an "alternative" terminal oxidase which donates

electrons to water but which is insensitive to cyanide. It is believed that this pathway plays an important role in growth and maintenance respiration, therefore, we have purified this complex from mitochondrial membranes and localized its constituents through immunological techniques. Antibodies to this pathway are being employed to (1) follow developmental control of respiration and (2) to allow the genes encoding this terminal oxidase to be cloned.

90. **MICHIGAN STATE UNIVERSITY DOE PLANT RESEARCH LABORATORY**
East Lansing, MI 48824

Sensory Transduction in Plants
K.L. Poff — $139,000

The primary objective of this project is to understand the mechanisms of environmental information via light and gravity reception. We are studying the blue light photoreceptor pigment system(s), which control(s) numerous light responses such as phototropism in flowering plants, and, in addition, we are studying the mechanism for the perception of gravity in geotropism. For these studies, we are using specific inhibitors and mutants as probes to dissect the initial steps in the transduction sequences. A screening procedure using a pulsed light stimulus has been devised and used to isolate 35 mutants of *Arabidopsis* with an altered phototropic response. Geotropism for these photo-minus strains falls into three classes: normal, impaired, and lacking. The photo-minus, geo-normal phenotype should represent an alteration early in the phototropism pathway and could arise from an altered photoreceptor pigment. A second screening procedure has been devised and used to isolate 105 mutants of *Arabidopsis* with an altered geotropic response. One of these mutant strains shows altered shoot geotropism, normal phototropism, and normal root geotropism. Based on this strain, one can conclude that shoot geotropism has at least one mutable element early in the transduction sequence which is not critical for root geotropism. We are continuing to study the convergent pathways for phototropism and geotropism 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.

91. **MICHIGAN STATE UNIVERSITY DOE PLANT RESEARCH LABORATORY**
East Lansing, MI 48824

Developmental Regulation of Gene Expression in Plants
N.V. Raikhel $125,000

The main aim of our research is to understand the DNA sequence elements and cellular processes that control tissue-specific gene expression. The overall approach will consist of the following stages: a) characterization of genomic sequences for WGA and rice lectins, b) analysis of the promoter sequences of these genes, c) mutagenesis of lectin gene promoters, and d) transformation experiments to test expression of specific gene constructs.

The differential localization of lectins among closely related species in the Gramineae provides a unique opportunity to elucidate the cell-specific expression of these proteins. Study of the differential expression of lectins in essentially analogous tissues, but in different cell types, will aid our understanding of the relationship between closely related proteins from different species. These questions cannot be addressed in systems where a gene product is generally distributed throughout an organ. Thus, we can ask questions not only concerning regulation of expression in particular organs, but we can address more precisely the level of lectin expression, especially in different tissues and cell layers in the same organs.

92. **MICHIGAN STATE UNIVERSITY DOE PLANT RESEARCH LABORATORY** -
East Lansing, MI 48824

Physiological and Molecular Genetics of Arabidopsis
C.R. Somerville $191,000

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 primarily regulated at the postranscriptional 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 of the overall organization of the membranes. We are currently exploring ways of exploiting the availability of the mutants to clone the genes affected.

93. **MICHIGAN STATE UNIVERSITY DOE PLANT RESEARCH LABORATORY**
East Lansing, MI 48824

Molecular Basis of Disease Resistance in Barley
S.C. Somerville $150,000

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. Our approach has been to determine, by mutational analyses, the number of host components which mediate race-specific resistance and the number of pathogen determinants of avirulence. Barley mutants with an intermediate level of susceptibility have been recovered from a mutagenized resistant line. Partially virulent variants of E. graminis have been identified from a mutagenized avirluent race. Neither fully susceptible barley mutants nor fully virulent E. graminis variants have yet been recovered, suggesting that more than one component distinguishes resistance from susceptibility in barley and avirulence from virulence in E. graminis. Our conclusion is that the "gene-for-gene" hypothesis, in its simplest formulation, does not adequately describe barley-E. graminis interactions.

94. **MICHIGAN STATE UNIVERSITY DOE PLANT RESEARCH LABORATORY**
East Lansing, MI 48824

Molecular Biology of Toxicogenic Plant Pathogens
J.D. Walton $110,000

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 ultimate 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, in order to understand the molecular genetic events by which new races of pathogens arise. 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.

95. **MICHIGAN STATE UNIVERSITY DOE PLANT RESEARCH LABORATORY**
East Lansing, MI 48824

Developmental Biology of Nitrogen-Fixing Algae
C.P. Wolk $185,000

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 our recently developed methodology for introducing cloned genes into nitrogen-fixing cyanobacteria by conjugation from *Escherichia coli*. Derivatives of *Anabaena* 7120 and *Nostoc* 29150 unable to fix nitrogen under aerobic conditions were isolated. The heterocysts of certain of these mutants lack a single envelope glycolipid, whereas others appear defective in their envelope polysaccharides. These mutants are probably defective specifically in protection of nitrogenase from oxygen. DNA that repairs two of the mutants has been identified. To accelerate such studies, we are constructing transposons that would insert strongly expressed antibiotic-resistance genes randomly within the *Anabaena* chromosome. The frequency of transfer of genes is greatly reduced by *Anabaena* enzymes that destroy "foreign" DNA. We are finding that modification, in *Escherichia coli*, of DNA to be transferred permits unencumbered transfer of the DNA to *Anabaena*. 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 commercial, biological conversion of solar energy.

96. **MICHIGAN STATE UNIVERSITY DOE PLANT RESEARCH LABORATORY**
East Lansing, MI 48824

Environmental Control of Plant Development and Its Relation to Plant Hormones
J.A.D. Zeevaart $153,000

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. Our studies on stem growth

and flowering in long-day rosette plants, the so-called physiological dwarfs, have demonstrated that photoperiodic control of stem growth is based on the activity of enzymes that convert inactive gibberellins (GAs) to active GAs. We have found that the enzymes involved in the later steps of GA biosynthetic pathway in spinach are dioxygenases with a molecular weight in the 30 to 40 kilodalton range. Our aim is to find out at what level the photoperiod controls the activity of some of these enzymes.

Our studies on the biosynthesis of the stress-hormone abscisic acid (ABA), are proceeding on the basis of the hypothesis that stress-induced ABA is a breakdown product of carotenoids. In agreement with this, we have found that in roots which have a much lower carotenoid content than leaves, more molecular oxygen is incorporated into the ABA molecule than in leaves. The objective is to elucidate the entire pathway from carotenoids to ABA with xanthoxin as a possible intermediate, and then to determine which steps in the pathway are affected by the water status of the tissue.

97. UNIVERSITY OF MINNESOTA - Minneapolis, MN 55455

The mechanism of switching from an acidogenic to a butanol-acetone fermentation by Clostridium acetobutylicum

P. Rogers, Department of Microbiology $76,544

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. Having purified and characterized the aldehyde dehydrogenase, we are now purifying and studying the other key enzymes in the two pathways branching from butyryl-CoA producing either butyric acid or butanol. Kinetic constants and allosteric modifiers will be studied to determine enzyme activity control.

Prior to solvent formation the synthesis of five or six enzymes is induced 40 to 100 fold. Using mutagenesis with ethyl-methane sulfonate and selection for allyl-alcohol resistance, low acid production, or asporogenesis we have found a number of presumptive regulatory mutants for the switch to solventogenesis. The physiological and enzymic properties of these mutants is under study. The transformation and fusion of C. acetobutylicum protoplasts will be studied in detail in order to develop an efficient genetic system to define and classify regulatory mutants into complimentation groups. Gram positive plasmids already developed with antibiotic markers and shuttle properties will be used. These will be tested for transformation, replication, and expression in C. acetobutylicum. A new vector using cryptic C. acetobutylicum plasmids will be developed.

98. UNIVERSITY OF MINNESOTA - Navarre, MN 55392

Genetics of Bacteria That Utilize Carbon Compounds
R.S. Hanson, Gray Freshwater Biological Institute $64,464

Bacteria that grow on methane and methanol are considered useful for the production of amino acids and other products because they grow on simple, inexpensive substrates. In some bacteria that grow on methane, methane monooxygenase (MMO) represents over 20% of the total cellular protein. In some bacteria that grow on methanol, methanol dehydrogenase (MDH) represents 10 to 20% of the cellular protein. Both types of bacteria assimilate formaldehyde for the synthesis of cell material. Facultative methylotrophs can also grow on non-C_1 substrates. In Methylobacterium organophilum the enzymes responsible for the the oxidation of methane or methanol and the assimilation of formaldehyde are induced by growth on C_1 compounds. The genes encoding nine enzymes of C_1 metabolism are located in more then seven regions of the genome separated by 50 kilobase pairs or more. Eleven genes essential for the synthesis of active MDH have been mapped on three cloned fragments. The MDH structural gene has been identified and the nucleotide sequence of the gene and regulatory region has been determined. Messenger RNA (mRNA) homologous to the MDH structural gene has been isolated. The amount and stability of this mRNA from cells grown under different conditions is being compared. The function of the other genes in MDH expression is being analyzed. Genes encoding five protein subunits of MMO from Methylosinus trichosporium strain OB3B are being cloned to study expression of these gene products. We expect this information will permit us to understand how rapid synthesis of these gene products is achieved.

99. UNIVERSITY OF MINNESOTA - St. Paul, MN 55108

Molecular Approaches to Genomic Organization
I. Rubenstein, Dept. Genetics & Cell Biology
R.L. Phillips, Dept. Agronomy & Plant Genetics $84,000

The goal is to gain a fuller understanding or the macro-organization of the maize eukaryotic genome. The approach seeks to determine the chromosomal location of physical markers, the relationship of physical length of DNA to recombination values, and the linkage of molecular genetic markers to major genes involved in quantitative traits. Hybridization probes will be used that consist of cDNAs and genomic clones or genes of known function, genomic clones of highly repeated sequences, and cDNA clones prepared from the mRNAs of maize endosperm and suspension cell culture. The chromosomal location of each of the unique probes will be determined by restriction fragment length polymorphism using DNAs from monosomic plants. This

technique has been successfully used to locate the 27 kd water-soluble reduced glutelin zeins to chromosome 4. The chromosomal locations of highly repeated sequences will be determined by in situ hybridization. We hope to identify clones that will enable us to identify specific sets of chromosomes. The distribution of major genes in the genome for a quantitative trait, maturity, will be determined via linkages with RFLP markers.

100. **UNIVERSITY OF MISSOURI** - Columbia, MO 65211

Analysis of Cyanobacterial Photosystem II Genes by Cloning and Mutagenesis
L.A. Sherman, Biological Sciences Division $70,000

This project identifies and clones the genes coding for membrane proteins involved in cyanobacterial photosynthesis. The strains used for these studies, Anacystis nidulans R2 and Aphanocapsa sp., are both transformable. Aphanocapsa can be grown photoheterotrophically and is thus ideal for the isolation of photosynthesis mutants. This year we will continue the investigation of some novel membrane proteins. We have isolated proteins that appear to be regulated by changes in environmental conditions, either iron or light. Iron-regulated proteins include: a 36 kDa protein that is involved in iron-acquisition, and a 34 kDa intrinsic thylakoid chlorophyll-binding protein associated with PS II. Utilizing a lambda gt11 library we have cloned and sequenced the 36 kDa protein and are performing site-directed mutagenesis to determine the function of the protein. Insertion of a transposon into this gene and transformation of the mutated gene into a wild type host resulted in a strain which is unable to grow under low-iron conditions. We have obtained a 42 kDa carotenoid-binding protein that is induced upon growth in high light conditions, and cloned the gene for this protein. We have also isolated a carotenoid-binding protein that is localized exclusively in the plasmalemma. We have also cloned the 33 kDa extrinsic manganese-binding protein that is involved in oxygen-evolution. The gene has been cloned and sequenced and contains a 28 amino acid signal sequence. We are also studying the membrane composition of a chlorophyll b containing prochlorophyte. We are interested in analyzing this organism and comparing the chlorophyll-binding proteins from the prochlorophyte to those of cyanobacteria.

101. **UNIVERSITY OF MISSOURI** - Columbia, MO 65211

Genetics and Molecular Biology of Hydrogen Metabolism in Sulfate-reducing Bacteria
J.D. Wall, Department of Biochemistry $73,000

In anaerobic digestors or natural environments, the sulfate-reducing bacteria (SRB) play a pivotal role in methane generation, either providing hydrogen and acetate for methane formation or competing with the methanogens for those same substrates. The SRB are also the primary culprits in causing environmental metal corrosion costing millions of dollars each year and in producing poisonous sulfide sometimes costing lives. A key metabolite controlling the interactions of the SRB with other microorganisms in their environment is hydrogen. The number of enzymes capable of producing or consuming hydrogen in the SRB and their physiological functions remain obscure. Our laboratory is seeking to develop the genetics and molecular biology of the SRB with the aim of examining the hydrogen metabolism. Specifically a system of transformation and/or conjugation will be sought. Desulfovibrio desulfuricans ATCC 27774 has been found to be amenable to classical genetic manipulation, antibiotic resistant mutants as well as mutants altered in sulfate and hydrogen metabolism have been isolated. Most excitingly, this strain has been found to produce a defective bacteriophage apparently capable of generalized transduction. Additional mutants continue to be isolated which now can be genetically analyzed.

102. **MOUNT SINAI SCHOOL OF MEDICINE** - New York, NY 10029

The Respiratory Chain of Alkalophilic Bacteria
T.A. Krulwich, Department of Biochemistry $79,000

The long term goals of this study are a dissection of the respiratory chain of extremely alkalophilic bacilli with a view towards gaining insight into apparent, pH-dependent capacities to transduce energy with great efficiency. The structures of the complex III, cytochrome oxidase, and associated soluble cytochromes will be examined in detail. Attempts will be made to reconstitute the complex III into proteoliposomes so that mechanistic properties can be examined. The focus during the coming project period will focus on the completion of a purification procedure for complex III from obligately alkalophilic Bacillus firmus RAB. In addition, the properties and possible role(s) of a cytochrome c552 and b558 that have been purified this year will be probed using a combination of biochemical, biophysical and immunological approaches. Finally, we will use the immunological reagents developed during the above studies to determine which cytochrome specie(s) account for the marked elevation in membrane cytochromes that is associated with growth of facultatively alkalophilic OF4 at very high pH.

103. **NATIONAL INSTITUTES OF HEALTH** - Bethesda, MD 20892

Partial Support of GenBank: The Genetic Sequence Data Bank
J. Cassatt, National Institute of General Medical Sciences $40,000

GenBank, the Genetic Sequence Data Bank, is an internationally available repository of all reported nucleotide sequences greater than 50 nucleotides in length, annotated for sites of biological interest, and checked for accuracy. As of December 31, 1987 GenBank contained 10.1 million base pairs, comprising 11,000 sequences. The data bank is operated under contract to Bolt, Beranek and Newman, Inc. (BBN) of Cambridge, MA. Data collection, verification, entry, and annotation are performed under the direction of Dr. Walter Goad of Los Alamos National Laboratory; while distribution, user support services, and overall data bank management are performed by BBN. This resource, administered by the National Institute of General Medical Sciences, and co-sponsored by five institutes of NIH, two divisions of DOE, the U. S. Department of Agriculture, and the National Science Foundation, is of particular interest to geneticists and molecular biologists. A copy of the data base is available for a modest fee on computer-readable magnetic tape to anyone requesting it and is now available on IBM compatible floppy disks. Dial-up on-line access is also available. A software clearinghouse is provided on-line to supply information only on sequence analysis software available worldwide. The next hard copy edition of the data base will be available in June 1987, as an eight-volume set to be published by Academic Press.

104. **UNIVERSITY OF NEBRASKA** - Lincoln, NE 68588

Characterization of Plant Plasma Membrane Antigens
D.W. Galbraith, School of Biological Sciences $76,000

An understanding of the molecular architecture and developmental behavior of the plant plasma membrane is central to an understanding of plant growth and development and of the interactions of plants with the environment. We are characterizing some of the proteins and glycoproteins that are specifically located at the _Nicotiana_ plasma membrane, using monoclonal libraries prepared with plant plasma membranes and protoplasts as immunogens. We are particularly interested in the analysis of a subset of the antigens recognized by these antibodies that are expressed at the plasma membrane under heterotrophic but not photoautotrophic conditions. We are employing three approaches. In the first, we have constructed cDNA libraries in phage expression vectors corresponding to the different protoplast developmental stages. These are currently being screened with the monoclonal antibodies, in order to permit a characterization of the controls of gene expression involved in this developmental system, and to

compare possible congruencies to controlling systems found in the plasma membranes of heterologous organisms. In the second, using two-dimensional gel electrophoresis under denaturing conditions followed by Western blotting, we have purified one antigen to near homogeneity for sequence analysis. 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 heterologous chimaeric genes within or at the surfaces of transformed protoplasts, using a novel reporter gene system based on the E. coli beta-glucuronidase enzyme. We intend to use these techniques for the isolation of mutant protoplasts deficient in the control of secretion of proteins through the golgi/endoplasmic reticulum system, leading to an improved understanding of the mechanisms that control plant cell growth and division.

105. UNIVERSITY OF NEBRASKA - Lincoln, NE 68588

Viruses of Eukaryotic Green Algae
J.L. Van Etten, Department of Plant Pathology $74,000

We have isolated and partially characterized 30 large polyhedral dsDNA containing, plaque forming viruses which infect a unicellular, eukaryotic Chlorella-like green alga. One unusual feature of these viruses, whose genomes are about 300 kbp in size and contain various amounts of methylated bases [0.1% to 47% 5-methyldeoxycytosine (m^5dC) and 0 to 37% N^6-methyldeoxyadenosine (m^6dA)], is that infection with at least some of them results in the appearance of DNA methyltransferases and type II DNA restriction endonucleases. At least some, if not all, of these restriction-modification systems are virus encoded. The virus infected algae are the first source of type II DNA restriction endonucleases from a eukaryotic system. In addition, infection with some of these viruses results in the appearance of other DNA restricting enzymes; preliminary experiments indicate that some of these enzymes have specificities and properties distinct from type I, type II, and type III restriction endonucleases.

The objectives of this proposal are to isolate and characterize several of these DNA restriction endonucleases and related DNA degrading enzymes as well as some of the DNA methyltransferases formed during infection of the alga.

106. NEW YORK STATE DEPARTMENT OF HEALTH - Albany, NY 12201

Methane-Producing Bacteria: Immunologic Characterization
E. Conway de Macario, A.J.L. Macario, Laboratory of Immunology
M.J. Wolin, Laboratory of Environmental Biology & Field Services
Wadsworth Center for Laboratories & Research $3,000 extension

The long-term goal of this research is to elucidate the methanogenic flora of ecologic niches of scientific and biotechnologic interest (cultures, sediments, wastes, landfills, digesters). Immediate objectives include generation of polyclonal antibody probes for methanogens, and optimization of simple procedures for collecting and examining samples. Efforts focus on direct identification of bacteria avoiding culture-isolation, and on measuring their molecular markers in their fluid milieu. For the latter measurements direct and inhibition-blocking methods are applied, using a slide immunoenzymatic assay (SIA). This assay, along with others carried out on the same support (SIA-constellation) are used to characterize immunologically whole bacterial cells. Antigenic fingerprinting is done with the antibody probes and the results are correlated with those from analysis of the fluid milieu, and with microbiologic, chemical and engineering parameters. This research has yielded a considerable quantity of data, probes and methods useful in many ways to study methanogens. The work is entering a new phase in which this research's products will be instrumental in: increasing our knowledge of methanogens, their antigenic relationships, biochemistry and habitats; designing, operating and improving bioreactors; and implementing genetic and genetic engineering strategies for obtaining specialized, improved strains.

107. NORTH CAROLINA STATE UNIVERSITY - Raleigh, NC 27695-7612

Phosphoinositide Metabolism and Control of Cell Growth
W. F. Boss, Department of Botany $68,000

Polyphosphoinositides, a class of regulatory phospholipids, play a pivotal role in signal transduction in animal cells. The polyphosphoinositides function by providing a link between a stimulus and a cascade of calcium-mediated responses. Since calcium is involved in regulating plant growth and development, and since polyphosphoinositides are present in plant cells (Boss and Massel, Biochem. Biophys. Res. Comm., 132:1018-1023, 1985), the question arises as to whether polyphosphoinositides also are important in signal transduction in plant cells. Specifically, this project addresses the following questions: 1) Is there a correlation between plant growth responses and changes in phosphoinositide metabolism? 2) What is the nature of the enzymes involved in phosphoinositide metabolism and how do they respond to growth regulators, calcium, calmodulin, and other factors known to influence plant growth? 3) What is the precise sequence of events

involved in the proposed stimulus-response paradigm in plants? Carrot cells grown in suspension culture will be used for this study. The first year of the study will entail complete analysis of the carrot inositol phosphates and phosphoinositides. Rates of metabolism of the phosphoinositides and inositol phosphates will be monitored in vivo using both myo-2-[^{3}H]inositol and [$^{32}P_i$] to label the compounds over time under normal growth conditions. Having characterized the metabolic rates under normal conditions, we will be able to study changes in phosphoinositide metabolism in response to growth regulators and calcium.

108. NORTHWESTERN UNIVERSITY - Evanston, IL 60202

Genetics of Thermophilic Bacteria
N.E. Welker $61,000

The protoplast fusion procedure described by Chen, Wojcik, and Welker (J. Bacteriol. 169:994-1001 1986) was modified for use in plasmid and chromosomal DNA transformation of protoplasts of *Bacillus stearothermophilus* NUB 36. Protoplast transformation and transduction with generalized transducing phages TP-42, TP-43, and TP-46 were used to verify the order of the six markers established by protoplast fusion (*hom-1-thr-1-his-1-gly-1-pur-2-pur-1*). Linkages were established between *hom-1-thr-1-his-1*, *his-1-gly-1*; and *pur-2-pur-1*. The efficient transformation and transducing systems developed for NUB 36 can be used for fine structure mapping and protoplast fusion can be used to establish linkage between adjacent linkage groups or to order markers that span a relatively large segment of the chromosome. The genetic exchange systems will also be used to construct strains. NUB 3621 Rif^r Res^- Mod^- was transformed with plasmid pLW60 (Cm^r Km^r) DNA with an efficiency of 1 X 10^4 to 5 X 10^5 transformants (chloramphenicol-resistant)/ µg DNA. Plasmid pLW60 is a hybrid plasmid derived from plasmid pPL401 (plasmid pUB110 with the chloramphenicol-resistance gene from plasmid pC194 inserted into the *Eco* RI site) and cryptic plasmid pNW1. NUB 3621 Rif^r Res- Mod- (pLW60 Cm^r Km^r) grows well at 60 and 65°C in the presence of chloramphenicol or kanamycin. The development of efficient genetic exchange systems and a molecular cloning system in *B. stearothermophilus* will have a profound effect on investigations concerning the biochemical molecular, and genetic basis of thermophily.

109. **OHIO STATE UNIVERSITY** - Columbus, OH 43210

Basis of Competitiveness of Rhizobia
W.D. Bauer $79,000

Rhizobia are soil bacteria that symbiotically infect and nodulate leguminous plants to fix atmospheric nitrogen for their host. The agricultural benefit of this symbiosis depends on having the best available strains of rhizobia form most of the nodules on roots of host plants in the field. It has not been possible to achieve this consistently because rhizobia inoculated onto the seed at planting do not colonize and infect seedling roots well enough to compete against indigenous rhizobia. The project objective is to determine what factors contribute importantly to the competitive ability of inoculated rhizobia. Recent studies have shown that chemotactic motility and attachment to roots contribute to inoculum competitiveness, at least under laboratory conditions. The capacity for firm polar attachment, apparently mediated by pili, seems unnecessary for nodule formation and no more important than loose association for infection initiation, but can enhance root colonization by 10-fold and more. Motility and chemotaxis are likewise unnecessary for nodules formation, but substantially enhance efficiency of nodule formation, probably by allowing the bacteria to come in physical/chemical contact with the root in high enough numbers to overcome host response thresholds.

110. **OHIO STATE UNIVERSITY** - Columbus, OH 43210

Structure and Regulation of Methanogen Genes
J.N. Reeve and J.I. Frea $78,000

The goal of this project is to determine the structure and mechanism(s) of regulation of genes in methane producing archaebacteria known as methanogens. We have cloned and sequenced several methanogen genes from a range of methanogens including mesophilic species (*M. vannielii*, *M. smithii*) thermophilic species (*M. thermoautotrophicum*, *M. thermolithotrophicus*) and the extremely thermophilic species *M. fervidus*. The DNA sequences so far obtained strongly suggest that methanogen genes are organized into multi-gene transcriptional units (operons) and that ribosome binding sequences are needed for initiation of protein synthesis. Our current experiments are designed to determine if these conclusions, based on DNA sequence data, are valid. We are using S1 nuclease protection experiments, run-off transcription and Northern blot procedures to identify and characterize mRNAs synthesized *in vivo* and *in vitro*. We have purified DNA-dependent RNA-polymerases from methanogens and are using these enzymes in DNA-binding experiments to identify methanogen promoters. Genes which

encode the subunits of non-F_{420}-reducing hydrogenase in M. thermoautotrophicum and methyl coenzyme-M reductase in M. vannielii have been cloned and sequenced. We are currently determining the effects of varying growth conditions on the expression of these genes whose products are enzymes of central importance to methanogenesis. This should provide basic information describing parameters which directly regulate the efficacy of biological methane production.

111. OKLAHOMA STATE UNIVERSITY - Stillwater, OK 74078

The Structure of Pectins and Their Possible Role in Resistance of Cotton to Cotton Blight
A. Mort, Department of Biochemistry $54,000

Pectic polymers are an integral part of the primary cell walls of dicots, yet we still do not have a complete idea of their structure. A more detailed knowledge of their structure should allow us to understand better how pectins are involved in cell wall function. Pectins play a role in cell expansion, cell adhesion, disease responses, and perhaps cell differentiation and recognition. We are particularly interested in the role of pectins in resistance to cotton blight. By using a combination of classical chemical and enzymic methods with new methods developed in this laboratory (solvolysis of specific glycosidic linkages with liquid HF and degradation of uronic acids with lithium in ethylenediamine) we will characterize, as fully as possible, two major regions of the pectin polymers of cotton suspension culture cell walls. (1) We will determine the size distribution of the homogalacturonan regions and distribution of methyl esterification and acetylation sites within it. None of these characteristics has been determined yet for a primary cell wall pectin. (2) We will characterize the region which in sycamore cells, and all of the species we have tested is a repeating disaccharide of rhamnose and galacturonic acid with sidechains on approximately half of the rhamnose residues. We have found that at least one in three of the galacturonic acid residues are acetylated at O-3. We will characterize the sidechains of this region and look for linkages from it to other cell wall polymers.

112. UNIVERSITY OF OKLAHOMA - Norman, OK 73019

Metabolism and Bioenergetics of Syntrophomonas wolfei
M.J. McInerney, Department of Botany & Microbiology $67,000

Anaerobic hydrogen-producing syntrophic bacteria degrade fatty acids which are important intermediates in anaerobic degradation and methanogenesis. These bacteria grow very slowly and require the presence of a hydrogen-using organism to degrade fatty acids. These organisms are excellent models to study the biochemical aspects of mutualism as well as the energetics of slow growing organisms. We have developed methods to physically separate cells of the anaerobic, fatty acid degrader,

Syntrophomonas wolfei, from those of the hydrogen user by isopycnic centrifugation as well as to selectively lyse S. wolfei cells using lysozyme. These methods allow the physiological study of S. wolfei without significant contamination by cellular components of the hydrogen user. We have also obtained pure cultures of S. wolfei by selecting for a variant which uses crotonate. The specificity of the coenzyme A transferase activity in the pure culture differs from that of the coculture suggesting that the ability to use crotonate resulted from an alteration of this enzyme. Pure cultures of S. wolfei have molar growth yields 2 to 3 times higher than that of other crotonate-using organisms. This suggests that S. wolfei has a very efficient means for conserving energy which is expected since S. wolfei probably evolved such a system in response to the very small change in free energy associated with growth on butyrate. S. wolfei grown on crotonate accumulates large amounts of poly-beta-hydroxybutyrate (about 20-30 percent of the dry weight) and radioisotopic studies suggest that poly-beta-hydroxybutyrate is synthesized directly from the beta-oxidation intermediate rather than from acetyl-coenzyme A.

113. OREGON GRADUATE CENTER - Beaverton, Oregon 97006-1999

Enzymic Components of the Phanerochaete chrysosporium Lignin Degradative System
Michael H. Gold, Department of Chemical, Biological and Environmental Sciences $62,000

Lignin, a major component of woody plant cell walls, is the second most abundant natural polymer on earth after cellulose. The potential use of white rot fungi for transforming lignocellulose into useful fuels, chemicals, and pulps is a promising field of research. Recently, two extracellular enzymes--a lignin peroxidase and an Mn peroxidase--have been isolated from ligninolytic cultures of Phanerochaete chrysosporium, and the mechanisms and structures of these enzymes are being elucidated.

The objectives of this project are to identify other enzymes or alternative enzyme forms which are part of the lignin degradation system of this organism.

1. Pathways and enzymes involved in lignin demethoxylation and ring opening reactions will be sought. Biphenyl, diaryl ether, and other dimeric and monomeric lignin model compounds will be synthesized and used in metabolic experiments to identify the pathways involved. Cleavage products will be separated and analyzed by GCMS and spectrophotometry. Mycelial bound and/or soluble extracellular crude enzyme preparations will be monitored for the activities involved, and any identified enzymes will be purified and characterized.

2. Enzymic degradation of polymeric dyes and lignin: Using culture transfer techniques, crude cell fractionation and HPLC separation of all of the soluble extracellular proteins from mutant and wild type strains of P. chrysosporium, we intend to resolve all of the components of the lignin

degradative system. Lignin model compounds, polymeric dyes and radiolabeled lignin will be used to determine which enzymes are responsible for the cleavage of specific bonds and what is the minimal complement of enzymes required for the efficient depolymerization or transformation of lignin and lignocellulose.

114. **OREGON STATE UNIVERSITY** - Corvallis, OR 97331

Genomic Variation in Maize
C. Rivin, Dept. Botany and Plant Pathology $55,593 (20 months)

We are interested in the molecular basis and biological significance of genomic diversity in maize. The repetitive portion of the maize genome may be highly plastic. In a comparison of nuclear DNA from different inbred lines, we have shown that many repetitive sequences are quantitatively variable while others are constant. We have also found variability for cryptic, low copy number transposons. These polymorphisms are stable within inbred lines, but unexpected copy numbers are observed in the progeny of specific outcrosses, tissue culture cells, and in regenerated plants. The project objective is to investigate the molecular and genetic basis for this modulation. Two approaches will be taken: First, we will try to identify features of molecular organization, function and DNA modification that characterize those sequences that are subject to variation in contrast to those that are invariant. Second, we will examine the genomic changes themselves by quantitative DNA hybridization and Southern blotting to determine when in development, culture or regeneration the modulation occurs, how it affects the restriction and modification patterns of specific sequences, and whether the novel variants are stable in successive generations. These experiments will provide new and more comprehensive information on genome plasticity in maize and its implications for the genetic manipulation and molecular evaluation of this major crop plant.

115. **PENNSYLVANIA STATE UNIVERSITY** - University Park, PA 16802

The Role of Turgor Pressure and Solute Uptake in Plant Cell Growth
D.J. Cosgrove, Department of Biology $82,000

Prolonged cell expansion during plant growth requires integration of three distinct but interrelated processes: wall relaxation and synthesis, water uptake, and solute uptake (or synthesis). Wall relaxation reduces cell turgor pressure and thereby generates the gradient in water potential needed for water uptake. Our recent results with pea (Pisum sativum L.) and soybean (Glycine max Merr.) seedlings have shown that water uptake by growing stems is rapid and imposes little restriction on the rate of cell expansion. Thus it cannot be a major control point for growth. Our current focus is on the processes of wall relaxation and solute transport. Although it is commonly accepted that wall yielding depends on the mechanical wall stress generated by cell turgor, there are new indications that wall yielding might be insensitive to moderate turgor changes, when

turgor pressure is high. One major goal of this project is to examine in detail the dependence of wall yielding on turgor pressure. This is being done by detailed measurements of in-vivo wall relaxation, using a computer-assisted pressure microprobe and the new pressure block techniques. Our newest pressure-block results indicate that wall relaxation is more dynamic than predicted by current theory. A second major goal of this project is to determine the interrelationship between cell expansion and solute transport into expanding cells. We will selectively block either cell expansion or solute transport, and measure the effect of such blockage on the unblocked process. This work will provide insight into the basic cellular and physical processes controlling plant growth.

116. **PENNSYLVANIA STATE UNIVERSITY** - University Park, PA 16802

Enzymology and Molecular Biology of Lignin Degradation
M. Tien, Department of Molecular and Cell Biology
$122,000 (two years)

Lignin is an aromatic polymer which accounts for a large percentage of the energy that plants capture from the sun. Approximately 20% of all the carbons fixed by photosynthesis is transformed to lignin. Lignin is an integral part of all woody tissue, imparting it with structural rigidity. The biodegradation of lignin plays a key role in carbon recycling on Earth. Its biodegradation is brought about predominantly by wood-destroying fungi. Much of what is known about fungal degradation of lignin has been via studies with *Phanerochaete chrysosporium*. This fungus degrades lignin by initially depolymerizing it with extracellular enzymes. We have purified many of the extracellular enzymes produced by *P. chrysosporium*. We have characterized them as hemeprotein peroxidases which require H_2O_2, also secreted by the fungus, to catalyze their oxidations. The goals of our work are to understand the enzymology of the ligninases. We are performing steady-state and transient-state kinetic studies to characterize the intermediates formed during enzyme catalysis. We are also performing biophysical studies to better understand the environment at the active site of ligninase. To further understand the structure of the ligninase enzymes, we are also cloning them. cDNA and genomic libraries have been constructed and we have identified some of the ligninase genes. We are presently attempting to express them in active form in yeast. This will allow us to over-express the ligninase which will facilitate our enzymology studies. It will also allow us to use site-directed mutagenesis to understand catalysis at the molecular level.

117. UNIVERSITY OF PENNSYLVANIA - Philadelphia, PA 19104

DNA Sequences Encoding Chlorophyll a/b Binding Polypeptides
A.R. Cashmore, Plant Science Institute, Department of Biology
$107,000

Chlorophyll a/b (CAB) polypeptides form the major polypeptide components of chloroplast thylakoid membranes. We have characterized many of the nuclear genes in tomato encoding these CAB polypeptides. The photosystem II (PSII) CAB polypeptides can be divided into 2 classes (types I and II), the genes for which have been mapped to 5 distinct loci. We have also characterized genes for a photosystem I (PSI) polypeptide; from the DNA sequence we have demonstrated that the PSI polypeptides are substantially divergent from their PSII counterparts. Expression of the CAB genes is photoregulated. By Agrobacterium mediated transformation with in vitro modified genes we are studying the DNA sequences that mediate this photoregulation. We find for chimeric genes in transgenic plants that at least 1.6 kb of CAB 5' noncoding sequences are required for maximum levels of expression. The detailed nature of these cis-acting photoregulatory sequences are presently being studied.

118. UNIVERSITY OF PENNSYLVANIA - Philadelphia, PA 19104

Factors Governing Light-Driven Electron and Proton Translocation in Protons Across Membranes
P.L. Dutton, Department of Biochemistry & Biophysics $91,000

Membrane redox proteins (e.g., photosynthetic reaction centers, ubiquinol-cytochrome c oxidoreductases, and various terminal oxidases) separate charge across the membrane coupled to electron transfer. These kinds of enzymes represent the primary battery of energy conversion systems in virtually all forms of life. The goal of this research is to develop experimental methods to study these enzyme systems in planar arrays so that the individual redox-linked charge-separating steps are resolved and can be studied individually. It is important that the planar arrays can be placed between electrodes and the electrical responses measured directly rather than using the traditional indirect methods. Planar arrays can be quantitatively and systematically manipulated by applying electric fields. We have concentrated on reaction centers from photosynthetic bacteria to develop strategies for deposition of the protein on solid electrode supports in ordered arrays. Flash-activation of such films followed by measurement of current or voltage in the presence of applied fields is

opening up new and promising views of the early events in the reaction center. A further complementary line of work is based on the use of modified enzymes: all of the above enzymes contain quinones functional in key positions, and methods are being developed to replace them with alternatives that are electrochemically and systematically varied. We intend to bring (to all the enzymes) the invaluable capability to activate via light flashes or voltage pushes.

119. RUTGERS UNIVERSITY - New Brunswick, NJ 08903

Cellulase - A Key Enzyme in Fermentation
D.E. Eveleigh, J.D. MacMillan, Dept. of Biochemistry & Microbiology
$67,000

A commercial source of cellulase has become increasingly more important in fermentable sugar production and in municipal refuse composting. The degradation of cellulose is accomplished by a multienzyme cellulase complex. Immunological tools are being used to study individual enzymes. Monoclonal antibodies specific for cellobiohydrolase (CBH I) from Trichoderma reesei have been used for the purification of CBH I, and in a direct ELISA assay (DSA) with labeled polyclonal antibodies for quantifying this enzyme in cellulase broths. Crude broth separation in and elution of individual bands from preparative isoelectricfocusing verified the quantification of percent CBH that was determined by the DSA. Biochemical and immunological methodology has been extended to the cellulase system of Microbispora bispora, a thermophilic actinomycete, whose cellulase enzyme complex consists of endoglucanases, CBH's and -glucosidases. Four endoglucanases and two CBH's have been purified. Monoclonal antibodies to the purified CBH II enzyme have been produced. Synergistic interaction among the purified components is necessary for cellulose degradation. The use of monoclonal antibodies to the individual enzymes as specific probes will help to further elucidate the mechanism of degradation.

120. RUTGERS UNIVERSITY - Piscataway, NJ 08855-0759

Corn Storage Protein: A Molecular Genetic Model
J. Messing, Waksman Institute of Microbiology $96,000

Seed storage proteins are the staple protein in feed of livestock. They represent a family of heterogenous proteins that accumulate during seed development. In corn, the bulk of them, which are collectively called zeins, are exclusively synthesized in the endosperm of the seed rather than the embryo. Their synthesis commences about 12 days after pollination and continues thereafter until close to maturity. Although these proteins are

collectively an important nitrogen source for the germinating seed, the absence of individual members is not lethal. Therefore, natural variants exist where individual genes are present or absent or modulated in their expression. These variations have been used to map a large number of them as single mendelian factors. We have developed new tools for the analysis of gene structure and function derived from bacteriophage M13 and are using them now to analyse the zein multigene family. The majority of them are encoded by many different genes that are frequently clustered and may have been derived from gene amplification and unequal crossing over of a repeated block structure central to their coding information. This block structure is high in glutamine, an important nitrogen acceptor, and repeated about 8 to 9 times in the central portion of the proteins from the zein-1 fraction. Other zeins, mainly from the zein-2 fraction that are high in other amino acids like proline and methionine, are encoded by only one or two genes per haploid genome. It is these zein genes with which we would like to study the regulation of their expression on the transcriptional and post transcriptional level.

121. **SALK INSTITUTE FOR BIOLOGICAL SCIENCES** - San Diego, CA 92138

Biosynthesis of Plant Plasmamembrane Polypeptides
C.J. Lamb, Plant Biology Laboratory
$152,000 (two years)

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 Mr 130-185 kDa elaborated from a single polypeptide species Mr 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. We now propose to identify molecular clones encoding polypeptide p50 sequences by screening cDNA libraries cloned in λgt 11 with appropriate antibodies or by protein microsequencing and synthesis of oligonucleotide probes. These molecular clones and extant antibodies will be used to study the biosynthesis and processing of the family of plasmamembrane glycoproteins based on polypeptide p50 with specific emphasis on the molecular basis for transport, post-translational modification and sortive trafficking through the endomembrane system to the plant plasmamembrane.

122. SMITHSONIAN INSTITUTION - Washington, DC 20560

Organization of Photosystem I and Photosystem II in the Photosynthetic Membranes of Phycobilisome - Containing Plants
E. Gantt, Botany Department, University of Maryland, College Park
$70,000

The competence of plants with changing light conditions is being investigated in phycobilisome-containing plants. The study is an integrated approach combing quantitative assessments of principal proteins of photosystem I, photosystem II, and phycobilisomes with physiological activity. Quantitative immunoelectrophoresis with specific antisera to selected apoproteins will allow correlation of physiological and structural changes as a function of light intensity. Concomitantly the structural arrangement of the photosystems in the thylakoids will be probed in vitro and in situ with protein cross-linking reagents and by immunocytochemistry. The stoichiometry of the photosystem reaction centers is being determined by photochemical techniques on a separate collaborative project. Recent results indicate that the energy flow from phycobilisomes to thylakoids occurs predominantly through the putative anchor polypeptide (ca. 94 kD). Partial N-terminal sequence analysis of 94 polypeptides from phycobilisomes of a eukaryote and a prokaryote had great homology with one another but not with other phycobiliproteins or linkers. Results from this research project are expected to greatly extend our understanding of how light acclimation occurs.

123. SOLAR ENERGY RESEARCH INSTITUTE - Golden, CO 80401

The Water-Splitting Apparatus of Photosynthesis
M. Seibert, Photoconversion Research Branch $120,000

The water-oxidizing complex of photosystem II (PS II) supplies the reductant ultimately used by algae and green plants to fix carbon during oxygenic photosynthesis. We are using a number of techniques to identify the components of the complex, to determine how the components are related structurally, and to understand the mechanism of O_2 evolution. New immunological studies have confirmed last year's finding that the Dl (Q_B) protein affects electron transport function on both sides of the PSII reaction center. The Scenedesmus LF-1 mutant (with a modified Dl protein) was found blocked in electron transport prior to Z (direct donor to PSII) possibly due to failure of the mutant to bind functional Mn. The three extrinsic proteins associated with the O_2-evolving enzyme complex (OEC) have been located on 8 nm high multimeric (tetrameric) protruding surface particles found on the lumenal side of appressed grana thylakoids by freeze-fracture electron microscopy. Smaller dimeric surface particles (6

nm high) that remain after extraction of the extrinsic proteins probably contain the rest of the OEC and perhaps components of the PSII core complex. These conclusions have profound implications with respect to current ideas about the structure of PSII. Anomalous O_2 production observed in damaged PSII preparations under steady-state and flash conditions was thought to be associated with S-state intermediates of the normal O_2-evolving process. Work this year showed that it is actually due to electron extraction from H_2O_2 by PSII with "free Mn" as a mediator in the absence of functionally active Mn.

124. SOUTHERN ILLINOIS UNIVERSITY - Carbondale, IL 62901

Regulation of Alcohol Fermentation by *Escherichia coli*
D.P. Clark, Department of Microbiology $70,000

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*. In addition we are investigating the regulation of other genes involved in anaerobic growth and fermentation. We have isolated mutations in the structural genes for alcohol dehydrogenase, the enzyme catalyzing the final stage of alcohol production. In addition we have isolated several regulatory mutations, some linked to the structural gene and others in a different region of the chromosome. The *adh* region has been cloned in order to facilitate the study of *adh* regulation. We have also isolated a comprehensive collection of operon fusions in which the *lacZ* structural gene is fused to promoters that are inactive in air but induced under anaerobic conditions. In addition we have operon fusions to both the structural and regulatory genes for alcohol dehydrogenase. Expression of these anaerobic genes is greatly affected by the nature of the carbon and nitrogen sources, the pH, the nature of the buffer, and the presence of alternative electron acceptors (e.g. nitrate, dimethyl sulfoxide, etc.). One common factor involved in the regulation of one major class of anaerobic gene seems to be the operation of the respiratory electron transport chain, however, the details remain to be clarified. Finally, we have devised a technique, proton suicide, which selects directly for mutants defective in the synthesis of acidic fermentation products. Several such mutants are presently being characterized.

125. **STANFORD UNIVERSITY** - Stanford, CA 94305

Host range and other symbiotic genes of Rhizobium meliloti
S.R. Long, Department of Biological Sciences $106,151

Plants which can establish nitrogen fixing symbiotic root nodules with Rhizobium bacteria provide a possible means of conserving energy which would otherwise be used in industrial ammonia synthesis. However, relatively few plants are able to do this. Our concern in this research proposal is the basis for Rhizobium host range. Previously, we had identified a cloned DNA segment from Rhizobium meliloti which bore host range determinants for the host plant, alfalfa. In the past year we completed the mapping and test of properties for 90 mutants in this genomic region. This has led us to the identification of at least 5 to 6 genes which affect nodulation. The sequences of four of these genes have been determined. We have also identified the RNA transcripts for the genes, and have defined the protein products. We have begun the microscopic investigation of how the mutants affect plant roots. Our current work is aimed at defining the biochemical basis for the action of host range genes, including the surface analysis of mutants.

126. **VIRGINIA POLYTECHNIC INSTITUTE & STATE UNIVERSITY** - Blacksburg, VA 24061

Enzymology of Acetone-Butanol-Isopropanol Formation
J.-S. Chen, Department of Anaerobic Microbiology $68,000

Butanol and isopropanol, which are characteristic products of Clostridium beijerinckii (formerly, Clostridium butylicum), are both important feedstocks for the chemical industry and ideal additives (as cosolvents and octane number enhancers) in transportation fuels. The industrial use of acetone-butanol-isopropanol ("solvents") fermentation is limited by several factors: the easy loss of the solvent-producing ability, a low product concentration due to butanol toxicity, and the lack of an effective way to regulate product ratio. This project will elucidate the molecular properties of enzymes specifically involved in the formation of acetone, butanol, and isopropanol. The study will generate information that may lead to rational approaches to alleviate some of the limitations. We showed that in butanol/isopropanol-producing C. beijerinckii, a NADP-specific butanol-ethanol-isopropanol dehydrogenase is responsible for the formation of both butanol and isopropanol, and it differs in almost all aspects from the butanol-ethanol dehydrogenase found in C. beijerinckii strain producing butanol. During solvent production, the conversion of acetoacetyl CoA into acetoacetate is a key step which shunts acetoacetyl

CoA into acetone/isopropanol formation, and properties of the enzyme catalyzing this reaction may be important for attempts to regulate the product ratio. In C. beijerinckii, the presence of an acetoacetyl CoA hydrolase (deacylase) as indicated, and it suggests new possibilities for regulating the metabolic pathways. We also found that activities of solvent-forming enzymes rose sequentially during the metabolic transition. These enzymes and the control mechanism for their expression are being further studied.

127. **VIRGINIA POLYTECHNIC INSTITUTE & STATE UNIVERSITY** -
Blacksburg, VA 24061

Enzymological Studies of One-Carbon Reactions in the Pathway of Acetate Utilization by Methanogenic Bacteria
J.G. Ferry, Department of Anaerobic Microbiology $43,000

The general pathway of acetate conversion to methane in Methanosarcina thermophila is known and several of the enzymes have been purified and characterized. Acetate is activated to acetylCoA catalyzed by acetate kinase and phosphotransacetylase followed by carbon-carbon bond cleavage thought to be catalyzed by a enzyme complex with carbon monoxide dehydrogenase activity. The methyl group is ultimately transferred to Coenzyme M and the carbonyl to the nickel site in the complex. Methylcoenzyme M is reductively demethylated to methane with electrons derived from the oxidation of the bound carbonyl. A ferredoxin is the immediate electron acceptor of electrons leaving the complex. A corrinoid protein in the complex is thought to catalyze methyl transfer, electron transfer, or both. All of the above proteins have been purified except the corrinoid protein. The synthesis of acetate activation enzymes and components of the carbon monoxide dehydrogenase complex are repressed when the organism is grown on alternate substrates implying regulation. Our laboratory is investigating the catalytic mechanism of the purified proteins using physical, chemical, immunological, and molecular genetic techniques. Electron paramagnetic resonance techniques are used to probe the iron and nickel redox centers of the ferredoxin and carbon monoxide dehydrogenase complex. Biochemical characterizations of all proteins will include amino acid analysis and N-terminal sequencing, kinetic parameters, physical and other properties. Antibodies will be obtained for genetic analysis and to determine the cellular location utilizing affinity-gold techniques. Other components of the pathway will continue to be purified and characterized including the corrinoid protein. Major emphasis will be placed on the isolation of genes encoding enzymes of the pathway. The genes will be sequenced to obtain structural information and will also be used to investigate the organization of genes and their regulation.

128. WASHINGTON UNIVERSITY - St. Louis, MO 63130

Genetic Engineering with a Gene Encoding a Soybean Storage Protein
R.N. Beachy, Department of Biology $74,000

The β-conglycinins are soybean seed storage proteins and are made up of three subunits, α', α , and β. Genes encoding the α' and β-subunit proteins have been isolated and characterized. Each of the genes have been transferred to petunia and tobacco cells via a disarmed Ti-plasmid of Agrobacterium tumefaciens. Each is regulated in much the same way as it is regulated in soybeans; i.e., each gene is expressed only in developing seeds and in a temporal fashion consistent with its expression in soybean plants. A series of promoter deletion mutations have been made in the α'-subunit gene and the expression of the mutant genes was assessed in transgenic plants. This work was recently reported in the PNAS, USA 83, 8560-8564 (November, 1986). To continue this work we created a number of chimeric transcriptional promoters made up of fragments taken from the promoter for the 35S transcript of cauliflower mosaic virus, from the α'-subunit gene, and the β-subunit gene of β-conglycinin. Chimeric promoters will drive the expression of storage protein genes or the chloramphenicol acetyl transferase (CAT) gene. The goal of these experiments is to identify the sequences of the seed promoters that are responsible for the temporal regulation of these genes. Additional mutations to be made will include alterations of specific nucleotides, and alterations of spacing between DNA sequences, that are implicated to control the expression of the β-conglycinin genes.

129. WASHINGTON UNIVERSITY - St. Louis, MO 63110

Processing and Targeting of the Thiol Protease, Aleurain
J.C. Rogers, Division of Hematology-Oncology $84,500

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. From analysis of the gene, the N-terminal 1/3 of the protein, abut 140 amino acids, with no detectable homologies to other known protein sequences, may represent sequence contributed by a transposable element. In aleurone tissue, the mRNA is increased by gibberellic acid and decreased by abscisic acid, but is expressed at high levels in leaf and root tissues. The amino acid sequence and cathepsin H homology suggests that the protein is both glycosylated and secreted. Using our cDNA clone in bacterial expression systems, we will make different fusion proteins containing the

"protease" domain, the "transposed" domain, and the complete pre-protein for aleurain. These will be used to induce specific antibodies in rabbits. With these antisera we will be able to identify the protease in different barley tissues, and to characterize the pre- and mature forms of the enzyme. The ultimate cellular target of the enzyme will be characterized in leaf tissue. The antisera will allow purification of the enzyme from leaf tissue, as an initial approach to characterizing its proteolytic substrate specificity and to understanding more about its function in different barley cells.

130. **WASHINGTON UNIVERSITY**- St. Louis, MO 63130

Separating the Influx and Efflux Components of Net Uptake of NO_3- and NH_4+ With the Use of Isotope Fractionation Data
G. Shearer, D. Kohl, Department of Biology $90,000 (2 years)

It is of interest to be able to separate the influx and efflux components of net nitrate and ammonium uptake into cells. Separating influx from efflux with presently available techniques requires short term measurements. Here we propose to evaluate and use a method for separating influx and efflux in steady state systems using isotopic fractionation data. NO_3- or NH_4+, after entering a cell, will either leave the cell or be chemically transformed. The three relevant processes (influx, efflux and chemical transformation) each will have an associated rate constant and kinetic N isotope effect. The observed N isotope effect on the disappearance of nitrate or ammonium ion from the external medium (β_{obs}) will be a function of the isotope effects on the individual steps of the reaction sequence and the ratio of efflux to influx. We propose to measure β_{obs} under a series of experimental conditions which alter the net uptake (influx minus efflux) of NO_3- or NH_4+. We further propose to measure the value of the appropriate ratios of kinetic isotope effects in whole cells of *Anacystis nidulans* and of *Pseudomonas florescens*, and the kinetic isotope effect for the chemical transformation of the NO_3- or NH_4+ with isolated enzyme preparations. This will allow us to separately assess the influx and efflux components of net uptake.

131. **WASHINGTON UNIVERSITY** - St. Louis, MO 63130

Hydroxyproline-Rich Glycoproteins of the Plant Cell Wall
J.E. Varner, Biology Department $123,000

The cell walls of plants, particularly of dicots, characteristically contain glycoproteins rich in hydroxyproline. It has been presumed that the plant glycoprotein(s)-extensin(s)-has an important structural/developmental role. The goal of this project is to check this presumption. Towards this end we have 1) isolated and purified to homogeneity one hydroxyproline-rich glycoprotein from aerated carrot slices, 2) characterized the conformation of this glycoprotein as an extended polyproline II helix -- it is a rod 80 nm long and visible by electron microscopy, 3) isolated and purified the mRNA for carrot extensin, 4) made cDNA against the mRNA, and 5) used the cDNA to isolate the gene for a carrot extensin. This gene has been sequenced and the amino acid sequence derived from the base sequence. The transcripts from this gene increase in abundance following wounding (slicing and aeration of the carrot root), treatment with ethylene and treatment with carrot cell wall-derived elicitors. Another, non-homologous, transcript coding for a 33 kilodalton peptide rich in proline and poor in leucine also accumulates following wounding. This second transcript encodes several repeats of - His Lys Pro Pro Val - and - Try Thr Pro Pro Val. Current work includes the study of 1) the control of the expression of the 33 kd protein, 2) the chemistry of the reactions responsible for insolubilizing the extensins in the cell wall, 3) further characterization of an hydroxyproline-rich protein that is also rich in proline, threonine and lysine from developing maize pericarp and 4) development of a tissue print technique that allows rapid and inexpensive cellular localization of soluble extensins.

132. **WASHINGTON STATE UNIVERSITY** - Pullman, WA 99164

Regulation of Terpene Metabolism
R. Croteau, Institute of Biological Chemistry $80,000

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 intensive investigation of two model systems: (1) camphor metabolism in *Salvia officinalis* and (2) menthone metabolism in *Mentha piperita*. These systems allow probing the control of both biosynthetic and catabolic processes involved in monoterpene accumulation. The pathways of biosynthesis of both monoterpenes have been established. As the plant matures, both terpenoids undergo catabolism by a pathway involving conversion to a glycoside and

transport of this derivative 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. During the catabolic phase, the epidermal oil glands collapse as terpenes are removed from the extracellular cavity. Ultrastructural investigations indicate that the gland basal cell is the likely site of terpene conjugation during oil gland senescence. Foliar application of several growth regulators and bioregulators increases terpene yield by delaying the onset of oil gland senescence and by increasing the levels of enzymes responsible for producing the parent monoterpene types. 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.

133. **WASHINGTON STATE UNIVERSITY** - Pullman, WA 99164-6340

Enhancement of photoassimilate utilization by manipulation of the ADPglucose pyrophosphorylase gene
T.W. Okita, Institute of Biological Chemistry $60,000

For many plants, starch represents the principal storage form of CO_2 fixation. The production of starch is metabolically regulated by the activity of ADPglucose pyrophosphorylase, an allosterically regulated enzyme which catalyzes the first committed step of the starch biosynthetic pathway. Despite a wealth of knowledge concerning the physical and enzymatic properties of this central enzyme, little is known about its gene structure, its organization on the potato chromosomes, and the molecular factors that control its expression during tuber development. To fill this gap, an investigation of the relationship between ADPglucose pyrophosphorylase gene expression and the tuberization process is proposed. Recombinant clones for pyrophosphorylase will be isolated from a λgt 11 tuber cDNA library by screening with potato tuber anti-pyrophosphorylase or with the rice seed pyrophosphorylase cDNA clone. Subsequently, genomic clones will be obtained through analysis of a potato gene library with the cDNA probe. The tissue-specificity of various isolated clones will be evaluated by standard hybridization-melting experiments employing poly(A+)RNA fractions derived from various plant tissues. With the identification of the tuber-specific gene, its coding and relevant flanking sequences will be elucidated. To identify cis-acting regulatory sequences, translational fusions with the chloramphenicol acetyltransferase coding segment will be generated and inserted into plants via Ti-DNA vectors. Resultant transgenic plants will be examined with a goal of determining those regions of DNA responsible for tissue-specific gene expression. This research will increase our understanding of the regulation of plant gene expression during somatic tissue differentiation and facilitate efforts to improve the catalytic conversion of photoassimilates into starch by molecular alteration of the allosteric site of ADPglucose pyrophosphorylase enzyme.

134. **UNIVERSITY OF WASHINGTON** - Seattle, WA 98195

Studies on the Control of Plant Cell Enlargement by Cellular Parameters
R. E. Cleland, Department of Botany $75,000

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. This is indicated by the fact that long-term in-vitro tension and 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.

135. **UNIVERSITY OF WASHINGTON** - Seattle, WA 98195

RNA Polymerase III Transcription in Higher Plants
B.D. Hall, Department of Genetics $71,000

Wheat germ is being used to develop a higher plant in vitro transcription system for the synthesis of pre-tRNA molecules. Whole cell extracts from wheat embryos are fractionated by methods which yielded active RNA polymerase (polIII) systems from yeast, Xenopus, and human cells. A presumptive transfer RNA-specific factor is partially purified and identified using a DNA binding assay based on gel electrophoresis. The assay detects specific binding of transcription factor III C (TFIIIC) to the internal promoter sequences of tRNA genes as shown with TFIIIC from yeast (alias tau). Binding activity was detected in several fractions after chromatography of wheat germ whole cell extracts on phosphocellulose and DEAE-sepharose. The fractions showing binding activity were added individually to a cell free yeast transcription system depleted for factor tau. None of the fractions was able to stimulate in vitro transcription of a tRNA gene. However, polIII isolated from wheat restored transcriptional activity of a yeast system lacking polIII. An active yeast system is used to monitor the removal of inhibitory components in the wheat extracts. For

the inhibition of the active yeast system by wheat extracts RNases are only partially responsible as shown by mixing and degradation experiments. By means of thin layer chromatography it was shown that the ribonucleoside triphosphate substrates are not affected by the addition of wheat extracts. Affinity chromatography using DNA sequences specific for TFIIIC will make possible further purification and characterization of TFIIIC from wheat germ. Once isolated, the wheat TFIIIC will be combined with wheat polIII and DNA as the basis for a cell-free transcription system. Additional protein fractions from the wheat extracts will then be added as necessary to obtain specific and accurate tRNA synthesis *in vitro*.

136. UNIVERSITY OF WASHINGTON - Seattle, WA 98195

The *Rhizobium meliloti* Exopolysaccharide: Biosynthesis and Role in Nodulation
J. Leigh $50,000

The symbiotic association of *Rhizobium* with legumes results in a vital supply of inexpensive nitrogen for the growth of crops such as soybean and alfalfa. In a complex series of events, the bacterial symbiont, *Rhizobium*, induces the formation a specialized root structure, the nodule. *Rhizobium* then enters the cells of the nodule and fixes nitrogen. We are interested in how polysaccharides. produced by *Rhizobium*, induce and control events in the establishment of this association. We are following up on the recent observation that mutants of *R. meliloti* which fail to produce the acidic extracellular polysaccharide fail to enter nodules. The conclusion that the exopolysaccharide is required for nodule invasion is based on highly consistent results with a large and genetically diverse set of mutants. In addition, we have found that mutants which still produce the polysaccharide but without a certain structural feature (succinylation) also fail to enter nodules. We are studying the mutants biochemically to determine where they are blocked in the pathway of polysaccharide biosynthesis and whether the mutations affect the synthesis of other poly- or oligosaccharides. We are studying the interactions of the mutants and of the polysaccharide itself with the plant to determine the precise function of the polysaccharide in

nodule invasion. These experiments include investigating the effects of isolated polysaccharide on nodule invasion, carrying out nodulation experiments with temperature sensitive mutants in polysaccharide synthesis, inoculating plant roots with different bacterial strains simultaneously, assaying attachment of the mutants or of the polysaccharide to plant roots or extracted components of plant roots, and probing nodules with antibody to the polysaccharide.

137. **UNIVERSITY OF WISCONSIN** - Madison, WI 53706

Enzymology of Biological Nitrogen Fixation
R.H. Burris, Department of Biochemistry $37,317

The objective of this work is to clarify and extend our knowledge of the enzymology of biological N_2 fixation. This will be accomplished through the use of crude and purified preparations of nitrogenase from *Azotobacter vinelandii*, *Azospirillum* sp. and *Herbaspirillum* sp. and the application of ^2H and ^{15}N as stable isotopic tracers. Work will focus on exchange and isotopic fractionation by nitrogenase, ascertaining the stoichiometry of HD production from D_2 by nitrogenase under a wide variety of gas pressures, and determining the number of electrons accumulated on dinitrogenase before it initiates reduction of H^+, C_2H_2 or N_2. A membrane-leak isotope-ratio mass spectrometer will be employed for some of these studies. The isolated nitrogenase from *Azospirillum* sp. and *Herbaspirillum* sp. for their physiological properties and studying the N metabolism and control of nitrogenase in these intact organisms will be examined. Collaborative studies will be continued with several investigators when our facilities for mass spectrometric analysis of stable isotopes can be useful. We have done exploratory work on some of these problems.

138. **UNIVERSITY OF WISCONSIN** - Madison, WI 53706

Molecular Genetics of Ligninase Expression
D. Cullen, K. Kirk, Department of Plant Pathology and Bacteriology
$122,000 (two years)

Knowledge of ligninase biochemistry and its applications in biopulping, biobleaching, and effluent treatment have advanced considerably in the past 5 years. In submerged fermentations of *Phanerochaete chrysosporium*, ligninase secretion occurs late because of nutrient repression and levels are low relative to other secreted fungal enzymes, e.g. glucoamylase, cellulases, and proteases. During the same 5 years, the molecular genetics of filamentous fungi has advanced to considerable sophistication. Recently, the regulated expression and secretion of heterologous protein by filamentous fungi has been demonstrated. Because of the high levels of protein secretion by certain filamentous fungi, and because of the apparent permissiveness of filamentous fungal expression systems with respect to recognition of heterologous promoters, signal sequences, and intron splice sites, the use of these hosts in lieu of procaryotes or yeast seems especially suitable for ligninases. We seek to increase yields and affect the regulation of ligninases by *P. chrysosporium*. To this end, a transformation system for *P. chrysosporium* will be developed. Expression and regulation of ligninase genes will be investigated in *P. chrysosporium* and in a model fungus, *Aspergillus* nidulans.

139. **UNIVERSITY OF WISCONSIN** - Madison, WI 53706

Role of Transit Peptides in the Proper Localization of Nuclear-encoded Chloroplast Proteins
K. Keegstra, Department of Botany $75,000

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 the work proposed here is to understand how imported proteins get sorted to the proper location within chloroplasts. We wish to evaluate the hypothesis that the transit peptide of the precursor protein has a role in this sorting process. The import and sorting process 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. We currently are studying the precursors for ferredoxin and plastocyanin; these soluble proteins are located in the

stromal space and the thylakoid lumen respectively. We also have a precursor gene for LHCP, a thylakoid membrane protein, and precursor genes for proteins destined for the chloroplast envelope membranes. 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.

140. UNIVERSITY OF WISCONSIN - Madison, WI 53706

The Role of Proteolytic Enzymes in Degradation of Plant Tissue
A. Kelman and L. Sequeira $50,000

Most bacterial plant pathogens that degrade plant tissues have the ability to produce extracellular proteases. For many years primary research emphasis has been placed on the nature and function of pectolytic enzymes and, to a lesser degree of cellulases, in efforts to understand the mechanisms by which plant tissue is macerated. Recent studies on the importance of hydroxy-proline-rich glycoproteins (HPRG's) in the nature and function of plant cell walls have led to the question as to whether proteolytic enzymes are also involved in tissue maceration and act in concert with other cell wall degrading enzymes in the process. The primary objective of this research proposal is to determine whether proteolytic enzymes, in combination with other enzymes, are involved in the degradation of plant cell walls and thus may be essential for pathogenesis by certain soft rot bacteria. Proteolytic strains of two tissue macerating bacterial pathogens will be examined to determine whether the proteases that they produce have the ability to degrade HPRG's. Proteases will be purified and used to determine how depletion of HPRG's in cell walls is related to loss of tissue integrity and how proteases may enhance activity of other tissue-macerating enzymes. Genetic studies with protease-negative mutants will be designed to evaluate how loss of protease activity will affect tissue maceration processes.

141. **UNIVERSITY OF WISCONSIN** - Madison, WI 53706

Organization of the R Chromosome Region in Maize
J.L. Kermicle, Laboratory of Genetics $57,000

Genetic organization of the R region in maize is under study with a view to determining the number, kind and arrangement of components involved in the control of anthocyanin pigmentation. R alleles carried in diverse races are comprised of one or more functionally independent units (R genic elements) which differ in their spectrum of tissue-specific effects. Mutagenesis, with tests of complementation and recombination tests among the variants, defines the extent of these units. Tissue-specific and temporal differences between genic elements is mapped in relation to R mutational variation and flanking marker loci. Molecular characterization provides detail concerning the physical structure of regions of particular functional significance. Separate attention is being given to the pattern of recombination occurring when duplications and insertions are present.

142. **UNIVERSITY OF WISCONSIN** - Madison, WI 53706

Carbon Monoxide Metabolism by Photosynthetic Bacteria
P.W. Ludden, Department of Biochemistry
G.P. Roberts, Department of Bacteriology $70,000

The photosynthetic bacterium, Rhodospirillum rubrum, is capable of converting carbon monoxide to CO_2 and cellular material. Because carbon monoxide is a major industrial pollutant in this country and a product of the biological oxidation of CO is the product of H_2, a major industrial feedstock, this process has practical importance. The oxidation of carbon monoxide to CO_2 by microorganisms is a major component of the carbon monoxide cycle on earth. We have isolated the enzyme responsible for this process from Rhodospirillum rubrum. Carbon monoxide dehydrogenase is a iron-sulfur, nickel and zinc containing enzyme. The enzyme is quite stable to heat and amenable to purification, however, it is very labile to oxygen, and all experiments must be carried out anaerobically. We are studying the activities of this enzyme, its regulation and its induction by its substrate carbon monoxide. The enzyme is absent in cells which have not been exposed to carbon monoxide, but cells produce the enzyme at a maximal rate upon exposure to carbon monoxide for as little as ten minutes. Oxygen, a potent and irreversible inhibitor of the enzyme, represses the synthesis of this enzyme. If cells are grown in the absence of nickel, the enzyme is produced in an inactive form (apo-CODH). The apo-CODH can be activated in vivo by the addition of nickel chloride to the medium or in vitro by the addition of nickel chloride to the enzyme solution. The

ability to produce the enzyme in apo and holo forms allows us to study the role of nickel in this enzyme. Other metals such as cobalt and manganese will be added to the cells and to the purified apo enzyme to see if alternate forms of the enzyme can be produced. Once the amino terminal sequence of the purified CODH has been determined, an oligonucleotide probe for the CODH gene will be prepared and used to isolate the gene for CODH from Rhodospirillum rubrum. The path of CO assimilation into cellular material will be studied.

143. UNIVERSITY OF WISCONSIN - Madison, WI 53706

Starch Synthesis in the Maize Endosperm as Affected by Starch-Synthesizing Mutants
O.E. Nelson, Department of Genetics $73,551

The goal of this project is to investigate the steps necessary to effect starch synthesis in developing endosperms of maize with the primary experimental probes being the mutants in which this process is disrupted. We have given considerable attention to the presence of a soluble enzyme complex which can synthesize de novo phospho-oligosaccharides with Glc-1-P and Glc-1,6-bisP as substrates. If Glc-1,6-bisP is not present in the reaction mixture, it can be synthesized slowly from Glc-1-P, but its presence in the reaction mixture markedly accelerates the synthesis of phospho-oligosaccharides. The enzyme complex is capable of synthesizing Glc-1,6-bisP and Glc when given either Glc-1-P or Glc-6-P as a substrate. Glc-1,6-bisP can also be formed from Fru-1,6-bisP. The enzyme complex with amylopectin and Glc-1,6-bisP as substrates will add Glc-6-P to the nonreducing ends of the amylopectin molecules. There is also a starch granule-bound phospho-oligosaccharide synthase present in the developing endosperms, but it has been less intensively investigated than the soluble form. We hypothesize that these enzymes synthesize the primers to which the enzymes capable of synthesizing alpha-1,4 glucans add glucose molecules and that their ability to utilize Glc-1,6-bisP accounts for the phosphate groups esterified to some glucose moieties of the starch.

144. UNIVERSITY OF WISCONSIN - Madison, WI 53706

Carbon Isotope Fractionation in Plants
M.H. O'Leary $73,000

Plants fractionate carbon isotopes during photosynthesis in ways which reflect photosynthetic pathway and environment. The fractionation is a product of contributions from diffusion, carboxylation and other factors which can be understood using models which have been developed in our work. The object of our work is to use this fractionation to learn about the factors which control the efficiency of photosynthesis. Unlike previous studies, we do not rely principally on combustion methods, but instead develop more specific methods with substantially higher resolving power. We have recently developed a new short-term method for studying carbon isotope fractionation which promises to provide a level of detail about temperature, species, and light intensity effects on photosynthesis which has not been available until now. We are studying the isotopic compositions of metabolites (particularly aspartic acid) in C_3 plants in order to determine the role of phosphoenolpyruvate in C_3 photosynthesis. We are studying the relative roles of diffusion and carboxylation in nocturnal CO_2 fixation in CAM plants. We are developing new methods for studying carbon metabolism in plants.

145. UNIVERSITY OF WISCONSIN - Madison, WI 53706

Gas Exchange Characteristics of Leaves as Indicators of the Biochemical Reactions Limiting Photosynthesis
T.D. Sharkey, Biological Sciences Center $38,334

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 the leaf. These measurements are combined with measurements of metabolite levels and enzyme activities which can disclose the molecular events which limit or regulate the rate of photosynthesis. The purpose of this research is to identify the underlying molecular events which give rise to easily measured photosynthetic characteristics so that those processes which are most important in regulating or limiting photosynthesis in leaves can be identified.

We continue to focus our efforts on the biochemistry which underlies the anomalous loss of O_2 sensitivity in C_3 plants. Since a switch to low O_2 results in RuBP carboxylase deactivation, we have used changes in O_2 partial pressure to study the rates of activation and deactivation of RuBP carboxylase. We have observed that substantial deactivation of RuBP

carboxylase can occur without changing the rate of photosynthesis. Measurements with field grown plants have confirmed that C_3 plants can lose O_2 sensitivity under physiological conditions. A mutant strain of <u>Flaveria linearis</u> with reduced O_2 sensitivity was obtained from Dr. R. H. Brown, University of Georgia, and initial characterization has been carried out. The mutant work is a first step to understanding the molecular biology of O_2 insensitivity.

146. UNIVERSITY OF WISCONSIN - Madison, WI 53706

Molecular Mechanism of Energy Transduction by Plant Membrane Proteins
M.R. Sussman, Department of Horticulture $71,000

The focus of this project is a protein that converts chemical into electrical energy. This protein is known as a proton pump, or H^+-ATPase and is found only in the plasma membrane of fungi and higher plants. Its function is to generate a proton electrochemical gradient that is essential for the uptake of minerals and nutrients. This enzyme is composed of a single catalytic polypeptide of M_r=100,000 that contains amino acid sequence homology with other M_r=100,000 cation-translocating ATPases located in the plasma membrane of animal and bacterial cells. In this project, protein modification and sequencing techniques are being used to study the catalytic function of this enzyme. Radioactive probes that react covalently with essential amino acids are used to characterize the enzyme's two active sites: dicyclohexylcarbodiimide for studying the proton binding site, and N-ethylmaleimide, for identifying an essential cysteine located in the nucleotide binding site. The ATPase is phosphorylated at serine and threonine residues by an endogenous, calcium-activated protein kinase. Protein sequencing techniques are being used to locate phosphorylated residues present in a potential 'regulatory' site. Location of essential amino acids is determined by comparing protein sequence surrounding these residues with that for the entire protein as deduced from a DNA sequence derived from the higher plant structural gene. Results from this study of the enzyme's primary structure will be used to define a molecular mechanism for protein-mediated energy transduction.

147. YALE UNIVERSITY - New Haven, CT 06520

Molecular Cloning and Structural Characterization of the R Locus of Maize
S.L. Dellaporta, Department of Biology $72,000

Last year we reported on the isolation of a molecular clone of the R locus of maize using an Ac transposon tagging strategy. During the past year we have confirmed the identity of this clone and have begun a molecular analysis of several R alleles. Our main focus continues to be on the analysis of R-r, an allele containing both seed (S) and plant (P) components. Genomic blot analysis and gene cloning experiments suggest that the R-r allele may be organized as a triplication. In addition to (P) and (S), there appears to be a third cryptic component we refer to as (Q). We are attempting to clone the complete R-r allele by chromosome walking techniques to determine the molecular organization of R-r. The second objective of our research on R is to understand the mechanism of tissue-specific regulation of anthocyanin. We are characterizing several R alleles that condition different pigmentation patterns in plant and seed tissues. In order to determine the allelic differences among tissue-specific components we have obtained genomic clones and are performing DNA sequence analysis to regions of several tissue-specific components that may be responsible for these allelic differences.

148. YALE UNIVERSITY - New Haven, CT 06511

Membrane Vesicles as a Simplified System for Studying Auxin Transport
M.H.M. Goldsmith, Department of Biology $73,500

Indoleacetic acid (IAA), the auxin regulating growth, is transported polarly in plants. IAA stimulates a rapid increase in the rate of electrogenic proton secretion by the plasma membrane. This not only increases the magnitude of the pH and electrical gradients providing the driving force for polar auxin transport and uptake of sugars, amino acids and inorganic ions, but, by acidifying the cell wall, also leads to growth. We find that auxin uptake by membrane vesicles isolated from actively growing plant tissues exhibits some of the same properties as by cells: the accumulation depends on the pH gradient, is saturable and specific for auxin, and enhanced by herbicides that inhibit polar auxin transport. We are using accumulation of a radioactive weak acid to quantify the pH gradient and distribution of fluorescent cyanine dyes to monitor the membrane potential. The magnitude of IAA accumulation exceeds that predicted from the pH gradient, and in the absence of a pH gradient, a membrane potential fails to support any auxin accumulation, leading to the

conclusion that the transmembrane potential is not a significant driving force for auxin accumulation in this system. Since increasing the external ionic strength decreases saturable auxin accumulation, we are investigating how modifying the surface potential of the vesicles affects the interaction of the amphipathic IAA molecules with the membranes and whether protein modifying reagents affect the saturability and stimulation by NPA. These studies should provide information on the location and function of the auxin binding site and may enable us to identify the solubilized protein.

149. YALE UNIVERSITY - New Haven, CT 06520

Mechanisms of Potassium Transport in Plants and Fungi
C.L. Slayman, Department of Physiology, Yale University $99,242

High-affinity potassium accumulation by Neurospora occurs via an H^+-K^+ symport which is kinetically responsive to membrane potential, extracellular pH, and extracellular $[K^+]$. The system mimics many characteristics of K^+ transport in other fungi, in algae and in higher plants. Two carrier reaction schemes have been drawn, and their detailed properties are being calculated as guides to further experiments, which are planned using cell-perfusion and patch-electrode techniques, as well as isotope flux measurements. Experiments will be carried out both on Neurospora and on dissociated callus (meristematic) cells from the higher plant Arabadopsis thaliana. On protoplasts of these same cells, the possible operation of physiologically gated passive K+ systems (i.e., channels) will also be explored.

By separate experiments, in collaboration with Dr. Richard Gaber, we are attempting to isolate and sequence the gene for the high-affinity K^+ transport system in Neurospora, using as a probe the previously sequenced gene from Saccharomyces cerevisiae. If a suitable procedure can be developed for Neurospora, then a similar approach will be used on Arabadopsis and on Nicotiana.

Finally, transport regulation studies, which have grown out of optimization tests on K^+ transport, are being continued. Temperature-shifting of the Neurospora membrane leads sequentially to permeability increases in three distinct transport systems: a constant-field type of passive system (K^+ channels?), an ohmic system, and the primary proton pump. Temperature jump experiments, in conjunction with membrane current-voltage measurements, will be used to search for further transport components, as well as to obtain kinetic labels for metabolic events underlying permeability control.

150. YALE UNIVERSITY - New Haven, CT 06510

Transfer RNA Involvement in Chlorophyll Biosynthesis
D. Soll, Department of Molecular Biophysics & Biochemistry $85,000

Chlorophyll is the major pigment in photosynthesis. One molecule of chlorophyll is synthesized from eight molecules of δ-aminolevulinic acid, the universal precursor of porphyrins. Chlorophyll synthesis is regulated at the synthesis of δ-aminolevulinic acid, which is formed in the stroma of greening plastids from glutamate. This pathway differs from -aminolevulinic acid formation in bacteria, fungi or mammals, where it is synthesized from glycine and succinyl coenzyme A. The mechanism of δ-aminolevulinic acid synthesis is still the most poorly understood part of chlorophyll synthesis, although a solid body of evidence has accumulated during the past few years to show that, at least in barley and *Chlamydomonas*, it involves the reduction of glutamate to glutamate-1-semialdehyde and its subsequent conversion to DALA. Studies on the *in vitro* synthesis of δ-aminolevulinic acid in barley chloroplast extracts showed a specific chloroplast $tRNA^{Glu}$ to be involved in this process. Glutamate is attached to the tRNA via an aminoacyl bond by a chloroplast aminoacyl-tRNA synthetase. In the subsequent reduction of glutamate to glutamate semialdehyde the tRNA is required as a specific "cofactor". The goal of these studies is to clone and characterize the genes for the enzymes and tRNAs involved in this process and to investigate in detail the mechanism of action and regulation of these molecules. As experimental systems we will use the cyanobacterium *Synechocystis* 6803 and barley. These studies should uncover novel principles regarding the role of RNA as a cofactor in metabolic conversions.

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