DOE/ER-0381

ANNUAL REPORT AND SUMMARIES OF FY 1988 ACTIVITIES

DIVISION OF ENERGY BIOSCIENCES

SEPTEMBER 1988

U.S. DEPARTMENT OF ENERGY
OFFICE OF ENERGY RESEARCH
OFFICE OF BASIC ENERGY SCIENCES
DIVISION OF ENERGY BIOSCIENCES
(FORMERLY DIVISION OF BIOLOGICAL ENERGY RESEARCH)

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WASHINGTON, D.C. 20545

PROGRAM OVERVIEW OF THE

DIVISION OF ENERGY BIOSCIENCES

The Energy Biosciences program (recently renamed from Biological Energy Research) supports research in the plant and microbial sciences that provides fundamental biological insights and data. This information on biological mechanisms, principles and organisms is designed to be the underpinning for future biosystems for the conversion and conservation of energy. The work supported has the objective of gaining basic biological understanding rather than to develop or optimize specific commercial processes.

The scope of the program is broad (see below). The nature of the program includes the support of high quality, long term contemporary research. At one end of the program's spectrum of activity is research in the fields that are well developed, have a critical complement of practitioners and abound with good questions for which techniques and approaches are at hand. Alternatively there are fields and problems where there has been neglect despite perceived importance, or the problem area has been refractory with the available tools and thinking, or the experimental system requires an extensive investment of time and effort before dividends may be anticipated. Innovative projects are always being sought. Research projects supported with discretionary funds are subject routinely to a peer review process.

As a program that has grown in its nine year existence from $4 million to approximately $20 million, there has been a continuing challenge to build and sustain quality, maintain focus and concentration and maximally match research needs and opportunities with the resources. The last decade or so has seen a dramatic change in the nature of biological research. Not only has the character of research changed but the increase in the pace has been astonishing. Research technologies such as those of molecular biology and the powerful analytical techniques such as advanced mass spectrometry, nuclear magnetic resonance spectroscopy and others have revolutionized the way biological research is done. The questions that can be realistically addressed, plentiful before, are now much more numerous because of these developments. These powerful tools are providing many new fundamental understandings of the mechanisms of key biological processes. In terms of the scope of the program, an evolution has come about principally as a result of the opportunities generated by these newer capabilities. But gaining an understanding of the broad, long term basic biological problems continues to be the overall objective of the program. The challenge is to

acquire more insights and understanding of highly complex phenomena. For the Energy Biosciences program the areas of interest 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 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.

In addition to the above mentioned topic areas, there are a number of others. From time to time additional research topics are encompassed in the program. One worthy of mention here focuses on the potentialities of using renewable resources in totally different ways in the creation of new materials with attractive properties. While not a new idea, this does represent a departure through the use of an array of new techniques to alter biological molecules. One facet of devising new materials is developing interfaces between biologists and members of the materials sciences and chemical research communities in order to define the properties for materials that might be the ultimate objectives. This is just beginning to happen. Energy Biosciences has joined with the Division of Materials Sciences in beginning to support interdisciplinary work at the Center for Advanced Materials at Lawrence Berkeley Laboratory in such a venture (see project #74). The project is exploratory but the potential appears great. Industrial connections will be an important feature of this effort.

The work on enzymatic modifications of materials is one representation of the Energy Biosciences program in which the intent is to reach into areas that may not be heavily worked but offer potential. This also applies to studies on orphan organisms where the particular attributes present opportunities for insights not afforded elsewhere.

Fiscal year 1988 was an eventful year for Energy Biosciences (the name was officially changed from Biological Energy Research). It was a year in which Energy Biosciences represented the Department of Energy as a partner in the USDA-DOE-NSF Plant Science Center Program. This program had its origins in discussions among the three agencies with Office of Science and Technology Policy (OSTP) encouragement in 1986. The primary driving pressures were 1) plant sciences is a research area with great opportunities in respect to future biotechnology development but is held back by an insufficient research base; 2) a number of problems are so complex that interdisciplinary approaches seemed requisite for optimal progress. The collaborative review and decision making processes among the three agencies led to three projects being identified for funding, one at Cornell University, one at Michigan State University, and one at Arizona State University. Each project will have the designation "a unit of the USDA-DOE-NSF Plant Science Center program", however each is being funded by only one of the agencies according to the original agreement among the agencies. Also included in the program is the University of Georgia Complex Carbohydrate Center award that was begun last year. DOE is funding the University of Georgia center along with the one at Arizona State University which has as the focus studies on early events in photosynthesis using biophysical and molecular biological approaches.

The EB program last year experienced an extraordinary increase in the number of submissions of new proposals of which roughly 12% were funded (22 out of 182).

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

	Number of projects	FY 88 funding (in thousands of $)	Percent of total funds
University Grants & Contracts	130	10544	52%
Michigan State University Plant Research Laboratory	13	2400	12%
Plant Science Centers at Universities	2	2200	11%
National Laboratories Brookhaven National Lab Lawrence Berkeley Lab Los Alamos National Lab	15	2490	12%
Solar Energy Research Institute	1	125	0.5%
Oregon Graduate Center	2	1613	8%
Other Research Institutions	3	158	0.5%
Small Business Innovation Research (SBIR) contribution, Conferences & Miscellaneous	9	600	3%
Databases	2	120	1%
	177	20250	

Several items in the budget including $1.7 million for the Oregon Graduate Center, Small Business Innovation Research (SBIR), and several other smaller items are mandated by Congress or administration direction (11% of total) whereas the majority of the budget is discretionary. Some 75% of the budget is invested in university projects with 13% of the funds going to national laboratories and other non-academic labs.

The Energy Biosciences program traditionally has attempted to stimulate interest in research areas by sponsoring workshops. In FY 1988 one such workshop devoted to lignin structure, biosynthesis and degradation was held at the University of Illinois (18-20 April 1988). A summary document is in preparation. A report was recently completed from a workshop on plant chromosome isolation, manipulation and genome sequencing held at Los Alamos, New Mexico 13-14 November 1986.

Other conferences for which the EB program provided support include:

1. Summer Investigations into the Isolation, Cultivation, and Metabolism of Anaerobes Involved in Biodegradation, Marine Biological Laboratory, Woods Hole, MA.

2. Conference on the Molecular Basis of Plant Development, UCLA Symposia on Molecular Biology, Steamboat Springs, CO, March 26-April 2, 1988.

3. Conference on the Molecular Biology of Plant-Pathogen Interactions, UCLA Symposia on Molecular Biology, Steamboat Springs, CO, March 26-April 1, 1988.

4. Conference on the Genetics & Molecular Biology of Chlamydomonas, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, May 4-8, 1988.

5. Third Annual Penn State Symposium in Plant Physiology, Pennsylvania State University, University Park, PA, May 19-21, 1988.

6. Genetics and molecular biology of Robertson's Mutator: A workshop. Iowa State University, Ames, IA, June 10-12, 1988.

7. Toward a Broad Understanding of Photosynthesis-Multiple Approaches to A Common Goal, Stanford University, Stanford, CA, July 17-23, 1988.

8. Alaska Seminar on Molecular and Physiological Responses to Environmental Plant Stresses, University of Alaska, Fairbanks, July 23-28, 1988.

9. International Symposium on the Molecular Biology of Hydrogenases, University of Georgia, held at Helen, Georgia, September 19-23, 1988.

At a time when the competition for research funds appears to be intensifying, one of the resultant effects is that the traffic in research proposals increases significantly. A second order effect is that more reviews are sought for the increased proposal burden. In terms of efficiency, this is not a positive trend. Nevertheless this is a situation

with which the U.S. research community is faced. We in the granting agencies who are responsible for assuring that the funds that are available are spent as wisely as possible are deeply indebted to the hundreds of scientists who perform reviews out of professional responsibility with generous commitment of their time and energies. The system could not operate otherwise.

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

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or
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U. S. Department of Energy
Washington, DC 20545
(301)353-2873

SUMMARIES OF FY 1988 ACTIVITIES

SUPPORTED BY THE

DIVISION OF ENERGY BIOSCIENCES

1. ARIZONA STATE UNIVERSITY - Tempe, AZ 85287-1604

Antenna Organization in Green Photosynthetic Bacteria
R.E. Blankenship, Department of Chemistry $155,500 (two years)

The photosynthetic unit of all chlorophyll-based photosynthetic organisms consists of a collection of pigments that act as an antenna, absorbing light and transferring the energy to a reaction center where energy storage takes place by a series of chemical reactions. The green photosynthetic bacteria have an unusually large photosynthetic unit of up to 1,500 bacteriochlorophyll/reaction center. The majority of the antenna pigment molecules are contained in chlorosomes, ellipsoidal vesicles attached to the cytoplasmic side of the cell membrane. Additional antenna pigments and reaction centers are contained in integral membrane proteins. The project 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 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 $\leq$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. Current work includes time-resolved fluorescence spectroscopy on whole cells, membranes, chlorosomes, and pigment oligomers, using both the streak camera and single photon counting techniques. Experimental and theoretical studies on the spectroscopic properties of pigment oligomers are also being carried out.

2. **ARIZONA STATE UNIVERSITY** - Tempe, AZ 85287-1604

Center for the Study of Early Events in Photosynthesis*
R.E. Blankenship, Director; J.D. Gust, S.H. Lin, T.A. Moore, G.R. Seely, W.F.W. Vermaas, N.W. Woodbury $1,200,000 (30 months)

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

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

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

3. **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 $62,000

The primary focus of this project is an elucidation of anaerobic metabolism in the eukaryotic green alga. Under N_2, Chlamydomonas photometabolizes acetate at a rate of 22 umole per mg chlorophyll per hour. In the dark, acetate uptake in N_2 is not detected. The rate of acetate respiratory uptake in the dark is roughly doubled. Anaerobically, Chlamydomonas cells evolve H_2 and CO_2 per mole of acetate consumed to support the occurrence of an anaerobic and light-dependent citric acid and glyoxylate cycles. Recently developed methods for cellular fractionation of Chlamydomonas into protoplast, chloroplast and mitochondria make it possible to localize quantitatively the enzymes involved in these cycles. About 20% of the succinic acid dehydrogenase and NAD-malic acid dehydrogenase is located in the chloroplast fraction. NAD-isocitric acid dehydrogenase, a key enzyme of the citric acid cycle is totally located in the mitochondria. The NADP-

isocitric acid dehydrogenase is cytoplasmic. Isocitric acid lyase, a key enzyme of the glyoxylate cycle, is totally cytoplasmic. The specific activities of the chloroplastic succinic acid and malic acid dehydrogenases and cytoplasmic isocitric acid lyase are sufficient to satisfy the photometabolism of acetic acid via the glyoxylate cycle. NADH produced by stromal malic acid dehydrogenase is photo-oxidized by the photosynthesis electron chain via a NADH-plastoquinone reductase while the flavin reduced by the oxidation of chloroplastic succinic acid dehydrogenase is re-oxidized by a thylakoidal sequence.

4. **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.

5. **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. With this strategy, inbred lines derived from an F_2 form a stable 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, and combining ability. Presently, 198 markers have been mapped in two recombinant inbred families. The resultant map covers 100% of the genome and provides the means for rapid mapping of 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 have succeeded in identifying and expressing 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.

6. **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

The long term goal of this research effort is directed towards elucidating the molecular bases for changes in abundance and composition of pigment proteins in response to variations in growth irradiance. The primary organism under investigation is a unicellular marine chlorophyte, Dunaliella tertiolecta. which has a similar photosynthetic apparatus to that found in higher plants, however exhibits a higher degree of physiological plasticity with regard to photoadaptation than found in higher plants. The project aims at understanding how pigment synthesis and protein synthesis are coordinated and regulated so that functional pigment

protein complexes are formed or degraded in response to light intensity. Attention is focused on the level of control of protein synthesis by irradiance, namely at differentiating between transcriptional, translational and post-translational regulation of the apoproteins. A cDNA library has been constructed and genes which encode for light harvesting chlorophyll a/b proteins of photosystem II have been cloned in gtll. Gene probes will be used to assess changes in mRNA levels during transitions from high to low growth irradiance level. Simultaneously the effects of irradiance on pigment synthesis are assessed using specific inhibitors of chlorophyll biosynthesis and radiotracers. In collaboration with Dr. John Bennett in the Biology Dept, attempts are being made to transform D. tertiolecta in order to assess the effects of changes in the light harvesting properties on photosynthetic performance.

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

Regulation 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. It is mediated by combined activities of the cytochrome b/f and photosystem I 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 which are being isolated and characterized. The cDNA for these enzymes will be cloned and sequenced to yield protein sequence information. The mechanism through which kinase activity is controlled by ambient redox poise is unknown and will be studied by biochemical and genetic approaches.

These investigations will provide knowledge of mechanisms within the thylakoid that optimize overall photosynthetic productivity. Evidence is accu-

mulating that State transitions also have a role in protecting the thylakoid against photoinhibition and photodestruction.

8. BROOKHAVEN NATIONAL LABORATORY - Upton, NY 11973

The Physiology and Biochemistry of Cyanobacteria
H.W. Siegelman, Biology Department $200,000

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 the biliproteins. 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. Crystallization of phycocyanin and phycoerythrin is providing useful material for the determination of the three-dimensional structure of the biliproteins using synchrotron radiation. A stable complex of a molecule of phycocyanin and of phycoerythrin was isolated and is being characterized by gel permeation and neutron scattering. Energy transfer between the two biliproteins is maintained in the complex. 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.

9. BROWN UNIVERSITY - Providence, RI 02912

δ-Aminolevulinate Biosynthesis in Oxygenic Prokaryotes
S. Beale, Division of Biology & Medicine $88,243

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

tic pathway having &-aminolevulinic acid (ALA) as its first committed member. ALA is known to be formed by two distinct routes: by condensation of glycine and succinyl-CoA in animal, fungal, and some bacterial cells, and by transformation of the intact carbon skeleton of glutamate in plants, algae, and other bacterial cells. We are characterizing the reaction components for ALA biosynthesis derived from oxygenic prokaryotes, comparing them to their counterparts in plants, and studying the regulation of their activity in response to light and nutritional status. The potential of the prokaryotes for molecular genetic studies is being exploited by generating ALA auxotrophs, and identifying the enzymatic lesions by in vitro reaction complementation with purified, identified reaction components obtained from wild-type cells. Genetic complementation of the auxotrophic cells will be carried out by plasmid transformation with genomic libraries obtained from wild-type cells and carried in E. coli. The genes coding for the macromolecular reaction components will be isolated, identified, and made available for use as probes for studying the regulation of their expression during adaptation of the cells to light and nutritional status. The probes will also be evaluated for use in measuring expression of analogous genes in eukaryotic algae and higher plants.

10. CALIFORNIA INSTITUTE OF TECHNOLOGY - Pasadena, CA 91125

Genetics in Methylotrophic Bacteria
M.E. Lidstrom, Environmental Engineering Science $84,996

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 shown that the methanol oxidation system in AM1 requires 12 gene functions, at least 7 of which appear to be non-regulatory. We have begun studying these 7 functions in methanotrophs, and our data suggest that these are conserved in methanol and methane utilizing bacteria, at both genetic and functional levels. We are currently cloning promoter regions from these genes into broad host range promoter cloning vehicles using lacz at the reporter gene. These will be characterized and used to study transcriptional regulation in response to environmental parameters.

11. CALIFORNIA INSTITUTE OF TECHNOLOGY - Pasadena, CA 91125

Molecular Analysis of Ethylene-Insensitive Mutants in Arabidopsis
E.M. Meyerowitz, Division of Biology $95,000

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

Current efforts are focused on one dominant mutation, designated etr, which lacks a number of responses to ethylene which are present in the wild-type plant, including inhibition of cell elongation, promotion of seed germination, enhancement of peroxidase activity, and feedback suppression of ethylene synthesis by ethylene. Saturable ethylene binding in leaf tissue of the etr mutant is five-fold lower than that in wild-type tissue, indicating that the etr mutation directly affects the ethylene receptor. Through appropriate genetic crosses, the etr mutation has been placed on both the standard and RFLP linkage maps. A polymorphic lambda genomic clone which maps near the etr locus has been used to screen a cosmid genomic library. The cosmid clones obtained will be used to isolate additional overlapping clones from the library in an effort to clone the entire region of the genome containing the ETR gene.

12. UNIVERSITY OF CALIFORNIA - Riverside, CA 92521

Catalytic Mechanism of Hydrogenase from Aerobic N_2-Fixing Microorganisms
D.J. Arp $52,000

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 investing the catalytic functions of this enzyme and the changes in catalysis which occur upon exposure of the enzyme to different redox states and inhibitors. We routinely measure three activities of the enzyme: H_2 oxidation, H_2 evolution and isotope exchange. In general, exposure of the enzyme to oxidants results in a time dependent inactivation of all activities. The mechanisms of several inhibitors are also under investigation. For example, acetylene is a slow, tight binding inhibitor directed at the H_2 binding site while nitric oxide rapidly inhibits hydrogenase in a manner that is not competitive with either H_2 or the electron acceptor. The EPR spectrum of hydrogenase with acetylene bound was essentially unchanged from the spectrum of the enzyme as isolated. In contrast, treatment with NO resulted in generation of a spectrum typical of an iron-sulfur-nitrosyl complex. Eventually, these studies should lead to a determination of the number and types of redox centers in these hydrogenases and the role of these centers in catalysis.

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

Energy Capture and Use in Plants and Bacteria
P.D. Boyer, Molecular Biology Institute $95,000

The project continues to focus on the mechanism and function of the ATP synthase from plants and bacteria. The methodology developed using 2-azido-ATP for distinguishing the binding of adenine nucleotides at catalytic and noncatalytic sites, and their replacement during catalysis, is being applied to test the hypothesis that control of ATPase in chloroplasts results from ADP binding at catalytic sites without bound P_i. Such control, not an inactivation accompanying lack of protonmotive force, may function in vivo. Other experiments are directed toward resolving the controversy as to whether two or three catalytic sites participate during active photophosphorylation. These make use of ^{18}O techniques and rapid mixing measurements. The ^{18}O measurements can detect if two separate catalytic pathways may remain after covalent modification of one catalytic site. Measurements by rapid mixing techniques of the bound P_i and ATP committed to form medium ATP may provide a much needed measure of the total number of participating active sites on each enzyme. With the E. coli enzyme, tyr^{351} and tyr^{354}, that react with 2-azido-ATP at catalytic or non-catalytic sites respectively, are being replaced by various amino acids, using site-directed mutagenesis, to help define their role in binding and catalysis.

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

Restriction of Virus Infections by Plants
G. Bruening, Department of Plant Pathology $88,000

The productivity of a given plant line, in terms of biomass or food or fiber production, often is limited by the action of plant pathogens. An obvious and direct, and usually ecologically sound, approach to limiting the effects of a pathogen is to develop a plant line that is resistant to the pathogen. We are taking two approaches to understanding and implementing resistance against plant viruses. In "genotypic resistance," one or a few lines of an otherwise susceptible plant species exhibit resistance against a specific virus. The basis of genotypic resistance of the Arlington line of cowpea against cowpea mosaic virus (CPMV) has been associated, in the course of the previously supported research, with two inhibitors, one of the processing of CPMV polyproteins and the other of the translation of CPMV RNAs. We are purifying proteins that exhibit these activities and that have the expected virus specificity in their action and the expected correlations in their inheritance in cowpea crosses. These will be tested for their ability to mediate resistance to CPMV in cowpea cells. We propose to characterize the inhibitor(s) and the respective genes. The second aspect of this research is concerned with actually engineering resistance, against cherry leafroll virus and tomato ringspot virus (TomRV), and understanding the biochemical basis of the resistance based on the antiviral activity of a small satellite RNA of tobacco ringspot virus. In preliminary experiments we have demonstrated resistance against TomRV in transgenic plants that express the satellite RNA molecule.

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

Characterization and Biosynthesis of Complex Protein-Bound Carbohydrates
M.J. Chrispeels, Department of Biology $74,000

Plant glycoproteins contain two types of asparagine-linked oligosaccharide sidechains (glycans). Both types originate as high-mannose glycans in the endoplasmic reticulum when the proteins are first synthesized. Then, as the proteins pass through the Golgi complex, some glycans are modified by enzymes in the Golgi. These modifications result in a variety of complex glycans. We are studying the enzymes which are involved in these modifications and the sequence in which these various reactions occur. The purpose of this work is to understand how these particular complex carbohydrates are formed in plants. As part of this study, we are using transgenic plants to find out if the glycans of a bean protein (phytohemagglutinin) undergo the same modifications in tobacco as they do in the bean.

We are also studying 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.

16. UNIVERSITY OF CALIFORNIA - Davis, CA 95616

Characterization and Expression of Clostridium Cellulase Genes
R.H. Doi, Dept. of Biochemistry & Biophysics $78,500

The extracellular cellulase genes of the gram positive, spore forming Clostridium strain IY-1 are being used as model systems to study the genetics of an anaerobic microorganism. We have cloned an endocellulase gene whose product can hydrolyze native cellulose with the production of cellotetraose and soluble residues. The properties of the enzyme and its gene will be analyzed to determine its transcription and translation signals, the properties of its signal peptide, and the properties of the mature cellulase enzyme. We will analyze the base sequence of the gene including the regulatory and structural sequences. S1 nucleases mapping experiments will be carried out with mRNA from the natural host and from E. coli carrying the cloned gene to determine the transcription initiation site for the gene. From the base sequence and S1 nuclease mapping analyses we will determine the location of the promoter, the sequence of the promoter region, the Shine-Dalgarno sequence, the signal peptide sequence, and the amino acid sequence of the mature enzyme. This should provide much more comparative information about the transcription and translation signals, the signal peptide, and the amino acid sequence homology of this cellulase and other reported cellulases of anaerobic microorganisms. Since other reported cellulases do not hydrolyze native cellulose, the properties of this enzyme may differ significantly from other known enzymes. We will also analyze another cellulase gene that appears to code for an exocellulase. The same type of analysis will be carried out for this gene and its product. The transformability of Clostridium strain IY-1 will also be tested by use of plasmid pUB110, the Staphylococcus derived plasmid.

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

Transcription Control Elements and Manipulation of Chloroplast Genes
W. Gruissem, Department of Botany $116,690

A prerequisite for the manipulation of crop plants by introducing desirable genes into plant cells, or by transformation of chloroplasts, is our understanding of regulatory processes that are involved in the expression of genes. Our research project focuses on the molecular mechanisms of chloroplast gene expression in higher plants. Most monocistronic and polycistronic chloroplast transcription units contain proximal to their transcription start site promoter elements that dictate the relative transcriptional activity of these genes by their respective promoter strength. Most significantly, the relative transcriptional activities of most chloroplast genes are maintained during different stages of plant development. The experimental analysis of the promoter regions, together with the quantitation of RNA polymerase levels and the analysis of proteins other than RNA polymerase binding to these regions, will allow us to devise a model for this type of transcriptional regulation. Our work has also demonstrated that the differential accumulation of chloroplast mRNAs is, to a significant extent, regulated at the post-transcriptional level. The 3' inverted repeats flanking most chloroplast genes do not serve as efficient transcription terminators, but may have a role in the stabilization of the individual mRNAs. In addition to the structural component, our research also addresses the function of specific proteins which interact with the inverted repeat sequences, and which may be important for the developmental control of mRNA levels. To support the in vitro analysis of regulatory elements, we continue our work to construct stable chloroplast transformants.

18. **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 $71,596

Acetate conversion to methane and CO_2 by the methanogenic archaebacteria is a primary rate limiting step in anaerobic biodegradative processes in nature. However, the genetic study of these organisms has not been experimentally tractable due to the inability to grow and plate the organisms as single cells, and to extract high molecular weight DNA and RNA without shearing. The acetate-utilizing species, Methanosarcina thermophila TM-1, is being used for the proposed genetic and molecular studies because, unlike previously described acetotrophic methanosarcina that have a thick heteropolysaccharide cell wall, this species can be cultured in a unicellu-

lar form that has a protein cell wall lacking the heteropolysaccharide layer. These cells can be gently disrupted to obtain protoplasts or lysed to yield intact genomic DNA and RNA. Experiments are in progress to develop a gene transfer system in this bacterial species. Methods are being developed and refined for the efficient plating of M. thermophila on defined media, for chemical mutagenesis, and for the isolation of mutants defective in acetate utilization. Chromosomal DNA libraries have been constructed from M. thermophila and are being used to clone genes involved in the acetate utilization pathway (e.g. carbon monoxide dehydrogenase). Once cloned, analysis of the molecular mechanisms responsible for their regulatory control will be performed. These studies should aid our understanding of the pathway for acetate utilization in M. thermophila and serve as a model for elucidating regulatory mechanisms in the acetotrophic methanogens.

19. UNIVERSITY OF CALIFORNIA - Berkeley, CA 94720

Calcium Homeostasis in Barley Aleurone
R.L. Jones, Department of Botany $170,000 (two years)

This proposal describes experiments using gibberellic acid and Ca^{2+}-sensitive protoplasts of barley (Hordeum vulgare L. cv Himalaya) aleurone to study Ca^{2+} homeostasis. A method is described that permits the non-intrusive loading of Ca^{2+}-sensitive dyes into aleurone protoplasts. Using the Ca^{2+} indicator indo-1, cytoplasmic Ca^{2+} levels will be monitored as external Ca^{2+} concentrations are changed and α-amylase synthesis and secretion are stimulated. The transport of Ca^{2+} between compartments of the endomembrane system will also be studied. Experiments with membranes purified by density gradient centrifugation focus on Ca^{2+}-transporting proteins at the endoplasmic reticulum. The role of external Ca^{2+} versus internal stores of this element will also be investigated. We will use energy dispersive X-ray analysis to study mobilization of stored Ca reserves in the protein bodies to establish whether they contribute to cytoplasmic Ca^{2+} homeostasis. Our goal is to provide a comprehensive description of Ca^{2+} metabolism in the aleurone cell and to describe its role in enzyme synthesis and secretion.

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

Membrane Bioenergetics of Salt Tolerant Organisms
J.K. Lanyi, Department of Physiology and Biophysics $78,000

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

21. **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 $64,135

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 are being determined by an evaluation of the fitness of 7,000 individual Tn5-induced insertion mutants in both species and subsequent determination of phenotypes associated with these insertional mutations. Tn5 inserts randomly into the chromosome of P. syringae as shown by the normality of sizes of EcoRI, NotI and SfiI restriction fragments containing Tn5. Mutagenesis of E. herbicola is by pLAFR3::Tn5 containing the levan sucrase gene which is lethal to this species in the presence of sucrose, pLAFR3 carrying cells can therefore be counterselected and transposants selected directly. The epiphytic fitness (population size) of the Tn5-induced mutants of P. syringae are being determined indirectly on leaves, after subjecting them to fluctuating moist and dry conditions, by a leaf freezing assay since population size of ice nucleation bacteria on leaves and the mean freezing temperature of leaves

is directly related. About 1.2% of Tn5-induced mutants of P. syringae are deficient in epiphytic fitness as determined by this assay. About half of these mutants do not survive the stress of desiccation on leaves as well as the parental strain and often grow at lower rates or attain lower population sizes on leaves. The phenotype of at least some Tn5-induced mutants will be determined in vitro and will be associated with alterations in growth and survival on leaf surfaces and in other environments.

22. UNIVERSITY OF CALIFORNIA - Davis, CA 95616

Transposon Tagging of Disease Resistance Genes
R.W. Michelmore, Department of Vegetable Crops $77,000

Host plant resistance is usually the most ecologically desirable, least energetically expensive and most predictable form of disease control. However, developing resistant plants by classical breeding is slow, if suitable genes are available. Also, pathogens frequently mutate to render resistance ineffective. Resistance genes should be amenable to manipulation at the molecular level; cloning such genes, however, is problematic as neither their patterns of expression nor their products are known for any plant. We are therefore developing a transposon mutagenesis system to identify genes for resistance to the fungal pathogen Bremia lactucae in lettuce. The cloned genes will be used to study the biochemical and genetic basis of specificity and variation in plant disease.

Our strategy depends on manipulating chimeric, heterologous transposons to generate mutations in resistance genes and then isolating the mutated genes using the transposon as a tag. Transposition will be controlled by an inducible promoter. The position of the transposon will be analyzed using plant selectable markers. Recloning will be aided by a bacterial selectable marker with the transposon.

We are currently determining the movement of several transposon constructs in lettuce. Many transgenic lettuce plants have been generated containing the transposon Ac from maize, cloned into the 5' region of a streptomycin resistance gene (courtesy of J. Jones, Advanced Genetic Sciences). Transposition will be studied by examining R_2 seedlings for streptomycin resistance and confirmed by Southern analysis. The transposon Tam3 from snapdragon has also been introduced and its movement is being studied by Southern analysis.

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

Characterization of a Defective Interfering RNA that contains a Mosaic of a Plant Viral Genome

T.J. Morris and A.O. Jackson, Department of Plant Pathology $75,500

Plant viruses represent a major class of plant pathogens responsible for the induction of plant stress. Our research offers a unique opportunity to identify viral RNA sequences involved in such fundamental processes as virus replication, encapsidation and the degree of expression of disease symptoms. The proposed research focuses on the molecular characterization of a unique class of symptom modulating RNAs which are associated with a small RNA plant virus of the Tombusvirus group. We have demonstrated that these symptom modulating RNAs represent linear deletion mutants of the helper virus that interfere with it and modulate disease expression. This discovery marks the first definitive report of a defective interfering RNA (DI RNA) in association with a small RNA plant virus. This contrasts with the common association of similar DI particles with most animal virus infections where they have proved to be valuable models for studying symptom modulation and virus persistence. In order to develop a more detailed understanding of this newly discovered class of DI RNAs, we are investigating the molecular structure of several DI RNAs and developing a transcription system for producing biologically active DI RNAs in vitro. Subsequent mutagenesis studies using DI RNAs is planned as is the evaluation of the expression of DI sequences in transgenic plants.

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

The Bioenergetics of Salt Tolerance in Cyanobacteria

L. Packer and I.V. Fry, Department of Physiology/Anatomy $78,000

The imposition of salt stress elicits a series of complex responses, both in the short term (shock) and in the long term (recovery/adaptation). These responses occur at structural and functional levels, overcoming the physical and energetic disruptions caused by the cell's interaction with, and adaptation to, salinity. This project seeks to understand the mechanisms that enable the photosynthetic cyanobacteria to survive under these conditions. Exposure of cells to sodium chloride shock elicits altered gene products redirecting the 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 turnover

during the initial exposure to salt and after adaptation; 3) ESR spin probe techniques to monitor energetic parameters (internal volume, pH, transmembrane potential gradients) and their roles in maintaining cellular function during salt exposure; 4) ESR-oximetry to measure intracellular O_2 levels due to the activity of photosynthesis and/or respiration; 5) and determination of ultrastructural changes, in conjunction with changes in glycogen and lipid composition to assess physiological responses to salinity. This investigation will determine the temporal sequence of the energetic, enzymatic and structural changes that accompany the inhibition and the recovery/adaptive phases encountered during exposure to salt.

25. **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 $83,000

This project is directed at understanding the molecular properties and biogenesis of phytochrome in light-grown tissue. We have shown earlier that the predominant phytochrome species in green Avena tissue behaves as a Mr-118,000 polypeptide under appropriate electrophoretic conditions. Current efforts are designed to generate antibodies and cDNA clones for this polypeptide. A number of clones identified as positive in cDNA expression libraries by a combination of screening with antibodies and hybridization probes to phytochrome are being further examined by Northern blot and sequence analysis. In addition, efforts to isolate phytochrome genes from the green alga Mesotaenium have been initiated. Preliminary Southern blot analysis of Mesotaenium genomic DNA indicates that existing phytochrome clones can be used as heterologous probes for this purpose.

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

Nucleotides and Energy Metabolism in Corn Roots

J.K.M. Roberts, Department of Biochemistry

100,000 (FY87 funds/two years)

Hydrolysis of nucleoside triphosphates (NTP) drives biosynthesis and transport in cells. To date, most attention has been given to adenine nucleotides; metabolism of other nucleotides must be unraveled if we are to understand the energetics of lipid, protein and cell wall synthesis in plants. In aerobic cells, NMP and NDP (except ADP) are converted back into NTP primarily using ATP (derived from oxidative phosphorylation) either

directly, via nucleoside mono- or disphospho-kinases, or indirectly via enzymes such as phosphoglycerate kinase. Because these enzymes are present at high activity, [NTP]/[NDP] should be similar for all nucleotides (with none being > [ATP]/[ADP]). However, HPLC analysis of root extracts produced a contrary result: over a wide range of metabolic rates [ATP]/[ADP] [CTP]/[CDP] > [UTP]/[UDP] > [GTP]/[GDP]. We have previously described another inequality between nucleotides: [NTP]/[NDP] observed in vivo by ^{31}P-nuclear magnetic resonance spectroscopy > [NTP]/[NDP] seen in extracts. Our experiments indicate that the aforementioned results can be attributed to the existence of bound nucleotides (notably NDP) in corn roots. First, differences between in vivo NMR results and results from extract analyses reflect fundamental differences between what is being measured. NMR sees only freely mobile metabolites; the distinction between such metabolites and immobilized nucleotides is abolished in cell extracts. Second, the low [GTP]/[GDP] relative to other nucleotides could result from proportionately higher GDP binding in cells compared to other NDPs (e.g. on protein synthesis elongation factor), which would make GDP less accessible to kinases. Thus, enzymes in plant cells do not necessarily see the same amounts of nucleotides seen by biochemists in cell extracts.

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

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

B. Staskawicz, Dept. of Plant Pathology $83,000

Bacterial blight of soybeans, a disease caused by Pseudomonas syringae pv. glycinea (Psg), provides a model system to elucidate the molecular genetic and biochemical events that determine recognitional specificity and the subsequent induction of disease resistance in a plant-bacterial interaction. The phenotype of disease resistance in this plant-pathogen interaction is typified by the plant defense response known as the hypersensitive reaction and can be observed as a rapid necrosis of leaf mesophyll cells near the invading pathogen and the subsequent inhibition of bacterial growth. Past studies have demonstrated that natural variation for the ability to induce the HR exists in different strains of Psg. Thus one strain of Psg may cause disease (i.e. be virulent) on one cultivar of soybean, but may induce a HR (be avirulent) on another cultivar. This pattern of interaction conforms with the gene-for-gene hypothesis as a single dominant resistance gene (Rpg1) has been identified in the cultivar Harosoy that corresponds to the avirulence gene avrB identified in race 0 and race 1 of Psg.

The avirulence gene, avrB, has been shown to be induced both during the infection process and during growth in minimal media and is repressed in

complex media. Research is being carried out to determine the genetic and biochemical regulation of avrB gene expression by characterizing the promoter region of this gene by determining the initiation of transcription and the minimum number of nucleotides necessary for induced expression. Furthermore, we have identified several mutations in the Hrp gene cluster that prevent the expression of the avrB gene. These regions are currently being characterized at the molecular level to determine their role in the induction of a race specific defense reaction.

28. UNIVERSITY OF CALIFORNIA - Berkeley, CA 94720

Characterization of embryo-specific genes
Z.R. Sung, Departments of Genetics and Plant Pathology $57,987

The long term objective of the proposed work is to understand the molecular mechanism of growth and differentiation during embryogenesis. It is generally believed that development is a result of temporal and spatial differentiation of gene expression. In order to study the molecular mechanism of gene regulation in embryogenesis, we isolated genes preferentially expressed during embryo development from somatic embryos of carrots. The temporal pattern of protein and mRNA expression of 4 genes have been characterized. In order to study the function and regulation of these genes, we have isolated the genomic clones corresponding with these genes and are in the process of determining their nucleotide sequence. The expression of these genes in cells impaired in embryo development is being investigated to identify mutants impaired in these genes. Moreover, the expression of the embryogenic genes in cell lines blocked at varying morphogenetic stages reveals the physiological and developmental conditions under which the genes express.

29. UNIVERSITY OF CALIFORNIA - Santa Cruz, CA 95064

Tonoplast Transport and Salt Tolerance in Plants
L. Taiz $65,000

Active transport across the vacuole plays an important role in the adaptation of plants to saline soils by sequestering potentially toxic ions from the cytoplasm. Two proton pumps have been identified on plant tonoplasts which provide the primary driving force for ion accumulation, and H+ATPase and H+-PPase. We have been characterizing the structure and function of the vacuolar H+-ATPase. We have used antibodies to two of the major subunits, 60 kDa and 70 kDa, to screen carrot lambda gt11 cDNA libraries. Full length cDNAs for each of the two subunits have been cloned and se-

quenced. A search of the current protein database indicated similarities between deduced amino acid sequence of the 70 kDa subunit and the beta subunit of FoF1-ATPases. In particular, the putative catalytic regions appear to be highly conserved. Future work will focus on the 60 kDa subunit. We will also use cDNA probes to study regulation of vacuolar ATPase (V-ATPase) mRNAs by salt. Genomic libraries of salt tolerant species are being constructed, including the extreme halophilic bacterium, Halobacterium halobium (there is evidence that the plasma membrane ATPase of H. halobium resembles the V-ATPase). These will be screened using our carrot cDNA probes. We plan to compare the primary structures of the vacuolar ATPase subunits in glycophytes and halophytes.

30. UNIVERSITY OF CALIFORNIA - Berkeley, CA 94720

Analysis of the proteins essential for Agrobacterium mediated DNA transfer to plant cells

P. Zambryski, Division of Molecular Plant Biology $99,500

Transformation of plant cells by A. tumefaciens is due to the transfer of a DNA segment, the T-DNA, from the Ti plasmid of the bacterium into the nuclear genome of the plant cell. The T-DNA element does not encode products for its movement; these are encoded elsewhere on the Ti plasmid in the virulence (vir) region. The vir region is the master switch for the T-DNA transfer process since vir genes are regulated to be expressed only when Agrobacterium is in the presence of susceptible plant cells. Thus, to ultimately understand how T-DNA transfer occurs, it is critical to characterize the vir protein products which mediate the event. The proposed research will focus on defining those vir products that are produced at high levels, and thus are likely to play a structural role in T-DNA transfer. We have previously shown that the T-DNA intermediate is a single stranded (ss) molecule, the T-strand. Since the T-strand is unlikely to be transferred as a naked DNA molecule, and it must somehow be guided through bacterial and plant cell membranes, we will assay for two types of vir proteins: DNA binding proteins and membrane associated proteins. For example, a non sequence specific, ssDNA binding protein may act to coat the T-strand to protect it from degradation. Or, other (DNA binding) proteins may act to target the T-strand to the bacterial or plant cell membranes, or ultimately to the plant nucleus. Vir specific membrane proteins must somehow move the T-strand-protein complex through the bacterial and plant cell membranes. Potentially a specific channel is formed to allow T-strand transfer. Biochemical, genetic, and immunological methods will be used to determine the DNA binding or membrane specific localization of vir proteins.

31. **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 $105,020 (17 months)

This research program studies the stomatal responses to carbon dioxide and their interactions with other environmental signals modulating stomatal movements. Recent work on guard cell physiology and biochemistry has provided new information on the mechanisms underlying the stomatal response to carbon dioxide. Experiments with radioactive carbon dioxide demonstrated the operation of the photosynthetic carbon reduction pathway in guard cell chloroplasts, providing a biochemical interpretation for previous observations on a red light-induced medium alkalinization by guard cell protoplasts, coupled to oxygen evolution. Using the electro-physiological technique of patch-clamping, we recently characterized a red light-stimulated, electrogenic proton pump at the guard cell plasmalemma. Pump activity was inhibited by CCCP and vanadate, implicating a proton pumping ATPase, and by DCMU, indicating that the proton pump was modulated by guard cell chloroplasts. These findings support the notion that the observed stomatal responses to light and carbon dioxide in the intact leaf are modulated by specific metabolic properties of the guard cells. The regulation of proton pumping and photosynthetic carbon fixation in guard cells is under current investigation.

32. **CASE WESTERN RESERVE UNIVERSITY** - Cleveland, OH 44106

Cloning and Analysis of Genes Regulating Plant Growth
C.D. Town, Biology Department $73,000

The long term aims of this work are to isolate and characterize genes involved in the control of proliferation of plant cells. This project adopts a novel approach by using tumors as a starting material. Plant tumors, like animal tumors, do not respect normal growth controls in vivo and, in contrast to normal tissue, are able to grow in vitro without added hormones (auxin and cytokinin). We have induced tumors on the model plant Arabidopsis thaliana using ^{60}Co gamma rays, and shown that they grow in the absence of hormones when transferred to culture. Individual tumors show interesting differences in morphology and degree of differentiation, in growth rate, and in their response to exogenous hormones, all suggestive of variations in hormone metabolism. We propose that these tumors arise by radiation-induced genetic changes which activate the expression of either the growth hormone genes themselves, or of other genes involved in the control of cell proliferation, in direct analogy to the activation of oncogenes in animal cells by radiation, chemicals or other genotoxic

agents. We are using recombinant DNA techniques and plant cell transformation to isolate and study these mutated growth-controlling genes and their normal counterparts. The availability of such genes will further our understanding of growth control in plants and could contribute to the development of new varieties of crop plants with improved yields, nutritional value and environmental tolerance.

33. 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 $80,037

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 six or seven regulatory genes among these, using a nifH::lac fusion. Four of the regulatory genes are required for expression of nifH. Two of these, nifR1 and nifR2, have sequences homologous to ntrC and ntrB of enteric bacteria. A third, nifR4, has sequence homology in the C-terminal region, to the ntrA genes of Rhizobium and Klebsiella. Constitutive expression of nifR4 in R. capsulatus, from a plasmid clone, complemented a nifR4 chromosomal mutant but not a nifR1 mutant. Moreover, both oxygen and ammonia regulation of nitrogenase were maintained under these conditions. These results are consistent with a model requiring both nifR1 and nifR4 for nitrogenase gene expression; they rule out our earlier suggestion that nifR1 is needed only to turn on nifR4. Current efforts are focused on the purification of RNA polymerase and the products of nifR1, nifR2 and nifR4 to study nif gene transcription in vitro, particularly with the goal of determining the role of DNA supercoiling in transcription.

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

Anaerobic Metabolism of Aromatic Compounds by Phototrophic Bacteria
C.S. Harwood, Department of Microbiology and J. Gibson, Section of Biochemistry, Molecular and Cell Biology $52,675

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 objective of this project is to elucidate anaerobic routes of benzoate and 4-hydroxybenzoate metabolism by the phototrophic bacterium, Rhodopseudomonas palustris. Our data suggest 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 theses studies can then be used to develop models which can be tested with nonphototrophic anaerobic cultures and consortia. Studies include the identification of the number and specificity of enzymes involved in benzoate and 4-hydroxybenzoate metabolism, the identification of cofactors and electron carriers involved in each pathway and a precise identification of the products formed. Mutants that are blocked in aromatic metabolism have been isolated and will also be used to corroborate the biochemical data. These mutants will be used, together with physiological approaches, to identify compounds that regulate the expression of genes for aromatic degradation. This work will provide basic information about the biochemistry and regulation 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.

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

Structure and Function of the Self-incompatibility Proteins of Brassica oleracea
M. Nasrallah, Division of Biological Sciences $72,000

The phenomenon of self-incompatibility is known to occur in many flowering plants and is utilized in commercial hybrid seed production in economically important crops such as Brassica oleracea, B. campestris and B. napus. In the "Brassicaceae" we have established that self-incompatibility is a cell-cell interaction mechanism between one pollen grain and one papillar cell. The main objective of this project is to gain an understanding of the

molecular events that precipitate the incompatible response when genetically identical pollen and papillar cells are brought into contact upon self-pollination for example. Having cloned the gene that encodes the stigma S locus specific glycoproteins and made fusion protein constructs, monoclonal antibodies directed against different segments of the polypeptide moiety can now be raised in order to analyze aspects of structure/function relationships and establish a basis for the allele-specific recognition. Basic questions such as the role of the oligosaccharide and sugar residues, expression of the S gene in the anther and pollen, and localization of the interacting molecules are crucial to the understanding of the mechanism of self-incompatibility in this family and perhaps in other angiosperms.

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

Mechanisms of Inhibition of Viral Replication in Plants
P. Palukaitis, Department of Plant Pathology $74,000

Bio-control of plant viruses to improve crop yields depends upon our ability to understand the mechanisms of replication of viruses in plants and the mechanisms by which plants interfere with the replication and movement of viruses. The RNAs of a broad host-range virus, cucumber mosaic virus (CMV), have been cloned and some specific antisense RNA fragments synthesized from such clones and introduced along with the CMV RNA into tobacco protoplasts by electroporation were shown to be capable of inhibiting the replication of CMV RNA. One of these fragments is being introduced into the tobacco genome via a Ti-plasmid vector, and the transgenic plants will be tested for the ability to restrict the replication of CMV in planta. In addition, CMV strains capable of overcoming plant resistance (inhibition) to virus replication, are being examined to localize those nucleotide sequence changes that correlate with the resistance-breaking phenotypes. Localization of these domains and the determination of their function(s) in virus-host interactions are essential to an understanding of the mechanisms of both virus replication and inhibition of virus replication in plants. This will be instrumental for developing bio-control strategies for pathogens such as viruses, which reduce plant biomass produced for conversion to fuels and chemicals.

37. **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 $84,000

The goal of our program is to provide a mechanistic understanding of the cellular and molecular aspects of freezing injury/cold acclimation in winter cereals from a perspective of the structural and functional integrity of the plasma membrane. Recent studies have focused on dehydration-induced mesomorphic phase transitions in the plasma membrane that result in the loss of osmotic responsiveness of isolated protoplasts. Studies of the cryobehavior of liposomes formed from plasma membrane lipids reveal that the differential propensity for dehydration-induced lamellar $\rightarrow H_{II}$ phase transitions is a consequence of differences in the lipid composition per se. Because there are no lipid species that are unique to the plasma membrane of non-acclimated or acclimated rye leaves, this differential behavior is the result of concentration-dependent lipid-lipid interactions. Therefore, studies of the lyotropic and thermotropic phase behavior of plasma membrane lipids are in progress. Studies of the influence of hydration on the liquid-crystalline phase transition temperature (T_m) of phosphatidylcholine species found in the plasma membrane has been completed: dehydration increases the T_m's by 60 to 70°C. Studies of the thermotropic behavior of the cerebrosides have been completed: the T_m is 56°C. Studies of lipid-lipid interactions in mixtures of phospholipids, phospholipid-cerebrosides, phospholipid-steryl glucosides are in progress. In addition, a theory for membrane destabilization at low water contents relating water content and intermembrane force resulting in changes in the intrabilayer compressive lateral stresses has been developed. Studies of ATPase activity and protein composition of the plasma membrane demonstrated the equivalence of freezing and hypertonic stress.

38. **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 $70,000

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 distin-

guishable 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. Results of our genetic studies also indicate that the ability to metabolize maackiain is important for the virulence of this fungus towards chickpea. Two of the genes for pisatin demethylase have been cloned, and one has been sequenced. These genes are being exploited to help characterize the demethylase they encode as well as other phytoalexin detoxifying enzymes that may be homologous to it.

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

Studies of the Genetic Regulation of the Thermomonospora Cellulase Complex

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

$63,000

Two (E2 and E3) of the five major cellulases (E1-E5) produced by the thermophilic soil bacterium, Thermomonospora fusca contain carbohydrate and thus are bacterial glycoproteins. Since little is known about bacterial glycoproteins, the composition of the sugar portion of each glycoprotein and the nature of its linkages to the protein are being studied. The DNA sequences of the cloned cellulase genes (E1, E2, E4, and E5) are being determined and will be compared to look for homologies with each other and with other cellulase genes. The gene for E2 has been cloned into Streptomyces lividans and the transformed cells produce appreciable amounts of active enzyme even though the enzyme produced in S. lividans does not appear to contain carbohydrate. Proteolytic cleavage of the enzyme produced by S. lividans gives a fragment which has full activity on carboxymethyl cellulose but no longer binds to Avicel. A small fragment which binds to Avicel but does not have CMC activity has also been found. A

cosmid clone that codes for the xylanase induced by growth on xylan was isolated and the xylanase gene present in it was subcloned into plasmid pBR322.

40. CORNELL UNIVERSITY - Ithaca, NY 14853

Microbial Ecology of Thermophilic Anaerobic Digestion
S.H. Zinder, Department of Microbiology $87,000

The objective of this project is to provide an integrated understanding of the ecology of microbial populations in a thermophilic (58°C) laboratory-scale bioreactor converting a lignocellulose waste to methane. Special attention is focused on formation and breakdown of acetic acid, the precursor of two-thirds of the methane produced by the bioreactor. 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 the 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; 4) the demonstration that the thermophilic Methanothrix has an apparent K_m for acetate uptake near 0.1 mM, lower than that described for any other aceticlastic methanogen; 4) the isolation in axenic culture of the acetate-oxidizing rod-shaped (AOR) member of thermophilic two-membered coculture which converts acetate to methane using interspecies hydrogen transfer; 5) the demonstration that the AOR can also grow on H_2-CO_2 and produce acetate, the reverse of the reaction it carries out in coculture; 6) the demonstration of high levels of CO dehydrogenase activity in extracts of the AOR grown axenically on H_2-CO_2 and in the acetate oxidizing coculture, while levels of formyltetrahydrofolate synthetase and of folates were found to be extremely low; 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 the biodegradation of acetate and benzoate.

41. **UNIVERSITY OF DELAWARE** - Lewes, DE 19958

Metabolic Mechanisms of Plant Growth at Low Water Potentials
J.S. Boyer, College of Marine Studies $94,000 (15 months)

In higher plants, growth is more frequently limited by water than by any other environmental resource. The project objective is to identify the underlying molecular mechanisms causing growth limitations by focusing on the process of cell enlargement. Studies so far have shown that, in localized growing regions, turgor in most of the cells is completely maintained when water potentials are low enough to inhibit growth. However, gradients in water potential decrease between the vascular tissue and the enlarging cells, which inhibits water entry and therefore growth. A few hours later, the extensibility of the cell walls decreases and the conductance of the tissue for water diminishes, implying that metabolic changes eventually occur. We found a 28kD protein accumulating in the cell walls under these conditions. The protein does not accumulate in the mature tissue of soybean stems nor in the roots, where growth continues unabated. The correlation between the appearance of this protein and the growth response suggests that the protein could play a role in growth at low water potentials.

We explored how gradients in water potential originate in the enlarging tissue. They were not caused by high concentrations of solutes in the cell walls but rather by the yielding of the walls to turgor. This caused a low water potential in the cells and a negative pressure in the walls. The gradients are present because the enlarging cells must overcome frictional resistances to water flow from the vascular system to the enlarging cells. With this understanding, subsequent work will focus on the metabolic causes of the decreased wall extensibility.

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

Molecular Studies of Functional Aspects of Higher Plant Mitochondria
J.N. Siedow, Department of Botany $115,080 (two years)

The marked sensitivity of mitochondria isolated from cms-T lines of maize to a toxin (BmT toxin) derived from the fungus, Bipolaris maydis, race T is associated with a unique mitochondrially-encoded 13 kDa protein (ORF-13). The goal of this research is to characterize the mechanism by which ORF-13 and BmT toxin interact to permeabilize biological membranes. This work will take advantage of the ability to express ORF-13 in E. coli, and the recent use of site-directed mutagenesis (carried out in C. S. Levings' laboratory, N.C. State University) which has led to amino acid modifications of ORF-13 that result in polypeptides which no longer respond to Bmt

toxin. These modified ORF-13 proteins will be analyzed for their ability to bind labeled BmT toxin in an attempt to characterize what features of the ORF-13 molecule are involved in toxin binding and what ones contribute to permeabilizing the membrane. In addition, ORF-13 expressed in E. coli will be solubilized, purified and reconstituted into phospholipid liposomes to better characterize the nature of its interaction with BmT toxin. This work will include studies of both ORF-13-containing E. coli membranes and phospholipid liposomes in experiments designed to elucidate the topographic orientation of ORF-13 in the membrane and the extent to which changes in ORF-13 secondary and quaternary structure appear upon interaction with BmT toxin. Understanding how ORF-13 and BmT toxin are able to bring about such dramatic effects on membrane properties could provide new insights into how proteins interact with biological membranes.

43. **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

$198,000 (two years)

This 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. This last statement has been challenged recently and over the last two years, we have studied the protein complement of guard cells, focusing particularly on the migration position of rubisco LS. ABA, presumably an endogenous signal for stomatal aperture size reduction, is being quantified in various leaf cells; these cells are derived from leaves in which water-stress was imposed by detachment and dehydration. In another project, we had begun characterization of PEPC in guard cells; that project is being delayed while we define better the assay conditions. (The reaction product OAA decarboxylates to pyruvate and thus is inaccessible to the typical indication procedures.)

44. UNIVERSITY OF FLORIDA - Gainesville, FL 32611

Ethanologenic Enzymes of Zymomonas mobilis

L.O. Ingram, Department of Microbiology and Cell Science $82,500

The production of ethanol in Z. mobilis, in yeasts, and in plant roots under anoxic conditions results from the combined activities of pyruvate decarboxylase and alcohol dehydrogenase. These enzymes serve an essential role by oxidizing NADH to allow the continuation of glycolysis while producing ethanol and carbon dioxide as waste products. Fermentation provides much less usable energy for maintenance and growth than oxidative metabolism and high levels of ethanologenic enzymes and glycolytic enzymes are required. The enzymes involved in the central catabolism of carbohydrates typically represent half of the soluble cell protein during fermentative growth. Our studies focus on the ethanologenic enzymes of Z. mobilis, an obligately fermentative gram negative bacterium being used for commercial ethanol production. These Z. mobilis genes share considerable amino acid homology with their counterparts both in maize and in Saccharomyces. In Z. mobilis, ethanologenic enzymes constitute 7% to 10% of the soluble cell protein. We are investigating the cellular mechanisms which allow such high levels of gene expression. Two isozymes of alcohol dehydrogenase are produced, ADHI which contains zinc and ADHII which contains iron. This second isozyme is unique in being the only known alcohol dehydrogenase to contain iron, all others containing zinc. ADHII is the dominant isozyme in Z. mobilis except under conditions of iron limitation and appears to be transcriptionally controlled. This enzyme appears optimized for fermentation and is activated by its product, ethanol. Z. mobilis occurs in nature in plant saps and nectars. The nutritional flexibility provided by isozymes with different metal ion requirements may result in a competitive advantage for growth and survival.

Pyruvate decarboxylase is the key enzyme which diverts carbon flow from glycolysis to ethanol production. The affinity Of this enzyme for pyruvate is much higher than that of most enzymes determining carbon flow into other fermentative pathways. Expression of high levels of Z. mobilis pyruvate decarboxylase and alcohol dehydrogenase I-I in bacteria such as E. coli dominates carbon flow, redirecting carbohydrate metabolism to ethanol production.

45. 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

$197,000 (two years)

The biochemical pathway of aromatic biosynthesis is not only crucial in higher plants as a source of aromatic amino acids, but it is a point of interface with a massive biochemical network for secondary metabolism. We seek to understand physiological, biochemical, developmental and genetic interrelationships within a single experimental system. Nicotiana silvestris and N. plumbaginifolia are under study at both the tissue culture and organismal levels. Strategies for isolation of regulatory-gene and structural-gene mutants in totipotent cells are being pursued. We have shown that an intact aromatic pathway which proceeds to L-phenylalanine and 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. Selected comparative work will be done in order to deduce what characteristics of the N. silvestris system can be generalized to higher plants. Analysis of this pathway should contribute heavily to an understanding of the biology of the plant because the pathway generates protein precursors, vitamins, growth regulators, many secondary metabolites, and medically significant pharmacological agents.

46. UNIVERSITY OF GEORGIA - Athens, GA 30602

The Metabolism of Hydrogen, Methane and Other Gases by Extremely Thermophilic Bacteria

M.W.W. Adams, Department of Biochemistry $82,899

Extremely thermophilic bacteria are a remarkable and unique group of microorganisms that grow optimally up to 105°C. They are a very recent discovery and have been isolated only from volcanic areas which include deep sea hydrothermal vents. Little is known of their metabolism and biochemistry yet they have enormous biotechnological potential. In this project we are studying the metabolism of hydrogen (H_2) by five extreme thermophiles grown under a variety of conditions in vitro. We will initially examine their hydrogenases, the enzyme responsible for catalyzing H_2 production and H_2 oxidation, using activity assays and analytical chromatographic analyses. One of the organisms will then be grown on a large scale (400 liters) and hydrogenase and related redox proteins will be purified. Their molecular and catalytic properties will be characterized

using various biochemical and electron and nuclear resonance spectroscopic techniques. A second aspect of this research is to determine whether any of these organisms can use methane (CH_4), nitrogen (N_2) or carbon monoxide (CO) as growth substrates. One (or more) of the enzymes involved will also be purified and their molecular properties will be determined. In mesophilic organisms, all of these gases are activated by thermolabile metalloenzymes. The central questions are, what novel metal centers have evolved in the enzymes of the extreme thermophiles for activating H_2, CH_4, N_2 and CO at 100°C and above, and what are the limits to their stability and catalytic activity? These gases play a central role in the commercial production of fuels and numerous chemicals thus extremely thermophilic enzymes have the unique potential to bridge the gap between biochemical catalysis and many industrial energy conversions.

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

Studies on Oligosaccharins: Carbohydrates Possessing Biological Regulatory Activities

P. Albersheim, Complex Carbohydrate Research Center $170,000

This project is concerned with the isolation and characterization of oligosaccharins, which are naturally occurring complex carbohydrates that possess biological regulatory activities. We have hypothesized that oligosaccharins, when released from the complex carbohydrates of cell walls, regulate various biological functions within plants. We are studying the following oligosaccharins: [1] Oligosaccharins isolated from plant cell walls that elicit phytoalexin (antibiotic) accumulation in plant tissues. Research in this area is emphasizing the involvement of microbial enzymes and a plant-derived inhibitor of microbial enzyme activity in the release of elicitor-active oligosaccharins from plant cell walls. [2] An oligosaccharin that may trigger the hypersensitive-resistance response in plants. We are purifying enzyme(s) secreted by pyricularia oryzae that release this oligosaccharin from isolated plant cell walls. [3] Oligosaccharins that may be able to induce flowers, roots, vegetative buds, or 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. At present, our studies with tobacco epidermal strips are emphasizing those oligosaccharins released from plant cell walls by endo-α-1,4-polygalacturonase that stimulate vegetative bud development and inhibit root development. Both these regulatory activities have been identified in fractions that are enriched for the pectic polysaccharide rhamnogalacturonan II.

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 a number of other laboratories to identify oligosaccharin activities in other biological systems.

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

Development of Methods to Structurally Characterize Complex Carbohydrates

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

$284,625 (two years)

This research focuses on the development of methods to aid in the structural characterization of complex carbohydrates. 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; this approach is aimed at obtaining the primary and eventually, the three-dimensional structures of these molecules. We are also developing computer software that predicts all possible ions resulting from a particular mass spectral analysis of a complex carbohydrate of known composition. This program will allow the quick, accurate and comprehensive analysis of mass spectral data. We have also recently developed a method for the sensitive detection and characterization of enzymes that cleave the glycosidic linkages of complex carbohydrates. Other methods we are studying that will aid in structural investigations include the development of chemical reactions 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.

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

The University of Georgia Complex Carbohydrate Research Center (CCRC)*

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

$1,000,000

The CCRC, with its multidisciplinary faculty and staff, was formed to serve as a national resource for basic research in complex carbohydrates. The CCRC will actively assist in defining the structures and studying the functions of plant and microbial carbohydrates. Research, training, and

service activities are components of this program. The research focuses on various aspects of carbohydrate science, including methods development, structural characterization, and function. The education function involves the training of graduate students, post-doctoral associates, and visiting scientists in the analytical methods used for studying carbohydrate structure. It is anticipated that 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 involves conducting routine analyses, including one-dimensional NMR and FAB-MS analyses, of carbohydrate samples provided by other scientists as well as collaborations with other scientists on more extensive research projects. These services and collaborative investigations are limited to non-proprietary research. Those interested in assistance or collaboration should write to:

Dr. Russell Carlson,
Technical Director
Complex Carbohydrate Research Center
Russell Laboratories
P.O. Box 5677
Athens, GA 30613

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

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

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

CarbBank is a computer program and database that is being developed that will, for the first time, enable scientists to systematically and rapidly search for the estimated 5,000-10,000 published complex carbohydrate structures. Incorporation of carbohydrate structures linked to literature citations in a computerized database (i) allows comparison of known and newly discovered carbohydrate structures, (ii) brings to scientists an awareness of structural heterogeneity, for example, the attachment of several different oligosaccharides to the same amino acid of a protein, (iii) leads to comparisons of carbohydrate structures between species, and (iv) assists in predicting carbohydrate structures based on limited structural information or properties of known molecules. CarbBank will enhance the research efficiency and capabilities of many scientists, not just those specializing in carbohydrates, and will make carbohydrate structures accessible to a wide spectrum of scientists.

The CarbBank program was written with extensive input from specialists in the various disciplines of carbohydrate chemistry in order to create an

environment that meets the needs of as wide a range of investigators as possible. The international CarbBank board of Overseers, with the special assistance of its Executive Committee has selected and approved some 50 potential curators in about 20 countries. Those curators who have agreed to participate have been asked to identify and enter into the database the structures and bibliographic information of approximately 100 complex carbohydrates per year. It is anticipated that CarbBank will be initially distributed in late 1988 when it will contain the structures and bibliographic information for about one-third of all published carbohydrate structures. It is proposed that all existing carbohydrate structures will be entered into CarbBank within three years. The database will be distributed quarterly on magnetic media (5.25 inch floppy diskette and 3.5 inch hardshell disk).

(Additional funding provided by the National Institute of General Medical Sciences.)

51. UNIVERSITY OF GEORGIA - Athens, GA 30613

Structural Studies of Complex Carbohydrates of Plant Cell Walls
A. Darvill, Complex Carbohydrate Research Center $205,000

The cell walls of a plant determine the plant's structure and morphology and act as a barrier to pests. Cell walls are also a source of complex carbohydrates with biological regulatory properties (oligosaccharins). This project involves the isolation and structural characterization of the complex carbohydrates that constitute approximately 90% of the walls of growing plant cells. These structural studies emphasize detailed analyses of two pectic polysaccharides, rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II). These polysaccharides have been shown to possess unexpectedly complicated structures. For example, RG-II contains at least 12 different glycosyl residues, including apiosyl, aceryl (3-C-carboxy-5-deoxy-L-xylosyl), KDO (3-deoxy-D-manno-2-octulosonic acid), and DHA (3-deoxy-D-lyxo-2-heptulosaric acid). The complete structures of oligosaccharide fragments of RG-II have been determined that, among the oligosaccharides, contain all of the known glycosyl residues of RG-II. Elucidation of arrangement of these oligosaccharides in RG-II is a goal of future work. Although RG-I contains only five glycosyl residues, like RG-II it exhibits tremendous structural complexity. RG-I is composed of a backbone of alternating rhamnosyl and galacturonosyl residues with side chains attached to 50% of the rhamnosyl residues. At least 30 structurally different side chains consisting predominantly of arabinosyl and galactosyl residues have already been identified. We are investigating the possibility that discreet families of side chains are attached to the RG-I backbone. We have recently isolated a cell wall fraction containing RG-I where greater than 90% of the rhamnosyl residues have side chains attached. We 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 structurally complex polysaccharides.

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 and cytoplasmic backgrounds, ploidy levels, and chromosome numbers crossed and backcrossed with different genotypes of diploid and tetraploid pearl millet, P. glaucum. Viability of pearl millet pollen has been maintained for over three years in $4^{o}C$ storage at pollen moisture content of 7% or less. The storage technique has application to other species and for germplasm preservation. Cytoplasmic effects for diverse cytoplasms have been observed for seed size, dry matter yields, and disease reactions. Significant progress is being made in producing a true-breeding apomictic pearl millet by gradually eliminating the chromosomes of wild species from pearl millet x apomictic wild species crosses. The overall impact is an increased, more efficient, and more reliable production of food, fiber, and forage.

53. **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 $93,000

The influence of high temperature stress (heat shock or HS), water stress, and heavy metals on gene expression are under investigation. The studies emphasize analysis of heat shock genes including structure and regulation of expression. The physiological/biochemical role(s) of heat shock proteins in cellular function is also under study, emphasizing the phenomenon of acquired thermotolerance. Additional members of the 15 to 18 kd and the 22 to 26 kd groups of HS genes have been sequenced. These studies included

the sequencing of some cDNAs related or identical to members of the 15 to 18 kd HS proteins isolated from poly(A)RNA of cadmium-treated soybean seedlings. Sequence analysis of several clones demonstrated that cadmium induced expression of either the same or identical genes as HS induces; additionally, cadmium induced expression of highly related genes not induced by heat shock. Cadmium was also shown to inhibit intron processing of a stress-induced mRNA which encodes a 26 kd protein. Colinear cDNA and genomic clones of a gene encoding a 70 kd HS protein were sequenced. This sequence was 65% identical to Drosophila gene and 84% identical to a maize gene. Unlike maize, Arabidopsis, and petunia genes which contain an intron at the identical position, the soybean gene contains no intron similar to genes encoding 70 kd HS proteins in Drosophila. Genes encoding the 83 kd group of HS proteins are being analyzed.

The activation of transcription of HS genes at the onset of HS (e.g., 40°C), their turn-off during continuous HS or upon return of soybean seedlings (cells) to normal temperatures (e.g., 30°C), and reactivation upon subsequent HS after 3 to 4 hr at 30°C are being analyzed using run-off transcription in isolated nuclei, gel retardation/foot-printing of 5' "regulatory" sequences, and expression of a reporter gene (-glucuronidase or GUS) under the control of a HS promoter in a perfect fusion at the ATG translation start of the GUS gene. These experiments have demonstrated that the HS promoter is many-fold more active than the CaMV 35S promoter and that its activity is much greater when a super-coiled construct is electroporated into tobacco protoplasts relative to a linear construct. These experiments serve as the focus of current research. Additionally, the acquisition of thermotolerance to an otherwise lethal HS treatment by prior exposure to an "optimum" HS is being analyzed as one approach to gaining insight into a possible function of HS proteins.

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

Soybean Ribulose Bisphosphate Carboxylase Small Subunit: Mechanisms and Determinants of RNA Turnover

R.B. Meagher, Department of Genetics $79,000

The ribulose bisphosphate carboxylase small subunit (SSU) genes, SRS1 and SRS4, synthesize 90% of the SSU mRNA detected in green soybean leaves. When etiolated seedlings are transferred to the light, transcription increases 32-64 fold within 24 hours but only a two fold increase is measured in the steady state levels of SSU mRNA. After four days of light induction the steady state SSU mRNA levels reach the levels found in leaves grown in continuous light (eight times higher levels of SSU RNA than is found in etiolated leaves). Far red light treatment of light grown seedlings followed by a few hours in darkness shuts down transcription to

the levels found in etiolated levels but again the steady state levels of RNA only change eight fold. This reproducible discrepancy between large transcriptional changes and small steady state RNA changes can only be explained if the SSU RNAs turn over more rapidly in the light than in the darkness. We have devised a model for translational linked turnover of SSU RNA to explain our hypothesis and have taken three experimental approaches to test this model. We will (1) continue to examine the molecular physiology of the light response of SSU transcription, RNA steady state and protein levels, (2) compare the physical structure of these RNAs under conditions of maximum and minimum turnover using in vivo chemical modification of SSU RNA and (3) we will examine potential sequences and structures in the soybean SSU RNAs, which might contribute to regulation of RNA stability, by examining chimeric gene constructs in transgenic plants.

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

Microbiology and Physiology of Anaerobic Fermentations of Cellulose
H.D. Peck, L.G. Ljungdahl, L. Mortenson, J.K.W. Wiegel; Departments of Biochemistry and Microbiology $455,000 (FY 87 funds/ 17 months)

This project involves the biochemistry and physiology of four major groups (primary, secondary, ancillary and methane bacteria) of anaerobic bacteria, that interact and convert cellulosic materials industrially to important feedstock chemicals and ultimately also to methane. The primary bacterium, Clostridium thermocellum, produces cellulolytic enzyme complexes designated cellulosomes and polycellulosomes with M_r ranging from 2 to 80 million. The cellulosome consists of at least 14 different polypeptides, some of which have endoglucanase activity or an activity resembling exoglucanase. Individually the polypeptides do not hydrolyze crystalline cellulose as does the intact cellulosome. The latter contains from 6 to 13% carbohydrates and some of the polypeptides appear to be glycoproteins. The polypeptides are now being separately purified, their composition, physical chemical, and enzymatic properties determined. C. thermocellum during growth secretes a carotenoid-like yellow pigment (YAS = yellow affinity substance), that facilitates the attachment of cellulosomes to cellulose. YAS has now been purified and its structure is being determined. Research on the secondary and ancillary bacteria includes clostridial acetogens and other clostridia, sulfate reducing bacteria (SRB) and Thermoanaerobacter ethanolicus. Aspects of metabolism are being studied which appear relevant for bioenergetics and the interactions of bacteria in consortia. Special attention is given to enzymes involved in the metabolism of hydrogen, formate, CO, and CO_2, the molecular basis of interspecies H_2-transfer and H_2-cycling, electron-transfer proteins, ATPase systems and enzymes of one-carbon metabolism. A common property of many of these enzymes is their contents of metal redox centers consisting of inorganic sulfur and one or

more combinations of (FE), (NiFe), (NiFeSe), (MoFe), (MoFeSe), and (WFeSe). The structures of the metal clusters and their roles in the catalytic processes of hydrogenases, formate dehydrogenases and carbon monoxide dehydrogenases are determined using several spectroscopic methods that involve also substitutions with different isotopes. One very recent result is the demonstration for the first time of a selenocysteine coordination to the active site nickel in the (NiFeSe) hydrogenase from Desulfovibrio baculatus. Another development of considerable importance is the finding that many bacteria produce two or more different proteins with e.g. hydrogenase or formate dehydrogenase activity. Thus different hydrogenases have been characterized in detail from the bacteria of interest: The (O_2-labile 12Fe bidirectional hydrogenases from C. pasteurianium and A. woodii; the O_2-labile 8Fe uptake hydrogenase from C. pastuerianium; the O_2-stable 12Fe hydrogenase from D. vulgaris and the O_2-stable (NiFe) and (NiFeSe) found in D. vulgaris, other SRB's and the methanogens. Although, the enzymes have different metal centers which determine to a large extent the catalytic properties, it is clear that the protein part of the enzymes strongly influence these properties. Therefore, it is necessary to obtain the primary structures of the proteins. This is being pursued using genetic techniques involving cloning and sequencing of DNA. The (NiFeSe)-hydrogenase from D. baculatus has been sequenced and work is in progress on other hydrogenases, formate dehydrogenases, and CO dehydrogenases. The enzymes involved with H_2, CO, CO_2 and formate metabolism are being localized in the bacterial cells using electron microscopic and immunological techniques. It is evident that they interact with membrane bound electron transfer proteins and ATPase systems. This work is especially directed toward the understanding of the generation of ATP coupled to vectorial electron transfer in acetogenic bacteria, which recently have been found to have the capacity to grow autotrophically using the newly discovered acetyl-CoA pathway. T. ethanolicus, that ferments many hexoses, pentose and also xylan to ethanol as a major product is investigated with regards to physiology and content of thermostable enzymes. This bacterium and other extreme thermophiles grow over a temperature span of 40°C or more. A biphasic growth response to temperature is observed. Preliminary evidence suggest that this phenomenon is due to the expression of enzymes at different temperatures. This is investigated using gel electrophoresis techniques and isolation of the enzymes.

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

Molecular Characterization of Phytochrome from Green Avena
L.H. Pratt, Department of Botany $60,800

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 thus plays a central role in biomass production, little is known about how it functions. Moreover, previous DOE-supported research led to the discovery that the phytochrome that predominates in light-grown plants is quite different from that which predominates in dark-grown, etiolated seedlings. Thus, the phytochrome that has been characterized is most likely not that which is operative in photosynthetically competent plant tissues. The goal of this research program is to learn more about this newly discovered type of phytochrome. In recent work, we have produced new monoclonal antibodies that are not only directed to this "green-plant" phytochrome, but that are also specific for it, recognizing phytochrome from etiolated plants either poorly or not at all. We are presently using these antibodies to investigate the possibility that the phytochrome found in a green plant is itself heterogeneous and it identify and isolate the DNA that encodes the phytochrome that is found in photosynthetically competent tissues. These investigations will contribute to an increased understanding of how green plants modify their photosynthetic capacity as a function of their light environment.

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

Nitrogen Control of Chloroplast Development
G.W. Schmidt, Department of Botany $80,000

A manifestation of nitrogen deficiency in vascular plants and algae is chlorosis, indicating that chloroplast biogenesis can be strongly restricted by direct or indirect effects of nitrogen assimilation products. Provision of nitrogen reverses the deficiency syndrome rather rapidly. To define the molecular basis of nitrogen responses we are using Chlamydomonas reinhardtii grown in a continuous culture system, as a model. Depending on the precise levels of ammonium provided, steady-state deficiency conditions are established such that the cellular levels of chlorophylls and xanthophylls are severely depressed. Also, under conditions which decrease the rate of cell division six-fold, the cells accumulate massive amounts of starch and become capable of synthesizing lipid triglycerides. Chloroplasts in nitrogen-deficient cells contain appreciable levels of carbon

assimilation enzyme and thylakoids with high electron transport activities. However, the light harvesting complexes are nearly absent and Photosystem I, especially, exhibits unusual characteristics. Studies of rates of protein synthesis by in vivo pulse-chase labeling and levels of RNAs encoded by the chloroplast and nuclear genomes have been initiated: the accumulation of transcripts for the nuclear light-harvesting apoproteins is dramatically altered qualitatively and quantitatively: there is no major effect on chloroplast RNAs but, in general, these are inefficiently utilized for protein synthesis until nitrogen is provided to the cultures. Supplying nitrogen results in an almost immediate release of chloroplast mRNAs from a translational arrest but the stimulation of the accumulation of nuclear transcripts for light-harvesting apoproteins does not occur until after a 1-2 hour lag. Current investigations are to more completely define the molecular basis for nitrogen-dependent synthesis of light-harvesting proteins and the constituents of the lipid and starch biosynthesis pathways.

58. UNIVERSITY OF GEORGIA - Athens, GA 30602

Transcriptional Analysis of the R Locus of Maize
S.R. Wessler, Botany Department $69,999

The R locus controls where, when and how much anthocyanins are expressed in at least 11 different tissues of the corn plant and seed. Enormous natural variation has been seen when the phenotypes of 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.

Over the past year we have determined that the genic elements (P), (S), and (Lc) all encode a very rare 2.8 kb transcript that is present in tissue displaying anthocyanin pigmentation. cDNA libraries have been constructed using mRNA isolated from tissues shown by Northern blots to be enriched for the R transcript. Full-length cDNAs will be sequenced and compared to each other and to the genomic sequences being determined at Yale (Dellaporta). In addition, these cDNAs will be a starting point in the eventual isolation of antibody that will be used with the cDNAs to localize R gene products and their kinetics of accumulation. This will then be correlated with the presence or absence of pigment.

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

Unraveling Photosystems
L. Bogorad, Department of Cellular & Developmental Biology $99,000

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 works. 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 experiments. 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 photoheterotrophically on sugar in dim light. However, one barrier to studying the molecular nature of mutations in its photosynthetic apparatus has been the lack of a convenient DNA complementation system. 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 study a photosynthesis deficient mutant we have isolated. A deletion within a gene for an antenna chlorophyll protein that is not a component of the minimal reaction center is the primary genetic lesion. In the absence of this gene product the photosystem II reaction center is almost completely lacking -- implicating it as a key element for assembly or stability. Surprisingly, the oxygen evolving complex and the major light-harvesting phycobilisome system, both closely associated with photosystem II functionally and physically, are unaffected. The role of the missing protein is being pursued and other mutants are being studied.

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

Structure and Function of Frankia Vesicles in Dinitrogen Fixation by Actinorhizal Plants
J.G. Torrey, Cabot Foundation, Harvard Forest $77,510

Frankia is a filamentous soil bacterium of the Actinomycetales that is capable of fixation of atmospheric dinitrogen both in the free-living state and within root nodules of a number of woody dicotyledonous plants in a symbiotic process. The bacterium is of special interest because of its genetic capacity to differentiate terminal swellings of the hyphal filaments called vesicles. Vesicles form in the free-living organism when deprived of combined nitrogen substrates under aerobic conditions. A multilaminate envelope surrounds the vesicle providing a barrier to direct exposure of the oxygen-labile nitrogenase enzyme that forms within

the vesicle. In root nodules, vesicles may or may not form, depending upon the structural configuration of the host plant cells, the ambient oxygen concentration surrounding the root nodule and the expression of host-microbial interactions under the control of the two genomes. Under varying stresses of nutrient availability and the changing gaseous environ ment, remarkable adaptations may occur in either or both partners of the symbiosis to optimize dinitrogen fixation.

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

Genetics and Chemistry of Lignin Degradation by Streptomyces
D.L. Crawford, Department of Bacteriology and Biochemistry $79,000

Current goals are to characterize catabolic mutants of S. viridosporus to determine the roles of extracellular cellulases and peroxidases in lignin solubilization, to clone lignin peroxidase and cellulase genes of S. viridosporus into S. lividans and then characterize the clones for lignocellulose degrading abilities, and to determine the effects of purified S. viridosporus lignin peroxidase on lignocellulose hydrolysis by purified cellulases. Recent findings confirm that a lignin-induced extracellular peroxidase of S. viridosporus is a lignin-oxidizing, heme-containing enzyme which carries out C-alpha/C-beta cleavage of phenolic and nonphenolic lignin substructure models. Peroxidase enhanced mutants are enhanced in cellulase activity as well as lignin solubilizing activity. The peroxidase gene has been cloned into S. lividans and is currently being characterized. The enzyme has been purified and is being characterized, and studies are being initiated to examine its role in lignin solubilization, a rate-limiting step in lignocellulose degradation by Streptomyces. This ongoing research is aimed at elucidating the mechanisms of lignin degradation by Streptomyces, with an ultimate goal of using genetic manipulation to construct improved lignin bioconversion strains.

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

Fatty and Aromatic Acid Catabolizing Bacteria in Methanogenic Ecosystems
M.P. Bryant, Department of Animal Sciences
$10,194 (three months)

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 monoaromatics. Anaerobic bacteria not requiring syntrophy are also of

interest. We previously described syntrophic species (1) -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 phosphatidylcholine 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.

63. UNIVERSITY OF ILLINOIS - Urbana, IL 61801

Photosynthesis in Intact Plants
A.R. Crofts, Dept. of Physiology & Biophysics
$202,000 (FY87 funds/ 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.

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

Mechanism of Proton Pumping in Bacteriorhodopsin
T.G. Ebrey $66,000

The purple membrane of Halobacterium halobium probably represents the simplest biological solar energy conversion system. Light absorbed by bacteriorhodopsin, a small protein whose chromophore is retinal, directly leads to the transport of protons across the cell membrane. The resulting chemiosmotic potential can be used to make ATP. An additional feature of the purple membrane is its ability to pump protons over a wide variety of salt 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 proposed experiments to help resolve the long standing confusion about the photocycle of BR and in particular about the relationship between the intermediates of the photocycle and the transport of protons across the membrane both as regards temporal correlation and quantum efficiency. We are also studying the effect of the large surface potential of the purple membrane on the proton pumping function of this membrane.

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

Studies on the Escherichia coli Respiratory Chain
R.B. Gennis, Department of Chemistry & Department of Biochemistry
$98,000

The aerobic respiratory chain of E. coli is responsible for providing the energy required by the cell for oxidative phosphorylation and for driving active transport. The respiratory chain essentially directs electron flow from the oxidation of organic substrates (e.g., succinate) to oxygen, which is reduced to water. The electron flow is coupled to proton translocation across the cytoplasmic membrane, thus generating a proton motive force. A central component of the electron transport chain which is responsible for the generation of a proton motive force is the cytochrome o terminal oxidase complex. This enzyme has been purified in our laboratory and the operon encoding the polypeptide subunits has been identified, mapped, and

cloned. The project supported by this grant is to determine the structure of this enzyme in the membrane and define the mechanism of proton translocation. The cloned operon has been sequenced and the polypeptide sequence of each of the four subunits has been deduced from the DNA sequence. Various mutations will be made to identify the role of each subunit within the complex. For example, individual subunits will be cloned separately to identify those which bind to the heme or copper prosthetic groups. Extensive use of genetics techniques will localize the amino acids required for catalysis and define their locations in the membrane. Biophysical and biochemical methods will be used in conjunction with this approach.

One focus will be to identify amino acid residues involved in ubiquinol binding. Work has been initiated on another enzyme, succinate dehydrogenase, which also interacts with ubiquinone. A similar molecular genetics approach will be used to define the quinone binding site in this enzyme and search for similarities with the quinone binding site in the cytochrome o complex.

66. UNIVERSITY OF ILLINOIS - Urbana, IL 61801

Cellular Energy Metabolism
M. Glaser, Department of Biochemistry $74,000

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 (MgATP + AMP $\rightleftharpoons$ MgADP + 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 phospholiplid synthesis coordinately decrease at the nonpermissive temperature. This appears to be due to changes in adenine nucleotide concentrations as a result of the inactivation of adenylate kinase. Thus, adenylate kinase may be one control point for determining the rates of macromolecular synthesis and cell growth. Adenylate kinase will be isolated and characterized from wild type and mutant strains. The structure and dynamic properties of the enzyme will be determined by x-ray crystallography and fluorescence spectroscopy. The cloned adenylate kinase gene will be used to vary the level of adenylate kinase in the cell in order to determine the effects on cellular metabolism and cell growth.

67. 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, Department of Microbiology $65,000

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

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

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.

68. 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 $103,760

The objective of this project is to elucidate the underlying mechanisms of the S-type of cytoplasmic male sterility (cms-S) in maize and to determine the molecular-genetic basis for both cytoplasmic and nuclear reversions to male sterility. 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 the reorganization that occurs upon spontaneous reversions to male fertility. Cytoplasmic reversion to fertility is being characterized at the

mtDNA level in the inbred line backgrounds M825, WF9, 38-11 and H95. The reversion event that occurred in the H95 nuclear background did so in the presence of a nuclear restorer gene (Rf) and is one of only two instances in which this is the case. New cases of cytoplasmic reversion have been established in the inbred line backgrounds 38-11, WF9 and M825 as well. The newly-arisen cytoplasmic revertants are being analyzed using the mtDNA probes previously employed in these studies. Additional mtDNA probes have been acquired recently and these are being used in studies of these new strains as well as in studies of the previously existing cytoplasmic revertant strains. In all cases studied, reversion is 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 reorganizations are not detected by all the mtDNA probes, however. The basis for the reorganization in only certain regions of the mtDNA genome is still unknown. Studies on transposition of spontaneously-occurring restorers and standard restorers of cms-S are being continued.

69. UNIVERSITY OF ILLINOIS - Urbana, IL 61801

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

Methanogenic bacteria are widespread in nature, and are found in diverse habitats wherever active anaerobic biodegradation of organic matter occurs. A new approach to the study of methanogenesis concerns the oxidation of alcohols by methanogens. This system, recently discovered by F. Widdel, provides a hydrogen-independent approach to the generation of electrons, enabling us to fractionate the system so as to by-pass the hydrogenases and their electron carriers. This approach provides another parameter through which to study the biochemistry of methanogenesis. We are isolating thermophilic alcohol-utilizing methanogens, mass culturing them, purifying and characterizing the 2-propanol dehydrogenase. We are defining the electron acceptors and are using this enzyme with out 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 tetrahydromethanopterin as well as the enzymology of this system. The recent determination of the structure of component B of the methylreductase system provides a new approach to an understanding of aceticlastic methanogenesis, and we propose to define the role of component B in this process.

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

Structure and Expression of Nuclear Genes Encoding Rubisco Activase
R.E. Zielinski $80,000

Rubisco activase is a soluble chloroplast polypeptide that is a ubiquitous regulator of rubisco activity. We have developed molecular probes for rubisco activase polypeptides and mRNAs. These tools will be used to help ask how the expression of rubisco activase is regulated, and what relationship activase expression has with the expression and function of rubisco. Our goal is to answer the following specific questions: (1) are steady-state levels of activase and rubisco protein and mRNA maintained in a fixed stoichiometry throughout the leaf cell life cycle; (2) is phytochrome the photoreceptor responsible for mediating the light-induced activase mRNA accumulation in barley; (3) what is the molecular basis of the activase-less, rca mutation of Arabidopsis thaliana; and (4) can the rca mutation be complemented by Ti plasmid-mediated gene transfer, using wild type activase, monocot activase, or activase that has been engineered in vitro to alter its interaction with ATP or rubisco? We will perform structural studies on cloned barley and Arabidopsis thaliana activase genomic DNA sequences. A long-range goal of these studies is to ascertain whether rubisco activase genes represent feasible targets for genetic engineering experiments whose aim is to alter the efficiency of photosynthetic energy conversion.

71. **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 $72,000

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. During the most recent program year the primary effort has been devoted to assembly of a new Raman microprobe system, with multichannel detection, optimized for our application; it was

jointly funded by the DOE University Research Instrumentation Program and the Institute of Paper Chemistry. The system has made possible acquisition of spectra in far shorter intervals of time than previously possible. Thus it will facilitate 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.

72. **IOWA STATE UNIVERSITY** - Ames, IA 50011-3223

A Study of Translational Regulation During Heat Shock
R.L. Hallberg, Department of Zoology $77,500

The objective of this project is to gain a better understanding of the mechanisms involved in the regulation of protein synthesis which occurs during hyperthermic stress in the ciliated protozoan Tetrahymena thermophila. Specifically, we wish to know how the translational machinery of these cells can, following exposure to heat shock protein inducing conditions, alternately discriminate between the selective utilization of heat shock protein mRNAs and a subsequent selective utilization of normal protein mRNAs. Recently we described two macromolecules, a ribosome associated protein and an RNA polymerase III - transcribed small molecular weight RNA, that become associated with the protein synthetic apparatus at the precise time that the cells change their ability to positively discriminate between heat shock protein and normal cell protein mRNAs. Using a combination of in vitro protein synthesis analysis and the identification of mutant strains with altered protein synthetic properties at elevated temperatures, we hope to ascertain the roles each of these macromolecules play in altering the protein synthetic properties of the cell. As an alternative and complementary approach to this work, we have recently introduced the T. thermophila gene for the small RNA into yeast and have found that it is expressed at high temperature. We will examine the effects the expression of this RNA has on protein synthesis in yeast. In addition, as the antiserum which we produced against the ribosome associated T. thermophila protein cross-reacts with a yeast protein, we are currently trying to clone the yeast gene for this protein so that we may better study its metabolism during heat shock.

73. UNIVERSITY OF KENTUCKY - Lexington, KY 40545-0091

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

G.M. Cheniae, Department of Agronomy $91,992

This project is directed towards mechanism(s) of photoinhibition of PSII Reaction Centers, conditions affecting kinetics, the quantum requirements and sites of photodamage, and the requirements for recovery of functional Reaction Centers and the associated water oxidizing complexes. These studies employ in vivo systems, PSII membranes, and PSII core complexes of higher plants. Presumptive evidence indicates that histidine residue(s) are involved in a transition of photoinhibition affecting Z>D>>>P680/ pheophytin a/Q_B to one affecting Z,F,P680 equivalently. Photodamage of pheophytin a may explain the latter observations. Western blot analyses of two radiolabeled (leucine or lysine) polypeptides resynthesized/assembled specifically during recovery of Z and D functions show they are D_1/D_2 polypeptides of PSII. Analyses of photoinhibition processes affecting photoactivation of the water oxidizing complex reveal that the site of electron donation by Mn^{2+} is more photosensitive and more highly correlated with photoactivation than the site of electron donation by diphenylcarbazide. During photoactivation, the high affinity Ca^{2+} required in O_2 evolution (2 Ca/Reaction Center) is ligated via kinetics of photoactivation. Other analyses indicate Mn^{2+} competitively inhibits ligation of Ca^{2+} at its high affinity site, histidine residue(s) constitute the Ca^{2+} high affinity site, and no high affinity Ca^{2+} is ligated by PSII membranes depleted of Ca^{2+} (and 17/24 kD polypeptides) unless the water oxidizing polynuclear Mn complex is intact in the membranes.

74. LAWRENCE BERKELEY LABORATORY - Berkeley, CA 94720

Enzymatic Synthesis of Materials

M.D. Alper, J.F. Kirsch, D. Soane, P.G. Schultz, D. Clark, M. Bednarski, H.W. Blanch, P. Smith, Center for Advanced Materials $70,000

The overall goal of this research, which is jointly funded by the Division of Materials Sciences, is the use of natural and engineered enzymes to synthesize new materials. These materials, by virtue of the unique stereochemical control exerted by enzymes and their ability to catalyze reactions at low temperature, can have structures and therefore properties that cannot be achieved using conventional synthetic routes.

Initial research efforts in pursuit of this goal are focused on the design of reaction conditions required for the enzymatic synthesis of polymeric materials; engineering of enzyme structure and activity to allow the binding and polymerization of novel monomers; characterization and processing of the polymer products of these reactions; and understanding the structure/function relationships of this new class of materials.

The genes for enzymes that can act in polymerization reactions are being cloned and expressed to provide systems for producing both natural and engineered enzymes. Substrate binding sites are being altered through site directed mutagenesis and other techniques to help understand enzyme substrate binding and provide a rational basis for the active site modifications required for binding of unusual substrates. For example, a system was developed to incorporate synthetic, unnatural amino acids at specific positions in an enzyme active site. This significantly increases the breadth and precision of the enzyme active site modifications that can be achieved. (This aspect of the project was funded jointly with the Office of Naval Research.) A variety of modified sugar and amino acid monomers are being synthesized for use in producing novel polymeric materials.

75. LAWRENCE BERKELEY LABORATORY - Berkeley, CA 94720

Light-Regulated Expression of Nuclear and Chloroplast Gene Expression
J.C. Bartholomew $51,000

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. In addition, numerous photosynthetic variants exist which have altered expression of genes encoding photosynthetic components. We have studied the growth of wild-type and bleached mutants of Euglena in the dark and light to compare their cell cycle properties. By varying the amount of light to which synchronized cells are exposed, we have shown that commitment to the cell cycle requires exposure to more than six hours of light. We propose that this is to allow the accumulation, through photosynthetic electron transport, of an initiating factor that will enable DNA synthesis to begin. Flow cytometry analysis also shows that once cells are committed to the

cell cycle, they complete the cycle in the dark, so mitosis is a light independent step. The levels of photosynthetic messenger RNAs (mRNAs) for two chloroplast-encoded genes -- psbA, which encodes the Photosystem II herbicide binding protein known as D1, and rbcL, which encodes the large subunit of ribulose 1,5-bisphosphate carboxylase -- and a photosynthetic nuclear-encoded gene -- rbcS, which encodes the small subunit of ribulose 1,5-bisphosphate carboxylase -- have been examined either in an alternating light-dark regime or in continuous light. The mRNA levels of all three genes have been found to accumulate in a cyclic fashion, with maximum accumulation occurring in the middle of the light period. Both logarithmic phase and stationary phase cells grown in a light-dark regime exhibit this cyclic accumulation, indicating that the accumulation is dependent only on light and is unrelated to the cell division cycle. In contrast, the levels of mRNA for a nuclear-encoded, non-photosynthetic gene, beta-tubulin, is shown to be cell-cycle regulated. -Tubulin mRNA accumulates in a cyclic fashion in light-dark synchronized and continuous light-grown logarithmic phase cells, and stays at relatively constant levels in stationary phase cells. 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.

76. LAWRENCE BERKELEY LABORATORY - Berkeley, CA 94720

Plant Hydrocarbon Biosynthesis
M. Calvin, J.W. Otvos, Chemical Biodynamics Division $65,000

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 includes 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 squalee 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.

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

Mapping Photosynthetic Genes in Prokaryotic and Eukaryotic Cells
J. Hearst, Chemical Biodynamics Division $251,000

The long term goal of this project is a complete understanding of the photosynthetic gene cluster from Rhodobacter capsulatus including the nucleotide sequence of the entire cluster, the identification of pigment biosynthesis genes and elucidation of their enzymatic activities, and an understanding of gene regulation of this cluster. We are engaged in physical studies of this cluster to determine the interrelations between the enzyme DNA gyrase, DNA superhelicity, genome organization, and photosynthetic gene expression. Recently, we have completed the nucleotide sequence of seven of the eight genes involved in Crt biosynthesis (crt A, B, C, D, E, F, I) in R. capsulatus. These genes are located on an 11 kb subcluster of the 46 kb photosynthesis region. The genes are organized into at least four transcriptional operons, based on the polarity of the deduced gene sequences. We have shown that DNA supercoiling may play an important role in the oxygen regulation of photosynthetic genes in R. capsulatus. DNA is negatively supercoiled by an enzyme called gyrase. We have found that the biosynthesis of Bchl and the LH (I, II)-Bchl complexes is significantly inhibited by the gyrase inhibitors novobiocin, coumermycin, malidixic acid, and oxolinic acid. The mRNA levels for LH (I, II), RC (L, M. H), Bchl biosynthetic enzymes, ribulose-bis-phosphate carboxylase and an open reading frame (ORF Q) located upstream of the puf operon were also immediately and markedly reduced by various concentrations of these inhibitors, while mRNA for Crt biosynthetic enzymes was less affected. These effects are comparable with those observed upon addition of proflavin, a transcriptional inhibitor, to the cultures. We suggest that DNA supercoiling is involved in the differential expression of photosynthetic genes in response

to oxygen in R. capsulatus. To investigate this possibility in more detail, we have developed an assay for local chromosomal superhelicity in vivo. Until now, superhelicity could be measured only for small plasmids using gel electrophoresis. The new assay measures the rate of photobinding of a DNA intercalator, psoralen, to chromosomal DNA in vivo. This rate is a function of the superhelical density of the DNA. Control experiments using aerobically grown cells treated plus or minus novobiocin indicate that the assay is sensitive to changes in superhelicity. An additional approach will be to look for specific chromosomal binding sites for DNA gyrase. A site located close to the photosynthetic gene cluster could govern regional superhelicity and thereby mediate photosynthetic gene expression. Our strategy will be to cleave the chromosome at sites of gyrase action by the sequential use of oxolinic acid, sodium dodecyl sulfate, and an appropriate restriction enzyme. The resulting large DNA fragments will then be analyzed by pulsed-field gel electrophoresis, a new technology that has opened the possibility of studying DNA molecules in the size range of 50 to 5000 kb in length.

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

Mechanism of Water Splitting and Oxygen Evolution in Photosynthesis
M.P. Klein, Chemical Biodynamics Division $167,000

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 P_{680} 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 Synechococus sp., we have determined that the manganese occur minimally as a binuclear complex with Mn-Mn separation of 2.7 Å. Additional Mn occurs at 3.3 Å. We have observed a Mn K-edge energy shift of ~ 1 eV upon advancing from the S_1 to the S_2 state, 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 reducing 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 provided the first direct evidence that the Mn centers are accessible to solvent water. Illumination at 190K followed by brief warming of PS II particles prepared with NH_3 produces an altered EPR signal whose ESE signals show modulations characteristic of ^{14}N providing evidence that NH_3 binds to Mn during the S_1 to S_2 state transition.

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

Tissue Specific Gene Regulation in Plants
F. Leach, J.C. Bartholomew, J.E. Hearst, Chemical Biodynamics Division $103,000

Our long-term goal is to understand how tissue-specific expression of a root-specific and a leaf-specific gene occurs in tobacco. In our original proposal, we suggested the study of the Ri T-DNA which show differential expression in these two tissues. We proposed to fuse the two promoters to the CAT gene and monitor the activity of the chimaeric constructs by transient protoplast transformation. In the past funding period we built all biological and genetic tools and mastered the techniques crucial to carry on the main experiments. In particular, we have established sterile cultures of plantlets, isolated roots, and suspension cultures of tobacco leaf calli. We have cloned the 5′ upstream regions of the leaf and root promoters and constructed transcriptional fusions to the CAT gene. Each of the 5′ regions has been further subcloned to yield overlapping subsets of fragments ending at the same 3′ nucleotide (just before the ATG codon) but with several combinations of 5′ end deletions. These sub-fragments have been fused to the newly available GUS gene in preparation for promoter analysis by deletion mutagenesis and S1 mapping experiments. We have successfully mastered techniques for the genetic transformation of plant cells using both a binary vector system derived from pBIN to obtain transgenic plants and direct protoplast transformation with naked DNA in view of testing for transient expression of our constructs. We have tested our capacity to prepare clean and intact tobacco root and leaf nuclei. We have developed a good isolation procedure by modifying a published technique. Filter-binding assays of nuclear proteins to the isolated root and leaf promoters have been undertaken. Although preliminary, none of the conditions tested have brought to light a repeatable difference in the DNA-binding qualities of the two promoter regions. As a consequence of the above experimental considerations, we are bringing the following modifications to our work. We have switched to GUS as a reporter gene because it is more sensitive and the product is easier to assay. We will test the possibility that the differing amounts of mRNAs from ORF12 and ORF15 in leaf and root tissues is due to post-transcriptional rather than transcriptional ones.

80. LAWRENCE BERKELEY LABORATORY - Berkeley, CA 94720

Photochemical Conversion of Solar Energy
L. Packer, R.J. Mehlhorn, I. Fry, J. Maguire, W. Nitchman, G. Khomutov, Applied Science Division $130,000

This project seeks to understand mechanisms of biological oxidation and energy conversion in microbial systems. Using cyanobacteria and B. subtilis, the role of biological oxidations, both photosynthetic and respiratory, in cell energetic conditions is investigated.

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

Further studies on the membrane assembly of subunits of succinic dehydrogenase derived from B. subtilis mutants and the role of the flavin and iron-sulphur redox centers in activity are being conducted by spectroscopic and low temperature electron paramagnetic resonance techniques.

81. LAWRENCE BERKELEY LABORATORY - Berkeley, CA 94720

Chemistry of Phycobiliproteins and Phytochrome
H. Rapoport, Chemical Biodynamics Division $51,000

A complete understanding of the chemistry and stereochemistry of phycobiliproteins and of phytochrome is sought to facilitate full understanding of the role of light in regulation of gene expression in green plants. The chemical structures of phycobiliproteins and phytochrome are being determined, including the detailed nature of the covalent attachments of chromophore to protein, by stereospecific synthesis of model

chromophores. Chromophore-peptides also are being synthesized to ascertain the effect of the peptide-protein on solution conformations and energy transfer.

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

82. LAWRENCE BERKELEY LABORATORY - Berkeley, CA 94720

Photosynthetic Membrane Structure and Photosynthetic Light Reactions
K. Sauer, Chemical Biodynamics Division $252,000

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 Forster inductive resonance transfer applied to pigment arrays of known geometry. For the cyanobacterial pigment C-phycocyanin new coordinates published based on revised X-ray crystallographic structure determinations make it clear that some delocalization should be occurring by the exciton mechanism. As a consequence there should be some significant excited state relaxation that is faster, and has been resolved in any measurements to date. We are now in the process of improving the sensitivity of our 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.

83. LEHIGH UNIVERSITY - Bethlehem, PA 18015

A Genetic Approach to Secretion and Hyperproduction of Cellulase by Trichoderma

J. Sands and J. Phillips, Department of Biology/Biotechnology

$80,000 (FY87 funds/ 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 morphological 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 cellobihydrolase 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 cellulase and not for protease, amylase and xylanase. At the non-permissive temperature heterogenous 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.

84. LOS ALAMOS NATIONAL LABORATORY - Los Alamos, NM 87545

Energy Metabolism in Methylotrophic Bacteria

J.A. Fee and C.J. Unkefer, Division of Isotope and Nuclear Chemistry

$115,000

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. During the initial period of the project, combined NMR/^{13}C labeling experiments revealed that pyroloquinoline quinone (PQQ) is synthesized in these organisms by combining glutamic acid and tyrosine to form the fused three-ring system of PQQ. A similar technical approach is being used to sort out the metabolic events of the so-called icl-pathway of carbon assimilation. Other 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.

85. LOS ALAMOS NATIONAL LABORATORY - Los Alamos, NM 87545

Metabolism of Cell Walls from Monocotyledenous Plants: ^{13}C N.M.R. Metabolic Analysis of Cell Walls Isolated from Proso Millet (Panicum miliaceum L. Cv. Abarr)

L.O. Sillerud and N.H. Fink, Division of Life Sciences $115,000

With the aid of high-resolution ^{13}C N.M.R. spectroscopy, we have characterized the structure and dynamics of plant cell wall polysaccharides. This study was extended to observe the metabolism of these complex Panicum miliaceum cell wall glucuronoarabinoxylans from cells grown on [1-^{13}C]- and [2-^{13}C]-glucose. The low natural-abundance of ^{13}C (1.1%) is advantageous in metabolic N.M.R. studies that follow the fate of specifically-labeled ^{13}C precursors because of the low natural abundance background signals. We were able to determine the relative rates of the pathways that contribute carbon atoms to the residues in the Panicum cell walls. The ^{13}C NMR study showed that 61.9% of the [2-^{13}C]-glucose label traveled via the nucleotide-sugar pathway and 38.1% via the pentose phosphate pathway. We observed 27.1% of the [2-^{13}C]-glucose encountering triose phosphate isomerase where the [2-^{13}C]-label moved to the C5 position and the [1-^{13}C]-glucose label to the C6 position. Very little flow of carbon from glucose entered the Kreb's cycle as indicated by the lack of significant label scrambling from C1 to C2 or C5 of glucose. On the other hand, the pentose phosphate pathway was clearly detectable through label movement from C2 of glucose to C1 of the pentoses. Furthermore, we observed some futile cycling of the ^{13}C-glucose prior to incorporation into the cell wall. Finally, we detected significant differences in the structure and metabolism of the cell wall hemicelluloses that depended on whether the carbon source was 4% sucrose or 2% glucose. We are extending

this study to monitor the metabolism of the cell wall in vivo as it is occurring in a perfusion system that measures oxygen consumption, pH and temperature during the course of the NMR experiments.

86. UNIVERSITY OF MARYLAND - College Park, MD 20742

Active and Passive Calcium Transport System in Plant Cells
H. Sze, Department of Botany $66,895

The ability of a cell to change its cytoplasmic Ca^{2+} levels rapidly has made this cation a key regulator of many biological processes. Cytoplasmic $[Ca^{2+}]$ is regulated by the balance of active and passive Ca^{2+} fluxes across the plasma membrane and organellar membranes. To understand the mechanisms and regulation of Ca^{2+} fluxes in plant cells, we have used isolated membrane vesicles to study Ca^{2+} transport systems in oat roots and carrot suspension cells. Cytoplasmic Ca^{2+} levels are kept low by several active transport systems. Ca^{2+} accumulation into plant vacuoles depends on a proton motive force generated by the tonoplast H^+-pumping ATPase. We have characterized this Ca^{2+}/H^+ exchange activity extensively; and have initiated studies to identify this antiporter. The tonoplast proteins are solubilized with octylglucoside and transport activity measured after reconstitution by detergent dilution. A C^{2+}-ATPase pumps Ca^{2+} into the endoplasmic reticulum, and is stimulated by calmodulin. A similar Ca^{2+}-ATPase may be associated with the plasma membrane. Ca^{2+} stored in the tonoplast vesicles can be released transiently by inositol 1,4,5-trisphosphate (IP_3), suggesting the presence of gated Ca^{2+} channels on the vacuolar membrane. IP_3 may be an important second messenger in plant cells, as it is produced in response to environmental stimuli. Future studies will focus on identifying these active and passive Ca^{2+} transport systems.

87. MASSACHUSETTS INSTITUTE OF TECHNOLOGY - Cambridge, MA 02139

Analysis of the Rhizobium meliloti Surface
E.R. Signer, Department of Biology $89,000

The formation by rhizobia of nitrogen-fixing nodules on the roots of leguminous plants presumably involves communication between bacteria 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 SU47 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. The structure of LPS from wild-type and mutants is also being determined. 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.

88. UNIVERSITY OF MASSACHUSETTS - Amherst, MA 01003

Cellulose Fermentation By Nitrogen-Fixing Anaerobic Bacteria
E. Canale-Parola, Department of Microbiology $78,000

The project objective is to conduct studies aimed at increasing understanding of i) the physiology of anaerobic cellulolytic nitrogen (N_2)-fixing bacteria, ii) the N_2-fixing system of these bacteria, iii) the effects that N_2 fixation has on cellulose fermentation, and iv) the physiological interactions between non-cellulolytic, N_2-fixing, commensal bacteria and cellulose fermenters in nitrogen-poor environments. Strains of obligately anaerobic cellulolytic N_2-fixing bacteria that we have isolated from freshwater environments, as well as known cellulolytic species that we have shown to fix N_2 will be investigated. Additional N_2-fixing cellulolytic strains with diverse properties will be isolated from terrestrial, fresh-water, and marine environments. Studies of these strains, including their morphology, fine structure, general physiology, and N_2-fixing systems, are intended to provide an understanding of the interrelations between nitrogen fixation and the fermentation of cellulose in biological systems in which these two complex physiological processes coexist within the same cell. Furthermore, these strains may be extremely useful in the direct conversion of biomass materials to ethanol and other fuels. Another objective of the research will be to establish diazotrophic cocultures of N_2-fixing, anaerobic, non-cellulolytic commensals and cellulolytic bacteria in order to study the effects of competition for cellulose depolymerization products on cellulose degradation.

89. UNIVERSITY OF MASSACHUSETTS - Amherst, MA 01003

Genetic Analysis of a Green Bacterial Photosynthetic Membrane
T. Redlinger and S. Robinson, Departments of Biochemistry and Botany
$120,000 (two years)

Chloroflexus is a facultative green bacterium. During aerobic growth, its photosynthetic apparatus is absent. A shift to anaerobiosis induces assembly of the photosynthetic system which consists of pigment protein complexes in the cytoplasmic membrane, and an extramembranous antenna system, the chlorosome. The reaction center of Chloroflexus is functionally similar to those of purple bacteria (and thus to chloroplast photosystem II) in terms of photochemical behavior, but is dissimilar in having a simpler polypeptide subunit structure. We will study the genetic regulation in Chloroflexus by constructing genomic libraries which will then be screened to isolate the genes encoding photosynthetic polypeptides. Oligonucleotides and heterologous DNA probes will be used for screening by nucleic acid hybridization; antibodies against purified Chloroflexus membrane components will be used for immunochemical screening. Isolated genes encoding photosynthetic components will be analyzed by sequence determination, and in terms of linkage relationships. RNA-DNA hybridization using RNA isolated from various growth conditions which modulate membrane assembly, will permit direct analysis of expression of isolated genes. Results from these experiments directly address questions of photosynthetic membrane assembly and structure. First, determination of the sequence of reaction center polypeptide genes will allow comparison with the purple bacterial and chloroplast gene sequences, and provide a first indication of how this simpler reaction center architecture supports its photochemical activity. Second, determination of the gene sequences encoding chlorosome polypeptides will provide a test of the post-translational processing hypothesis. Finally, studies of regulation using cloned gene probes will indicate mechanisms for the development of photosynthesis in response to environmental cues.

90. MEHARRY MEDICAL COLLEGE - Nashville, TN 37208

Respiratory Enzymes of Thiobacillus ferrooxidans
R.C. Blake II, Biochemistry Department $74,000

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

91. 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 $97,000

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.

92. **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
(Extension of FY 87 Funding)

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 are a) examining the distribution of ACP forms in a variety of photosynthetic species, in various tissues and under different environmental influence; b) preparing monospecific antibodies to each spinach leaf isoform to use as probes of their individual function; c) modifying 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. In addition we are examining the composition of the acyl-ACP pool in plant chloroplasts in order to evaluate potential rate limiting steps in lipid metabolism.

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

Cloning, Characterization and Expression of genes/cDNA encoding Lignin Peroxidases in Phanerochaete chrysosporium, a white-rot Basidiomycete
C. A. Reddy, Department of Microbiology and Public Health $66,880

P. chrysosporium is known to elaborate a number of extracellular lignin peroxidase isozymes that play an integral role in lignin degradation.

These proteins have also been implicated in the detoxification of recalcitrant environmental pollutants such as dioxins. Our current focus is to develop heterologous and homologous expression systems for cDNA/genes encoding lignin peroxidases of P. chrysosporium. Yeast and E. coli expression vectors have been constructed and use of these to obtain expression of lignin peroxidases in yeast or E. coli and characterization of the recombinant is an intensive focus of study. We have also succeeded recently in isolating and sequencing P. chrysosporium genes that encode lignin peroxidase H10 and a mutant (lig-) of this fungus that lacks the ability to elaborate lignin peroxidases. Furthermore, we have described a transformation procedure for this fungus. Hence, we are in a position to transform H10 gene into the lig- mutant and study the expression of lignin peroxidase. We propose to do appropriate manipulation of the transformant to increase the level of expression and study the regulation of gene expression at the molecular level.

94. MICHIGAN STATE UNIVERSITY DOE PLANT RESEARCH LABORATORY

East Lansing, MI 48824

Enzymatic Mechanisms and Regulation of Plant Cell Wall Biosynthesis

D. Delmer, Hebrew University, Jerusalem, Israel $115,000

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 a 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.

(Portion of work carried out at Hebrew University under sub-contract.)

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

Resistance of Crop Plants to Environmental Stress
A.D. Hanson $197,000

Plants have biochemical or metabolic adaptations to environmental stresses, as well as adaptations expressed at higher levels of organization. Were metabolic adaptations to stress better understood, they could be used in crop improvement, via conventional or recombinant DNA technologies. This project aims (1) to identify adaptive metabolic responses of plants to stress, (2) to find the enzymes and genes involved, and (3) to explore the effect on the whole plant of genetically modifying metabolic adaptations. We are working on two topics: betaine accumulation and lactate glycolysis. During water- and salt-stress, certain plants accumulate betaine. Much evidence indicates that betaine acts as a non-toxic cytoplasmic osmolyte during stress. Betaine is synthesized in the chloroplast by a two-step oxidation: choline → betaine aldehyde → betaine. The second step is catalyzed by a stromal, NAD-linked dehydrogenase. We have purified this enzyme to homogeneity, raised antibodies against it, and obtained amino acid sequence data. Several immunopositive clones have been isolated from a spinach leaf cDNA library in λgt11, and are being analyzed further. We have shown using ^{18}O-labeling techniques that the first step is most probably an oxygenase; purification of this enzyme has begun. Lactate dehydrogenase (LDH) activity is induced by hypoxia in roots and catalyzes lactate glycolysis, which is important during hypoxia. We have developed an improved purification for barley LDH. Preparatory to cDNA cloning, we have raised antibodies against highly purified protein and obtained amino acid sequences of tryptic peptides.

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

Action and Synthesis of Plant Hormones
H. Kende $244,000

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. Currently, we are trying to obtain amino acid sequences of ACC synthase as a first step towards cloning the gene for this enzyme. We are also screening expression libraries with ACC-synthase antibody in an attempt to isolate

ACC-synthase clones. We are also investigating the enzyme responsible for ethylene formation from ACC. We have characterized some of its properties in isolated vacuoles and found that it requires membrane integrity and probably a transmembrane ion gradient. The role of stress ethylene is being investigated in deepwater rice where low-oxygen stress during submergence induces ethylene biosynthesis. Ethylene, in turn, mediates the growth response of submerged plants. We are investigating the effect of ethylene on a number of biochemical processes that are related to the growth response. 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.

97. MICHIGAN STATE UNIVERSITY DOE PLANT RESEARCH LABORATORY

East Lansing, MI 48824

Cell Wall Proteins

D.T.A. Lamport $178,000

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

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

Regulation of Flowering
A. Lang $59,000

This project deals with various aspects of the regulation of flower formation. One part has been concerned with promotive and inhibitory, graft-transmissible, hormone-like materials (florigen and antflorigen). The evidence for florigen and antflorigen 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. This objective has been pursued, using extraction and diffusion techniques, and tissue explants capable of regeneration of flowers as bioassay. So far, however, no consistent results have been obtained. In another project it was found that some recently synthesized powerful inhibitors of gibberellin synthesis (A-Rest, Tetcyclacis) greatly reduced the gibberellin content in the plants and inhibited stem growth, but had 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 has 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. A third project dealt with regeneration of flower buds in explants from stems and inflorescence branches of various Nicotianas. Previously, this had been accomplished only in nonphotoperiodic (day-neutral) cultivars of N. tabacum (tobacco). We found that it can be accomplished also in short- and long-day genotypes. The response in these is spatially somewhat more limited than in the day-neutral ones, but in all genotypes it is maintained for quite extensive times, showing that once flower formation is initiated certain tissues acquire a persistent, although not irreversible floral determination state.

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

Interaction of Nuclear and Organelle Genomes
L. McIntosh $204,000

Our purpose is to genetically define the components of energy transduction in photosynthetic organisms and to employ molecular modification of specific polypeptides to explore their function. At a practical level, this means the isolation of genes involved in photosynthesis and respiration, analysis of their expression and structure/function studies of the polypeptides they encode. A model system for the study of photosynthesis was developed

employing the unicellular cyanobacterium Synechocystis 6803. Many "photosynthetic" genes have been cloned and sequenced from this organism. Some genes have been altered and reinserted into Synechocystis in order to analyze the influence of single amino acid changes on photosynthetic electron transport. The expression of these genes is also being investigated in relation to the mechanism of assembly of a competent photosynthetic apparatus. Plants contain a branched pathway for mitochondrial respiration; with a normal cytochrome pathway and an alternate pathway which is not coupled to ATP production. There is a fundamental lack of understanding concerning the manner by which plants utilize and regulate these two pathways of respiration. While work has advanced on the cytochrome pathway the alternate pathway has resisted elucidation until recently. We have now solubilized and isolated the alternate oxidase from higher plants. Both polyclonal and monoclonal antibodies have been raised to the alternate oxidase. These antibodies are being employed to further dissect the oxidase and to aid in cloning the genes encoding it.

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

Sensory Transduction in Plants
K.L. Poff $177,000

The primary objective of this project is to understand the mechanisms of environment information via light and gravity reception. We are studying the blue light photoreceptor pigment system(s), which control(s) numerous light responses such as phototropism in flowering plants, and, in addition, we are studying the mechanism for the perception of gravity in gravitropism. For these studies, we are developing a genetic system with which we can dissect the initial steps in the transduction sequences. Screening procedures have been devised and used to identify mutants of Arabidopsis with altered shoot phototropism, altered shoot gravitropism, and/or altered root gravitropism. Based on these strains, one can conclude that shoot phototropism and shoot gravitropism share many common elements, but that shoot gravitropism and root gravitropism are substantially separate pathways. The shoot photo-minus, gravi-normal phenotype should represent an alteration early in the phototropism pathway and could arise from an altered photoreceptor pigment. We are continuing to study these pathways for phototropism and gravitropism through mutant isolation, and genetic, physiological and biophysical characterization. This genetic approach should permit positive identification of the photoreceptor pigment, access into the transduction sequence, and eventual understanding at the molecular level of the events from photoreception to the bending response.

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

Developmental Regulation of Gene Expression in Plants
N.V. Raikhel $150,000

The first main objective of our research is to understand the cellular and molecular mechanisms that control developmentally and hormonally regulated, tissue-specific gene expression. Cereal and rice lectins provide an excellent system for studying differential expression in plant tissue. These proteins are synthesized only during formation of the seed and in the root tips of adult plants. Accumulation occurs in a tissue-specific manner in morphologically distinct cell layers of various embryonic organs (e.g. coleoptile, epiblast, coleorniza and radicle) at specific times during embryogenesis. Abscisic acid is also involved in the accumulation of cereal and rice lectins. Thus, expression of these proteins is controlled by a number of factors. Using the different cell-layer specificities found in related species in the Gramineae, we will learn why adjacent cell layers exhibit an on/off pattern of expression of similar proteins and whether expression in different cell layers is regulated by the same or different DNA sequences.

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

Physiological and Molecular Genetics of Arabidopsis
C.R. Somerville $239,000

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 post-transcriptional 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.

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

Molecular Basis of Disease Resistance in Barley
S.C. Somerville $165,000

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

The results of mutational analyses suggest more than one component distinguishes resistance from susceptibility in barley, and similarly more than one component determines avirulence in E. graminis. Our conclusion is that the "gene-for-gene" hypothesis, in its simplest formulation, does not adequately describe barley-E. graminis interactions.

As a step towards developing tools needed for a molecular analysis of avirulence in E. graminis, we have prepared a lambda library from race CR3, and have cloned the beta-tubulin in gene from this library. We plan to introduce into the beta-tubulin gene, by in vitro mutagenesis, mutations known to confer resistance to the fungicide Benomyl, and then to use the Benomyl resistance gene as a marker in transformation experiments.

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

Molecular Biology of Toxicogenic Plant Pathogens
J.D. Walton $150,000

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. 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.

105. MICHIGAN STATE UNIVERSITY DOE PLANT RESEARCH LABORATORY

East Lansing, MI 48824

Developmental Biology of Nitrogen-Fixing Cyanobacteria

C.P. Wolk $204,000

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. Cloned methylase genes within the donor are now used to protect the transferred DNA against restriction by Anabaena. Mutations that render Anabaena 7120 unable to fix N_2 under aerobic conditions have been repaired by addition of DNA from normal parental strain, thus leading to the identification of genes that are essential for aerobic fixation of N_2. We are also trying to achieve site-directed mutagenesis and viral transfer of DNA to Anabaena. To accelerate the identification of genes that are important for N_2-fixation, we have constructed transposons that should insert strongly expressed antibiotic-resistance genes together with the genes for the light-emitting protein, luciferase, randomly within the Anabaena chromosome. 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.

106. **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 $168,000

Plant growth and development are affected by environmental factors such as daylength, temperature, and moisture. Hormones often function as chemical messengers between the perception of an environmental factor and the morphological expression. The objective of this project is to characterize the hormones involved, how their synthesis and catabolism are regulated by the environment, and how these hormones act. In the long-day rosette plant, spinach, photoperiodic control of stem growth is based on dioxygenases which convert inactive gibberellins to an active one. The objective is to purify one of the light-regulated enzymes, to prepare antibodies against the protein, and then to determine at what level light controls the activity of this enzyme.

Studies on the biosynthesis of the stress-hormone abscisic acid (ABA) have demonstrated that three of the four oxygen atoms are derived from molecular oxygen. After incubation of stressed leaves in ^{18}O, one ^{18}O atom is rapidly incorporated in the carboxyl group, while only small amounts of ^{18}O are present in the ring oxygens of ABA. These results suggest that there is a large precursor pool which already contains two of the four oxygen atoms found in ABA. This precursor pool is probably made up of xanthophylls. Since the xanthophyll pool is large and the turnover low, the amount of ^{18}O incorporated in the ring positions of ABA would be small. The high incorporation of ^{18}O into the carboxyl group of ABA would be the result of oxidative cleavage of the central polyene chain of a xanthophyll. The fourth oxygen atom of ABA, in the carboxyl group, originates from water. This observation is also consistent with the hypothesis that xanthophylls are precursors of ABA, assuming that the cleavage reaction yields an aldehyde which is subsequently oxidized to a carboxyl group by a dehydrogenase.

107. UNIVERSITY OF MINNESOTA - Navarre, MN 55392

Genetics of Bacteria that Utilize Carbon Compounds
R.S. Hanson, Gray Freshwater Biological Institute $65,000

Bacteria that grow on methane and methanol are considered useful for the production of several products because they grow on simple, inexpensive substrates. In some bacteria that grow on methane or methanol, methane monooxygenase (MMO) and methanol dehydrogenase (MDH), each represent over 20% of the total cellular protein. In Methylobacterium organophilum, the enzymes responsible for 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 than 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 half life of this mRNA is approximately three minutes. A gene encoding a small (10 kD) polypeptide associated with MDH has been identified. 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. We have also described a thermophilic gram positive methanol utilizing bacterium. An NAD^+ linked MDH from this bacterium is being characterized.

108. 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 $104,015

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 butyr aldehyde dehydrogenase, we are now 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. We have isolated three classes of regulatory mutants that either under-produce or over-produce the enzymes for solvent formation. Two cryptic plasmids of C. acetobutylicum have been isolated and mapped with restriction endonucleases. Shuttle plasmids will be developed using E. coli plasmids and vectors and Streptococcal conjugative plasmids. The C. acetobutylicum gene for butyraldehyde dehydrogenase will be cloned and expressed in E. coli. Plasmid DNA containing C. acetobutylicum genes will be transferred into Streptococcus lactis and transferred by conjugation into various regulatory mutants of C. acetobutylicum. The genetics of the regulation of the fermentation will be analyzed.

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

Analysis of Cyanobacterial Photosystem II Genes by Cloning and Mutagenesis

L.A. Sherman, Division of Biological Sciences $71,000

This project identifies and clones genes coding for membrane proteins involved in cyanobacterial photosynthesis. The strains used for these studies, Anacystis nidulans R2 and Synechocystis PCC6803, are both transformable; Synechocystis can be grown photoheterotrophically and is thus ideal for the isolation of photosynthesis mutants. This year we will continue our investigation of some novel membrane proteins and initiate some important new projects. We have detected proteins that are regulated by changes in environmental conditions, either by iron-deficiency or light. We have cloned, sequenced, and mutagenized a gene (irpA) that codes for a protein involved in iron acquisition or storage. This year we will attempt to clone the genes for the 2 chl-binding proteins of the chl-protein complex (designated CPVI-4) that is strongly induced by iron-deficiency. We will also clone the gene for the GP35, a 35kD, iron-inducible protein that is glycosylated. We have purified GP35 and obtained a 25 residue N-terminal sequence of the protein. We will further analyze a mutant in the irpA gene which is unable to grow under iron-deficient conditions. We will also clone the gene for a second carotenoid-binding protein and compare the sequence with the 42 kD protein sequence obtained this year. We have also initiated a project with the chlorophyll b-containing prochlorophyte, Prochlorothrix hollandica. We have produced an antibody against the chl a/b protein from this organism and will use the antibody to clone the gene from our new lambda ZAP library. Finally, we will construct site-directed mutants in these cloned genes to determine amino acids and domains involved in specific functions.

110. UNIVERSITY OF MISSOURI - Columbia, MO 65211

Genetics and Molecular Biology of Hydrogen Metabolism in Sulfate-reducing Bacteria
J.D. Wall, Department of Biochemistry $67,000

In anaerobic digestors of 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. Key factors controlling the interactions of the SRB with other microorganisms in their environment are hydrogen metabolism and their tolerance of exposures to oxygen. 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 capable of generalized transduction. In addition we have observed that colonies of sulfate-grown cells are capable of rather long term survival after exposure to air and that liquid cultures also exhibit oxygen tolerance. These attributes will contribute to our continued generation and analysis of interesting mutants for the physiological investigation of hydrogen metabolism.

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

The Respiratory Chain of Alkalophilic Bacteria
T.A. Krulwich, Department of Biochemistry $80,000

The extraordinary amounts of respiratory chain components in membranes of extremely alkalophilic bacilli, the apparently efficient proton pumping by that chain, and the bioenergetic demands of life at high pH have focused us upon the properties of the respiratory chain in these organisms. During the coming year studies will continue on interactions between soluble acidic cytochromes from Bacillus firmus RAB and the membranes. A major effort will also be made to characterize a category of respiratory chain mutants among a larger group of pH conditional mutants of facultatively alkalophilic Bacillus firmus OF4. The pH conditional mutants, isolated this

year, have lost the ability to grow above pH 9 or 9.5, but can still grow at near neutral pH. Their properties should enhance our understanding of the interplay between specific properties of the membrane, respiratory chain, and other ion porters.

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

Partial Support of GenBank: The Genetic Sequence Data Bank
J.C. Cassatt, J. Peterson, National Institute of General Medical Sciences $20,000

GenBank, the Genetic Sequence Data Bank, is an internationally available repository of all reported nucleotide sequences greater than fifty nucleotides in length, annotated for sites of biological interest, and checked for accuracy. As of June 15, 1988, GenBank contained about 19 million bases comprising 20,000 sequences. The data bank is operated under contract to Intelligenetics Inc. of Mountain View, CA. Data collection, verification, entry and annotation are performed under the direction of Dr. Christian Burks at Los Alamos National Laboratory, while distribution, user support services and overall data bank management are performed by Intelligenetics. The project is enhanced through collaboration with the EMBL DNA Data Library at Heidelberg, and the DNA Databank of Japan. This resource, co-sponsored by the National Institute of General Medical Sciences, Division of Research Resources (of NIH), the National Science Foundation, the Department of Energy and the National Library of Medicine, is of particular interest to geneticists and molecular biologists. A copy of the database is available for a modest fee on computer-readable magnetic tape and floppy diskettes to anyone requesting it. Dial-up-on-line access, including Telenet access, is also available.

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

Identification of the Enzymatic Steps in Methanogenesis from Acetate: Studies on the Structural and Catalytic Properties of Carbon Monoxide Dehydrogenase from Methanosarcina barkeri
D.A. Grahame, National Heart, Lung, and Blood Institute $60,500

The objectives of the proposed research are, in Part I: To identify, purify, and characterize the enzymatic components involved in methanogenesis from acetate by reconstitution of the factors required for the partial reaction in which the methyl group of acetate is converted to the methyl moiety of 2-methylthioethane-sulfonate (methyl-S-CoM) and to demonstrate whether CODH is essential for methyl-S-CoM formation; and in

part II: To determine specific characteristics of CODH including the subunit location of the active site and the chemical properties of nickel in enzyme.

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

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

114. UNIVERSITY OF NEBRASKA - Lincoln, NE 68588

Characterization of Plant Plasma Membrane Antigens
D.W. Galbraith, School of Biological Sciences $79,000

An understanding of the molecular architecture and developmental behavior of the plant cell wall/plasma membrane interface is central to an understanding of plant growth and development. We are characterizing the process of biosynthesis of some glycoproteins that are specifically localized at the Nicotiana plasma membrane and that are expressed at the plasma membrane under heterotrophic but not photoautotrophic conditions. We are employing three approaches. Firstly, we have constructed monoclonal libraries using plant plasma membranes and protoplasts as immunogens. Using two-dimensional gel electrophoresis followed by Western blotting, we have identified the molecular nature of an antigen recognized by one of these antibodies as extensin, a hydroxyproline-rich glycoprotein. We have

found that biosynthesis of this antigen occurs within the endoplasmic reticulum/Golgi system and accompanies the initiation of heterotrophic growth and cell division by leaf protoplasts in culture. Secondly, we have constructed cDNA libraries in phage expression vectors corresponding to the different protoplast developmental stages. These are currently being screened in order to identify changes in gene expression associated with this developmental system. 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 surface of transformed protoplasts, using the E. coli beta-glucuronidase gene reporter system. We intend to use these techniques to compare the molecular signals required for operation of the endomembrane secretory pathway in heterologous systems, as well as for the isolation of mutant cell types deficient in the control of secretion of proteins through this pathway. This research should lead to an improved understanding of the mechanisms that control plant cell growth and division.

115. **UNIVERSITY OF NEBRASKA** - Lincoln, NE 68583

Viruses of Eukaryotic Green Algae

J.L. Van Etten, Department of Plant Pathology

(Extension of FY 87 Funding)

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

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

116. NEW YORK UNIVERSITY MEDICAL CENTER - New York, NY 10016

Anaerobic O-demethylation of Phenylmethylethers
A.C. Frazer and L.Y. Young, Department of Microbiology $71,000

Research on the process of anaerobic O-demethylation (AOD) of methoxylated aromatic compounds has both basic and applied significance. Aryl-O-methyl ethers are abundant in natural products, particularly as components of lignin. Methoxylated lignin monomers are present in anaerobic environments where they can be completely degraded by microbial food chains. AOD is an early necessary step in this process and involves the utilization of the O-methyl substituent as a C-one substrate. An understanding of the AOD reaction might suggest new ways in which chemicals could be derived from lignocellulosic materials. AOD is particularly intriguing because the biochemical mechanism by which the aryl-O-methyl ether bond is cleaved is not known, although methyl group transfer appears to be involved. Thus, novel biochemical information on an important biotransformation reaction will be gained from the research proposed. Recently, we have shown that AOD activity is inducible and we have developed an assay for detecting AOD activity in cell-free extracts of Acetobacterium woodii. AOD Activity is stimulated in vitro by the addition of ATP (1 mM) and pyruvate (30 mM), the K_M for vanillate being 0.4 mM. In collaboration with experts in protein purification, we now propose to purify the AOD enzyme and characterize the protein(s) and the enzymatic reaction involved.

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

Phosphoinositide Metabolism and Control of Cell Growth
W.F. Boss, Department of Botany $62,000

Polyphosphoinositides, a class of regulatory phospholipids, play a pivotal role in signal transduction in animal cells. The polyphosphoinositides have been shown to be present in plant cells. The question being addressed is whether or not they play a role in signal transduction in plant cells and if so, what is the metabolic pathway involved. Analysis of the plant phosphoinositides revealed that the ratio of PIP to PIP_2 in both the carrot suspension culture cells and soybeans was about 9 to 1. In addition, LPI and LPIP were present in relatively high amounts, and in some instances, were equal to the percentage of PIP recovered. Localization of the polyphosphoinositides in the cells was studied and the plasma membrane was found to be enriched in PIP, LPIP, and PIP_2 compared to endomembranes. In addition, the nuclear membrane contains PIP, LPIP, and PIP_2. While the absolute amount of the inositol lipids is low in the nuclear membrane the ratio of PIP to PI is similar to that found in the plasma membrane. IP_3 has been difficult to detect in plants. This is in part due to the relatively low levels of PIP_2. In addition, plant cells metabolize inositol to

glucuronic acid and other sugars which are used in cell wall synthesis. The compounds co-elute with the inositol phosphates on Dowex anion-exchange columns. We are currently studying the synthesis and metabolism of the phosphoinositides and inositol phosphates in the carrot cells.

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

Genetics and Biochemistry of Surfactant Synthesis in Rhodococcus sp. H13-A

W.R. Finnerty, Department of Microbiology $83,500

The synthesis of biosurfactant by Rhodococcus species H13-A and the development of recombinant DNA technology in this genus is under study. An Escherichia coli-Rhodococcus shuttle vector, pMVS301, has been constructed plus the development of efficient transformation in this genus (J. Bacteriol. 170:638-645, 1988). The 10.1 Kb recombinant plasmid encodes and expresses ampicillin-, thiostrepton-resistance in Rhodococcus and replicates in Rhodococcus or E. coli. A restriction map of pMVS301 demonstrated 14 unique restriction sites, some of which are useful for molecular cloning in Rhodococcus. An improved cloning vector (pMVS301K) has been constructed encoding ampicillin-, thiostrepton- and kanamycin-resistance. The utility of this vector was demonstrated by cloning H-13A genomic DNA into pMVS301K, which complemented E. coli histidine-auxotrophs. Biosurfactant synthesis by Rhodococcus is physiologically regulated by nitrogen-limitation in the presence of alkanes as the sole oxidizable carbon source. The biosurfactant is an anionic glycolipid containing glycerol, trehalose and 3-5 glucose monomers plus C35-C40- mycolic acids, normal saturated fatty acids and dicarboxylic acids. Current studies are directed to the molecular cloning and characterization of genes encoding glucosyl-and fatty acyl transferases in Rhodococcus H13-A.

119. NORTHWESTERN UNIVERSITY - Evanston, IL 60208

Genetics of Thermophilic Bacteria

N.E. Welker $62,000

Temperate and virulent phages, isolated from soil, carry out generalized transduction of several auxotrophic and antibiotic-resistance markers in B. stearothermophilus NUB36. Efficient conditions for the assay and propagation of a phage and transduction were established so that a transducing frequency of 1×10^{-5} to 7×10^{-4} was obtained for temperate phages TP-42 and TP-56. The transducing frequency for virulent phage TP-68 was two to three orders of magnitude lower. Contransfer analyses with the three

phages showed that hom-1 was linked to thr-1 and his-1 is linked to gly-1. The molecular weight of the TP-42, TP-56 and TP-68 genome, as shown by restriction analyses, was 18, 20, and 26 megadaltons, respectively. The difference in the size of the phage genomes was reflected in the different contransduction values obtained with the three phages. A chromosomal DNA protoplast transformation was developed in NUB36. In contrast to traditional transformation systems, the primary transformants were unstable partial or complete diploids. Some of these stabilized as haploid recombinants. Linkage was established between hom-1-thr-1-his-1, his-1-gly-1-pur-1, and pur-1-pur-2. The linkages established by transformation agree with that established by protoplast fusion and transduction. Protoplast fusion and protoplast transformation and transduction will be used to order markers that are not closely linked and to identify linkage groups and fine structure mapping, respectively. A highly efficient PEG-induced plasmid transformation system was developed for NUB3621 Rif Hsr Hsm. (pNW01) using plasmid pLWo5 Cm^r. pLWo5 was a recombinant plasmid derived from mesophilic plasmid pP1401 Cm^r Km and thermophilic cryptic plasmid pNW01. Transformation frequencies were strongly influenced by temperature, Ca^{++}, and molecular weight of PEG. The frequency of transformation was generally between 2 x 10^8 to 2 x 10^8 transformants per μg DNA. Plasmid pLWo5 also transformed competent cells of B. subtilis. Thus, plasmid pLW05 can be considered as a shuttle vector that can replicate both in mesophilic and thermophilic bacilli.

120. OHIO STATE UNIVERSITY - Columbus, OH 43210

Study of the Basis for the Competitiveness of Rhizobium japonicum in the Nodulation of Soybean

W.D. Bauer $80,000

The project objective is to determine the mechanisms by which bacterial chemotaxis and motility contributes to the competitiveness of rhizobial symbionts added as inocula to crop and forage legumes. Previous studies have established that impairment of chemotaxis and motility drastically reduces competitiveness as measured by relative occupancy of root nodules. Our recent studies have shown that hypermotile mutants of rhizobia are more efficient at nodule initiation than the wild-type bacteria. We will isolate and test additional hypermotile mutants to learn whether hypermotility is generally advantageous in nodulation and root colonization. We will also characterize these mutants microscopically and attempt to isolate clones corresponding to the genes responsible for hypermotility. Our studies have also shown that the flavonoid luteolin, present in alfalfa and other legumes, is a specific and potent chemoattractant for Rhizobium meliloti. Luteolin has been shown by others to be a specific and potent inducer of certain nodulation genes in the bacterial symbiont. We will

attempt to determine how the chemotactic and gene induction responses of R. meliloti to luteolin are interrelated. This will be done primarily by identification of the genes and transducer molecules required for the specific chemotactic response. We will also examine the role of specific chemotaxis in root surface colonization by comparing mutants lacking this chemotaxis with the parent.

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

Structure and Regulation of Methanogen Genes
J.N. Reeve $81,000

The goals of this project are to characterize gene structure and the regulation of gene expression in methane producing archaebacteria known as methanogens. We are focusing on histidine biosynthetic genes (hisA and hisI) and methyl reductase (mcrBDCGA) encoding genes from the mesophile Methanococcus vannielii and purine biosynthetic gene (purE) and methyl-viologen (MV) reducing hydrogenase (mvh) encoding genes from the thermophile Methanobacterium thermoautotrophicum. The primary structures and genomic organizations of these genes have now been determined. DNA-dependent RNA-polymerases have been purified from M. vannielii and M. thermoautotrophicum and used in DNA binding-footprinting experiments to identify promoter sequences. Sites of transcription initiation in vivo have been shown to correlate well with the site identified in vitro as RNA polymerase binding sites. Current work is aimed at determining how varying growth conditions regulate the transcription of these methanogen genes in vivo. Analysis of the primary structure of the MV-reducing hydrogenase encoding genes has indicated evolutionary relationships to the F_{420}-reducing hydrogenase of M. thermoautotrophicum and to a hydrogenase present in the eubacterium Desulfovibrio.

122. **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 $55,000

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 within it. As far as we can tell, there is no acetylation of this region in cotton. (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.

123. **UNIVERSITY OF OKLAHOMA** - Norman, OK 73019

Metabolism and Bioenergetics of Syntrophomonas wolfei
M.J. McInerney, Department of Botany and Microbiology $68,000

Anaerobic, hydrogen-producing syntrophic bacteria degrade fatty acids which are important intermediates in anaerobic degradation and methanogenesis. The physiology of one of these bacteria, Syntrophomonas wolfei, was studied in pure culture and in defined, mixed culture. In pure culture, S. wolfei degrades crotonate to acetate, butyrate, caproate and a small amount of hydrogen. Activation of crotonate and the production of butyrate are catalyzed by coenzyme A transferase activities which use or produce acetyl-coenzyme A, respectively. S. wolfei produces large amounts of poly-beta-hydroxybutyrate (PHB) when grown on crotonate. PHB synthesis occurs throughout the exponential phase of growth and is not affected by nitrogen limitation. PHB seems to be made directly from the beta-oxidation intermediate rather than from the condensation of two acetyl-coenzyme A molecules. We have developed a simple method to synthesize uniformly labeled-^{14}C-crotonate by acid hydrolysis of PHB purified from Bacillus subtilis grown with uniformly labeled-^{14}C-glucose. Studies on the effects of short-chain monocarboxylic acids on the growth and metabolism of S. wolfei showed that the build up of these molecules decreases both the rate of butyrate degradation and the growth of S. wolfei. These findings are important for the efficient operation of anaerobic digestors.

124. **OREGON GRADUATE CENTER** - Beaverton, OR 97006-1999

Biochemical Genetics of Lignin Degradation by P. chrysosporium
M.H. Gold, Dept. of Chemical and Biological Sciences $100,000

Lignin, a major component of a woody plant cell walls, is the biosphere's most abundant renewable aromatic polymer. The exploitation of white rot fungi for the biotransformation of lignocellulose into useful products is an important field of research. Phanerochaete chrysosporium, the best-studied lignin-degrading organism, secretes two extracellular heme enzymes--lignin peroxidase and manganese peroxidase--which appear to be largely responsible for the oxidation and depolymerization of lignin. The objective of this project is to understand the enzymology of lignin degradation on the biochemical and genetic levels. We are conducting spectroscopic and transient state kinetic studies on the oxidized intermediates of the peroxidases, and their interconversions, in order to characterize the catalytic mechanisms of these enzymes. We are also conducting spectroscopic studies on the metal centers of these enzymes in order to elucidate structure-function relationships.

In addition, we are studying the molecular genetics of lignin biodegradation. We have sequenced several cDNA clones for manganese peroxidase and genomic clones for both manganese peroxidase and lignin peroxidase are being isolated and characterized. We have also developed the first DNA transformation system for P. chrysosporium, utilizing auxotrophic marker strains. We intend to use this system to study the regulation of the lignin-degrading enzymes and, in conjunction with site-directed mutagenesis, to expand our studies of structure-function relationships of these enzymes at the molecular level.

125. **OREGON GRADUATE CENTER** - Beaverton, OR 97006-1999

Expansion of Bioconversion Technology at the Oregon Graduate Center
M.H. Gold, Dept. of Chemical and Biological Sciences $1,100,000

This expansion will establish a multidisciplinary program for bioconversion research at OGC. At least six independent laboratories will be established for the study of various aspects of the utilization and production of renewable resources. During the first year of this program we have added three new faculty and brief descriptions of their work are included here.

1. Characterization of Laccases from Lignin-Degrading Fungi
N.J. Blackburn

Many wood-rotting fungi secrete extracellular laccases as a component of their lignin degradative system; however, the role of these enzymes in

lignin degradation is not well understood. A major goal of this project is to elucidate the mechanisms of fungal laccases and their roles in lignin degradation. Since the catalytic activity of laccases resides in the copper atoms at the enzyme active center, our initial studies are focusing on the coordination chemistry of these copper centers, utilizing a variety of biophysical techniques. These studies are being coordinated with ongoing biochemical and genetic studies on laccases at OGC.

2. Molecular Biology of Wood Plant Cell Walls
   D.R. Corbin

Several lines of research are being pursued with the aim of understanding the molecular basis of cell wall structure and function in woody plants. We are investigating the molecular biology of cell wall structural proteins, specifically the hydroxyproline-rich glycoproteins (HRGP) of poplar and pine, representatives of angiosperms and gymnosperms, respectively. Our emphasis is on the identification of HRGP genes, elucidation of their structures via gene sequencing, and subsequent analysis of gene expression during development and lignification.

In an effort to understand the genetic control of lignification in tree species, we plan to clone poplar genes which encode those enzymes of the lignin biosynthetic pathway which may have key roles in determining lignin content and composition. The results of preliminary enzymological experiments with lignifying poplar callus cultures will determine which genes we select for further genetic study.

3. Oxidative Enzymes Involved in Cellulose Degradation
   V. Renganathan

Because of their potential applications in the conversion of cellulose to ethanol, the hydrolytic enzymes involved in cellulose degradation by various microorganisms have been subjected to detailed study. However, several cellulolytic fungi, including Sporotrichum pulverulentum (P. chrysosporium), Polyporus versicolor, and Monilia sp. produce extracellular cellobiose-oxidizing enzymes in addition to the hydrolases. Cellulose degradation by S. pulverulentum culture filtrates is stimulated by molecular oxygen, suggesting the direct involvement of oxidative enzymes in cellulose degradation. S. pulverulentum produces three different cellobiose-oxidizing enzymes, two of which have been characterized. One is a hemeflavoprotein-dependent oxidase requiring molecular oxygen for activity. The second enzyme is a flavoprotein-dependent dehydrogenase, requiring quinone as an electron acceptor. We are undertaking a detailed investigation of the structures and mechanisms of all three cellobiose-oxidizing enzymes with the objective of elucidating their roles in the degradation of lignocellulose.

126. **OREGON GRADUATE CENTER** - Beaverton, OR 97006

Chemistry of Ultrathin Membranes and Films
J.K. Hurst, D.H. Thompson, Department of Chemical and Biological Sciences $500,000

The objective of this project is to develop a focused research group to synthesize and physically characterize new organic amphiphilic compounds capable of forming durable ultrathin membranes and films. Molecular designs will be patterned after archaebacterial models; synthetic strategies are modular in concept, allowing rational and systematic investigation of relationships between molecular structure and film/membrane properties. Additional objectives include developing:

(i) An understanding of the molecular forces controlling microphase morphology.

(ii) Physical methods for determining ultrastructural organizations of dopant molecules and structural properties permitting their manipulation.

(iii) An understanding of mechanisms of interfacial and transmembrane dynamics of dopant molecules.

(iv) Novel methods for controlled interfacial deposition of metals and metal oxides.

Physical techniques to be used include a wide range of optical and magnetic spectroscopies, Langmuir-Blodgett film technologies, fast-kinetic methodologies, electron microscopy and low-angle x-ray spectrometry.

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

Genomic Variation in Maize
C. Rivin, Department of Botany and Plant Pathology $55,593
(FY 87 Funds/ 20 months)

We are interested in the molecular basis and biological significance of genomic diversity in plants. We have learned that 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 quite variable in copy number while others are constant. These polymorphisms are stable in inbred lines, but unexpected copy numbers are observed in the progeny of specific outcrosses, tissue culture cells and in regenerated plants. We have also observed variation in cryptic transposons of the Robertson's Mutator system under these circumstances. The project goal is to investigate the molecular and genetic basis for this modulation. Using southern blotting and quantitative DNA:DNA hybridization we are attempting to determine when during plant development, tissue 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 succeeding generations. These experiments will provide new and more comprehensive information on genome plasticity and its implications for the genetic manipulation of a major crop species.

128. 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 $84,000

Plant cell expansion requires coordination of three distinct processes: wall relaxation and synthesis, water uptake, and solute uptake (or synthesis). Wall relaxation reduces cell turgor pressure and thereby generates the reduced water potential needed for water uptake. Our studies with pea (Pisum sativum L.) and soybean (Glycine max Merr.) seedlings have shown that water uptake is rapid and is not a major control point for growth. Our current focus is on the processes of wall relaxation and solute transport, and how they are influenced by water stress. 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 wall relaxation in living cells, using a computer-assisted pressure microprobe and the new pressure-block technique. Our pressure-block results indicate that wall relaxation is more dynamic than expected. Rapid changes in wall yielding appear to compensate for minor fluctuations in cell turgor pressure, thus maintaining stable growth rates. 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. A third goal is to examine the basis for reduced cell expansion when plants are water stressed. Our results indicate that growth is retarded in part because of reduced turgor pressure, and in part because of reduced cell wall relaxation. The alteration in wall relaxation will be examined by in-vivo relaxation methods. These studies will provide insight into the basic cellular and physical processes controlling plant growth, and how they are perturbed by water stress.

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

Enzymology and Molecular Biology of Lignin Degradation
M. Tien, Dept. of Molecular and Cell Biology
$122,000 (FY 87 funds/ two years)

Lignin is an abundant, under-utilized renewable resource. Recent discovery of enzymes which degrade this polymer has intensified interest in the bioconversion of lignin. The goal of the proposed research is to characterize the lignin peroxidases (ligninases) of the wood-destroying fungus Phanerochaete chrysosporium. The research objectives address the fundamental questions on the structure and mechanism of the lignin peroxidase isozymes. More specifically, we will: 1) characterize the kinetics of the reactions of lignin peroxidase with veratryl alcohol and H_2O_2. Both are secreted by the fungus. Their role in lignin degradation will be addressed; 2) characterize the heme active site by electrochemistry, ^{1}H-nmr spectroscopy, and the use of mechanism-based inhibitors; and 3) express the lignin peroxidase cDNAs in E. coli to facilitate our biochemical studies. The rationale for the research is 1) an intimate knowledge of the mechanism of the lignin peroxidases is essential to optimize its utilization; 2) the role of the multiple lignin peroxidases needs to be answered. Physical and kinetic characterization will distinguish the role of the various isozymes. Molecular cloning already has and will yield valuable information on the structure of lignin peroxidase isozymes.

130. **UNIVERSITY OF PENNSYLVANIA** - Philadelphia, PA 19104

DNA Sequences Encoding Chlorophyll a/b Binding Polypeptides
A.R. Cashmore, Plant Science Institute, Department of Biology
$110,000

The polypeptide components of the light-harvesting chlorophyll a/b (CAB) binding protein complex are encoded in nuclear genes and their expression is regulated by light. We have been studying the nature of the cis-acting DNA sequences that mediate this photoregulation. A promoter fragment from a Nicotiana plumbaginifolia CAB gene (Cab E) was fused to the bacterial gene for chloramphenicol acetyl transferase (CAT). The chimeric gene was introduced into tobacco plants where it showed photoregulation. By 5'-terminal and internal deletion studies we have begun to characterize the nature of the promoter sequences that mediate this photoregulation. We have demonstrated the presence of both positive (PRE) and negative (NRE) regulatory elements, that modulate the level of expression mediated by the Cab E promoter. We have also characterized an additional light regulatory element (LRE) that confers photoregulated expression when fused to the constitutive nopaline synthase promoter. Studies aimed at further characterizing these regulatory elements are in progress.

131. **UNIVERSITY OF PENNSYLVANIA** - Philadelphia, PA 19104

Factors Governing Light-Driven Electron and Proton Translocation in Protons Across Membranes

P.L. Dutton, Department of Biochemistry and Biophysics $87,000

Membrane Redox proteins 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. This research is successfully developing 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. This grant is funding vanguard studies on reaction centers from photosynthetic bacteria to develop strategies for deposition of the protein on solid electrode supports in ordered arrays; good progress is being made. Flash-activation of such films followed by measurement of current & voltage in the presence of applied fields ls revealing unique 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; this has now been accomplished with the photosynthetic reaction center. The product of this work will be a molecular level understanding of how biological reaction center protein effects and stabilizes charge separation, and how electrons move through the protein matrix.

132. **PURDUE UNIVERSITY** - West Lafayette, IN 47907

Biosynthesis and Assembly of Cell Wall Polysaccharides in Cereal Grasses

N. Carpita, Department of Botany & Plant Pathology $72,000

We will investigate the biosynthesis and assembly of mixed-linkage β-glucans and glucuronoarabinoxylans (GAX) comprising the hemicellulosic matrix of the primary cell walls of maize tissues and other cereal grasses. Golgi membranes that participate in biosynthesis and transport of these matrix polymers has been enriched away from tonoplast, endoplasmic reticulum, and most of the plasma membrane. A combination of specific endoglucosidases and chemical linkage analysis coupled to radiogas proportional counting will be used to determine the chemical structure of nascent GAX and β-glucans synthesized in vivo, and requirements for the

faithful synthesis of these polymers in vitro with the enriched Golgi membranes will be explored. During the course of this project, use of ^{13}C nuclear magnetic resonance spectroscopy will be evaluated to observe non-invasively the genesis of these soluble precursor hemicelluloses. The goal of this project is to provide biochemical information necessary to examine expression and regulation of specific polysaccharide synthase systems during plant cell development.

133. **RUTGERS UNIVERSITY** - New Brunswick, NJ 08903

Cellulase - A Key Enzyme for Fermentation Feedstocks
D.E. Eveleigh, J.D. Macmillan, Dept. of Biochemistry & Microbiology
$119,776 (20 1/2 months)

Biomass can be fermented to a range of useful products. For efficiency a well characterized and effective cellulase is necessary to gain fermentable sugars from cellulosics (forest by-products and agricultural wastes and municipal refuse). Two cellulases have been further characterized, the classic from Trichoderma reesei and a thermally stable complex from the actinomycete Micro-bispora bispora. Thus there is no direct assay for one of the major cellulase components, cellobiohydrolase (CBH). Immunologic (monoclonal antibody - MAb) approaches have been used for the cellobiohydrolases of each system. With T. reesei, rapid purification has been achieved through MAb affinity chromatography and also a highly sensitive MAb assay for CBH in crude cellulase broths has been developed. For M. bispora, the monoclonal antibodies towards CBH have been prepared and are being used for selection of clones from a M. bispora gene library. Characterization of the other M. bispora cellulase components via rDNA protocols has indicated five endoglucanases in comparison to four found by classical biochemical characterization. One gene (MB1.CEL1) has been sequenced and shows certain homologies with other cellulases from Cellulomonas and Trichoderma. Both immunologic and cloning approaches have been used successfully to yield novel insights into these cellulase complexes.

134. **RUTGERS UNIVERSITY** - Piscataway, NJ 08855-0759

Corn Storage Protein: A Molecular Genetic Model
J. Messing, Waksman Institute $98,000

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 or the reduction of single members is not lethal. Therefore, natural variants exist between different inbreds of corn where individual genes are 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 analyze the zein multigene family. The majority of them are encoded by many gene copies that are frequently clustered and may have arisen from gene amplification and unequal crossing over a repeated block structure central to their coding information. This block 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. We are using these unique zein genes now as reporter genes to study mechanisms of how genes are overexpressed either by gene dosage or by increased accumulation of RNA levels.

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

Biosynthesis of Plant Plasmamembrane Polypeptides
C.J. Lamb, Plant Biology Laboratory
$152,000 (FY 87 funds /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.

136. **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
$72,000

Acclimation of plants with changing light conditions is being studied in phycobilisome-containing plants. The study is an integrated approach combining quantitive assessments of principal proteins of photosystem I, photosystem II, and phycobilisomes with physiological activity. The structural arrangement of the photosystems within the thylakoids are being probed by use of cross-linking reagents. Correlations are being made with specific antisera and gold-protein-A labeling. The stoichiometry of the reaction centers and the antennae is being determined by photochemical techniques. Recent results indicate that the polypeptide composition of photosystem I is highly conserved in red algae, cyanobacteria, and green plants. Photosystem I complexes consisting of four polypeptides (62-68 kDa) have been obtained by cross-linking from cyanobacteria. A phycobilisome-core polypeptide (94 kDa), the terminal pigment involved in energy transfer to thylakoids, has an amino acid composition different than that of a thylakoid-94-kDa polypeptide. This suggests a greater complexity of the phycobilisome-thylakoid interface than anticipated.

137. **SOLAR ENERGY RESEARCH INSTITUTE** - Golden, CO 80401

The Water-Splitting Apparatus of Photosynthesis
M. Seibert, Photoconversion Research Branch $125,000

The light reactions of photosystem II (PSII) in concert with the dark reactions of the water-oxidizing complex supply reductant ultimately used by algae and green plants to fix carbon during oxygenic photosynthesis. The primary interest of this laboratory has been in relating PSII structure to function. Comparison of surface-enhanced Raman scattering properties of PSII-enriched membrane fragments from Scenedesmus wild type and the non-O_2-evolving LF-1 mutant led to the conclusion that the D1 (Q_B) protein (a

component of the PSII reaction center complex) contains a site that binds high-affinity Mn, functional in water oxidation. Additional biochemical studies have shown that the "high-affinity Mn binding site" is composed of two distinct components, one of which cannot be detected in LF-1. The missing Mn-binding component explains why LF-1 does not bind as much Mn as the wild type, cannot be photoactivated, and does not reach S_2 of the S-state cycle. Amino acid blocker experiments show that the missing Mn site in LF-1 is associated with histidine residue(s) on the D1 protein. PSII reaction center (D1/D2/cytochrome b-559) complex isolated from spinach by the Nanba/Satoh procedure was found to be unstable when unfrozen and exposed to room light. Simple spectral and photophysical properties of the reaction center complex were related to its functional competence. A procedure was developed to improve the stability of the isolated reaction center, and this stabilized material was shown to contain the full complement of high-affinity Mn binding site observed in PSII membrane fragments. This work indicates that the site required for binding all high-affinity Mn is restricted to the three proteins that compose the PSII reaction center complex.

138. SOUTHERN ILLINOIS UNIVERSITY - Carbondale, IL 62901

Regulation of Alcohol Fermentation by Escherichia coli
D.P. Clark, Department of Microbiology $84,452

The purpose of this project is to elucidate the way in which the fermentative synthesis of ethanol is regulated in the facultative anaerobe Escherichia coli. We are also investigating the control of other genes required for fermentation and anaerobic growth. We have isolated both structural and regulatory mutations affecting the expression of alcohol dehydrogenase, the enzyme responsible for the final step in alcohol synthesis. Some of these regulatory mutations also affect other anaerobically induced genes. The adh gene has been cloned and is presently being sequenced. The ADH protein is one of the largest in E. coli and requires approximately 3000bp of DNA for its coding sequence. We have also isolated mutations affecting the fermentative lactate dehydrogenase. In consequence it is now possible to construct E. coli strains defective in the production of any one or more of their normal fermentation products (i.e. formate, acetate, lactate, ethanol and succinate). Examination of our collection of anaerobically controlled gene fusions has shown that many can be switched on in air upon treatment with cyanide or certain other inhibitory agents. We are investigating the basis for this effect at present.

139. **STANFORD UNIVERSITY** - Stanford, CA 94305-5020

Host range and other symbiotic genes of Rhizobium meliloti
S.R. Long, Department of Biological Sciences $222,933 (two years)

The Rhizobium bacteria are able to recognize and invade legume plants. Their consequent ability to fix nitrogen in root nodules allows the plants to grow without energy-expensive nitrogen fertilizer. We are researching the basis for the symbiosis between alfalfa (Medicago sativa) and Rhizobium meliloti. We have identified and completed molecular analysis (at the DNA sequence, transcript and protein levels) of four genes needed for recognition of the host plant. One of these, nodH, appears to be required for nodulation of some plants in the alfalfa group, but not for others. Furthermore, the symbiotic requirement for nodH is affected by the physiology of the host plant, for example the exposure of roots to light. Microscopic studies of plants inoculated with host range gene mutants shows that the block to nodulation occurs very early in the symbiotic process. The defect of cells with host range gene mutations cannot be relieved by co-inoculation with cells normal for these genes but mutated in the common nod genes, even at high cell concentrations. This indicates that the products of common nod genes and of the host range nod genes interact within the same bacterial cell. We are pursuing analysis of protein action of the existing genes, and also have defined and begun molecular analysis of two new genes which are adjacent to the original four host range genes.

140. **VIRIGINIA POLYTECHNIC INSTITUTE & STATE UNIVERSITY**
Blacksburg, VA 24061

Enzymology of Acetone-Butanol-Isopropanol Formation
J.-S. Chen, Department of Anaerobic Microbiology $71,000

Acetone, n-butanol, and isopropanol ("solvents") are important industrial chemicals and fuel additives. Several Clostridium species produce butanol as a major product, and Clostridium beijerinckii (including strains formerly known as Clostridium butylicum) produces all three compounds, with some strains producing mainly butanol and isopropanol. Industrial solvent fermentation is limited by several biological factors. Efforts aimed at alleviating these limitations require an understanding of these organisms and their solvent-producing machinery. This project focuses on the elucidation of the molecular properties of solvent-forming enzymes and the control mechanism for the expression of solvent-producing genes. We have shown that a primary alcohol dehydrogenase is present in acetone/butanol-producing C. beijerinckii, whereas a structurally distinct primary/ secondary alcohol dehydrogenase is present in butanol/isopropanol-producing C. beijerinckii. The primary/secondary alcohol dehydrogenase has been puri-

fied, and its kinetic and structural properties have been characterized. This enzyme could serve as a site for the regulation of the product ratio. In solvent production, the conversion of acetoacetyl CoA to acetoacetate is a key reaction. In C. bejerinckii, acetoacetyl CoA hydrolase activity is present in addition to coA transferase activity for this reaction, which suggests additional possibilities for the regulation of the product ratio. Purification of the acetoacetate-forming enzymes is in progress. Using solvent-forming enzyme activities as an indicator, the transition from acid production to solvent production is now detected two generations before solvent production is detected. The result will help identify conditions pertinent to the initiation of the solventogenic process.

141. **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 $78,458

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 an 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. Membrane components are implicated in electron transport from the ferredoxin to methylcoenzyme M. 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. 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 electron transfer components. 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.

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

Unravelling Lignin Formation and Structure in Living Plants
N.G. Lewis, Departments of Biochemistry, Wood Science and Forest Products $82,000

Vascular plants have evolved with a unique capacity to produce lignin, a complex phenylpropanoid polymer which performs essential structural and defense functions in plants. Because of its intractable nature, little is known about lignin bonding patterns in situ. Consequently, representations of lignin structure rely heavily upon the analysis of isolated lignin-derived materials and synthetic DHP polymers produced in vitro from E-monolignols by the action of H_2O_2 and peroxidase. Recently we were able to demonstrate that the bonding patterns of these phenylpropanoid polymers could be investigated in situ, by first growing T. aestivum plants in the presence of specifically-labeled (C-13 enriched) lignin precursors, and then examining the resulting intact plant tissue by solid state ^{13}C nuclear magnetic resonance spectroscopy. We are now developing this methodology to probe lignin bonding patterns in woody plant tissue, as well as establishing changes that the lignin macromolecule undergoes during delignification.

Mechanisms controlling lignin monomer transport into the cell wall, and subsequent initiation of lignification are not understood. In this study, 3we are identifying the mechanisms by which a) hydroxycinnamic acids are esterified to cell walls, b) Z-monolignols and their glycosides are formed in Fagus grandifolia and c) monolignols, or their conjugates, are transferred from the cytoplasm to the cell wall.

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

Genetic Engineering with a Gene Encoding a Soybean Storage Protein
R.N. Beachy, Department of Biology $76,000

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-subunits were introduced into transgenic tobacco and petunia plants via a disarmed Ti-plasmid in Agrobacterium tumefaciens. Each of the genes was expressed only in developing seeds in a temporal manner consistent with its expression in soybean seeds (e.g. the α'-gene is expressed early and the β-gene is expressed late in seed development). The DNA-sequence element that causes the elevated level of expression of the α'-gene, relative to the β-gene, was located between nucleotides -257 and -60 upstream (5') of the start of transcription of the α'-gene. This sequence element has the capacity to alter the level of gene expression of the 35S promoter, a strong constitutive promoter, and causes enhanced transcription from this promoter in

developing seeds transgenic plants (The EMBO J. 7,2:297.302, 1988). We are extending the characterization of the DNA sequence elements located 5′ to the α′ and β-subunit genes by identifying those that bind protein factors isolated from nuclei of seeds. Because it appears that there are several different DNA sequence elements, each of which bind different protein factors, each element will be tested for in vivo function by constructing chimeric reporter genes that contain one or more of the sequence element. The goal of these experiments is to identify the sequences of the α′ and β-subunit gene promoters that control the quantitative and temporal expression of these genes.

144. **WASHINGTON UNIVERSITY** - St. Louis, MO 63110

Processing and Targeting of the Thiol Protease, Aleurain
J. C. Rogers, Division of Hematology-Oncology $127,124

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, about 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.

145. **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 (FY87 funds/ two years)

It is of interest 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. We are evaluating a method for separating influx and efflux in steady state systems using isotopic fractionation data. Nitrate or ammonium, after entering a cell, will either leave the cell or be chemically transformed. The three relevant processes (influx, efflux and chemical transformation) each have an associated rate constant and kinetic N isotope effect. The observed N isotope effect on the disappearance of nitrate or ammonium from the external medium (βobs) is a function of the isotope effects associated with these processes and on the ratio of their rates. We are measuring variation of βobs in Anacystis nidulans incubated under a series of experimental conditions which alter the net uptake (influx minus efflux) of nitrate and ammonium. We will then measure the values of the appropriate kinetic isotope effects. This will allow us to separately assess the influx and efflux components of net uptake.

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

Hydroxyproline-Rich Glycoproteins of the Plant Cell Wall

J.E. Varner, Biology Department

$107,000

The cell walls of plants, particularly of dicots, characteristically contain glycoproteins rich in hydroxyproline. It is presumed that the plant glycoprotein(s)-extensin(s) has an important structural/developmental role. We are checking this presumption. Towards this end we have 1) isolated and purified one hydroxyproline-rich glycoprotein from aerated carrot slices, 2) characterized this glycoprotein as an extended polyproline II helix -- it is a rod 80 nm long and visible by electron microscopy, 3) isolated 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. The transcripts from this gene increase in abundance following wounding, 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 - Tyr Thr Pro Pro Val. We have characterized an hydroxyproline-rich protein that is also rich in proline, threonine and

lysine from developing maize pericarp and we have developed a tissue print technique that allows rapid and inexpensive cellular localization of soluble extensins. Current work is centered on the chemistry responsible for insolubilizing extensins in the cell wall.

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

Regulation of Terpene Metabolism
R. Croteau, Institute of Biological Chemistry $82,000

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

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

D-Erythroascorbic Acid: Its Preparations, Chemistry, and Metabolism (Fungi and Plants)
F.A. Loewus, Institute of Biological Chemistry
$146,820 (two years)

In plants, L-ascorbic acid (AA) is closely associated with developmental processes of growth although functional aspects of this association need to be elucidated. Products of AA metabolism include L-threonic, L-tartaric, and oxalic acids. Yeasts and fungi contain an AA-like constituent which

has been identified as D-erythroascorbic acid or D-glycero-pent-2-enono-1,4-lactone (EAA) but AA is absent or present in very low amount. EAA has been isolated from Saccharomyces cerevisiae, Lypomyces starkeyi, and Neurospora crassa. Methods for the preparation of EAA in quantity sufficient for metabolic studies in yeasts, fungi and plants are underway in collaboration with Paul A. Seib, Department of Grain Science, Kansas State University, Manhattan, KS 66506. Eventually, specifically tagged, radiolabeled EAA will also be prepared. Particular attention will be given to the possible role of EAA as a precursor of oxalic acid formation in fungi in analogy to that of AA in oxalate-accumulating higher plants. In this regard, model studies will be undertaken on the peroxidative cleavage of EAA to oxalate and D-glyceric acid, and its putative role as a lytic agent during fungal attack by oxalate-producing pathogens. Efforts will also be directed toward elucidation of EAA biosynthesis which offers a possible model for AA biosynthesis in higher plants. The presence of EAA rather than AA in yeasts prompts interesting questions such as: Is EAA a substitute for AA in certain biological systems? Is it an antagonist of AA? What are its nutritional properties? Are yeasts a potential source of EAA on a commercial basis? Are there uses for EAA in technical and biotechnical processes? Results from this study will be used to examine such issues.

149. **WASHINGTON STATE UNIVERSITY** - Pullman, WA 99164-4660

Isocitrate Lyase and the Glyoxylate Cycle
B.A. McFadden, Biochemistry/Biophysics Program $74,500

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

We have completed cloning and sequencing the isocitrate lyase gene of Escherichia coli and will communicate our results for publication. In this publication, the sequences of the subunit of isocitrate lyase from E. coli and castor bean will be compared. In the project period the amino acid sequences of four active-site peptides from the E. coli enzyme will be elucidated. Mutagenesis of the E. coli gene will be directed towards functional residues that are conserved in the castor bean enzyme to test our postulated catalytic mechanism.

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

150. 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 $62,000

Starch biosynthesis is regulated by the gene activation and expression of ADPglucose pyrophosphorylase, starch synthase and branching enzyme as well as by the allosteric behavior of ADPglucose pyrophosphorylase. In spite of the pivotal role of ADPglucose pyrophosphorylase in controlling the level of photosynthate into starch, little is known about its gene structure and the factors that control its expression during the development of storage tissues. Our research has focused on the pyrophosphorylase genes expressed specifically in the storage organs of rice endosperm and potato tubers. Recombinant DNA clones have been isolated for both the mRNA transcript and genomic sequences of rice. From analysis of a near full length cDNA clone, the encoded protein displays a molecular size of 55,000 and shares significant homology with the ADPglucose pyrophosphorylase of Escherichia coli, particularly around the putative catalytic and allosteric sites of the primary sequence. Analysis of genomic clones revealed that the pyrophosphorylase gene possesses a complex structure; it spans over 7,000 base pairs (bp) and contains at least 9 introns varying in lengths from 75 bp to over 2000 bp. Current efforts are centered at elucidating the complete DNA sequence of this gene. Studies have been initiated on the pyrophosphorylase from potato tuber. The in vitro translation product is 54,000 daltons, about 4,000 daltons larger than the mature protein. Efforts are currently directed at the construction of a tuber cDNA library and identification of pyrophosphorylase DNA sequences. Results from these studies will lead to the elucidation of the tuber specific gene and identification of cis-acting regulatory elements responsible for the expression of this gene during somatic tissue differentiation. Overall, this research will facilitate efforts to improve the catalytic conversion of photoassimilates into starch by molecular alteration of the allosteric site of ADPglucose pyrophosphorylase enzyme.

151. UNIVERSITY OF WASHINGTON - Seattle, WA 98195

Studies on the Control of Plant Cell Enlargement by Cellular Parameters
R.E. Cleland, Department of Botany $77,000

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 calicum 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.

152. UNIVERSITY OF WASHINGTON - Seattle, WA 98195

RNA Polymerase III Transcription in Higher Plants
B.D. Hall, Department of Genetics $72,000

Wheat embryo nuclei prepared by the procedure of Guilfoyle et al. (Plant Mol. Biol. 7:95 [1986]) are active in Pol III transcription, as these authors have shown. Pre-tTNA formation by wheat nuclei is inhibited 10 to 20 fold by heparin at a concentration of 0.125 µg/ml, indicating the occurrence of transcription reinitiation during the incubation. By the addition of 0.5% Triton X-100, chromatin is released from nuclei. Upon incubation in a suitable reaction mixture, pre-tRNA and 5S rRNA transcripts are produced and reinitiated from the chromatin. Neither whole nuclei nor isolated chromatin are stimulated in their transcription by added amounts of RNA polymerase III isolated from wheat germ. In order to establish a template-dependent Pol III system from wheat, high salt extracts have been made of wheat embryo nuclei and of chromatin derived from the nuclei. Neither extract is active with added tDNA template. When added to an active Pol III system from yeast, these wheat nuclear extracts cause inhibition. The inhibitory activity appears not to be a hydrolase of any type (DNAase, RNAase, phosphatase) but rather has its effect by preventing pre-initiation complex formation between yeast τ factor and DNA. Further characterization of this inhibitor is in progress.

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

Rhizobium meliloti Exopolysaccharides Required for Nodule Entry
J.A. Leigh, Department of Microbiology $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 of a specialized root structure, the nodule. Rhizobium then enters the cells of the nodule and fixes nitrogen. We are interested in an extracellular polysaccharide, produced by Rhizobium, which is required for nodule entry. We have found that R. meliloti mutants which produce the polysaccharide but fail to enter nodules, actually produce an abnormal form of the polysaccharide which lacks succinate. Recently we have discovered a second polysaccharide which can be produced in place of the first polysaccharide and also satisfy the requirement for nodule entry. We are studying the genetic regulation of the production of the two polysaccharides. Using a variety of approaches, we hope to determine the exact function(s) of the polysaccharides in nodule entry.

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

Enzymology of Biological Nitrogen Fixation
R.H. Burris, Department of Biochemistry $79,487 (two years)

We have demonstrated, with the aid of $^{15}N_2O$, that N_2 is a competitive inhibitor of N_2O when N_2O serves as a substrate for nitrogenase. Acetylene is a noncompetitive inhibitor of N_2O, but N_2O is a competitive inhibitor of acetylene reduction. N_2O at 40 atm almost completely inhibits H_2 production by nitrogenase, whereas it was shown earlier that 51 atm N_2 still allows 27% of the electron flow from nitrogenase to go to H_2 production. There always is a burst in H_2 production when nitrogenase activity is initiated regardless of whether the substrate is N_2 N_2O, C_2H_2 or azide. The moles of H_2 in the burst corresponded closely to the moles of Mo in the dinitrogenase of the reaction mixture. Azospirillum brasilense, A. amazonense and A. lipoferum differed both qualitatively and quantitatively in their response to various amino acids. The nitrogenases of A. brasilense and A. lipoferum are inhibited completely by 2 kPa (about 0.02 atm) of O_2. In contrast to "switch off" by NH_4^+, the effect of O_2 does not appear to involve a covalent modification of dinitrogenase reductase. Covalent modification is controlled by the draG (activating) and draT (inactivating) genes. The genetic system has received considerable study in Rhodospirillum rubrum and we have initiated studies of the system in the azospirilla. The draG gene from A. lipoferum was identified on a 10 kb EcoRI fragment and homology was demonstrated to R. rubrum. However, the

position of the genes on the DNA differs markedly between R. rubrum and A. lipoferum. Isotope discrimination favors ^{14}N over ^{15}N in the root nodules by leguminous plants but we have been unable to obtain convincing evidence that rapidly-fixing Azolla Anabaena azollae associations have a higher ^{15}N concentration than slowly-fixing associations.

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

Molecular Genetics of Ligninase Expression
D. Cullen and K. Kirk, Department of Bacteriology
$122,000 (FY87 funds/ two years)

Knowledge of ligninase biochemistry and its applications in biopulping, biobleaching, and effluent treatment have advanced considerably in the past five 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 five 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 is being developed. In this project, we have cloned and sequenced several lignin peroxidase genes from P. chrysosporium. and we are currently studying their expression and regulation in a model system, Aspergillus nidulans.

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

Role of Transit Peptides in the Proper Localization of Nuclear-encoded Chloroplast Proteins
K. Keegstra, Department of Botany $77,000

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. Past efforts have focused on the precursors for ferredoxin and plastocyanin; proteins located in the stromal space and the thylakoid lumen respectively. Genes for precursor proteins destined for the chloroplast envelope membranes are being isolated and characterized. The role of the transit peptides is being examined by generating hybrid precursor proteins containing the transit peptide from a precursor destined for one location fused to the mature peptide destined for a different location. In vitro import followed by chloroplast fractionation is used to determine whether the transit peptide influences the ultimate location of the polypeptide.

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

The Role of Proteolytic Enzymes in Degradation of Plant Tissue
A. Kelman and L. Sequeira $54,000

Recent studies have emphasized the importance of hydroxy-proline rich glycoproteins (HPRG's) in the structure and function of plant cell walls. Most studies on tissue maceration have been concerned with the role of pectic enzymes, but the possibility exists that degradation of cell walls also involves the action of proteases on the HPRG's acting in concert with the other cell-wall degrading enzymes. The primary objective of this research project is to determine whether the proteolytic enzymes produced by many soft rot bacteria and strains of Erwinia carotovora in particular have an important role in the degradation of plant cell walls and thus may be directly or indirectly involved in pathogenesis. Proteolytic enzymes will be purified and characterized. Purified enzymes will be used to determine how depletion of HPRG's in cell walls is correlated with loss in tissue integrity and how proteases may enhance the activity of other tissue-macerating enzymes. As one phase of this project, transposon mutagenesis has been used to introduce Tn5 mutations into a rifampicin-resistant strain of E. carotovora subsp. carotovora. Mutants deficient in protease and pectate lyase have been obtained and will be used in further studies to analyze patterns of tissue maceration and degradation of specific cell wall components.

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

Organization of the R Chromosome Region in Maize
J.L. Kermicle, Laboratory of Genetics $122,000 (two years)

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 of more functionally independent units (R genic elements) which differ in their spectrum of tissue-specific effects. Mutagenesis, with tests of complementation and recombination 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.

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

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

Rhodospirillum rubrum is capable of carbon monoxide oxidation to CO_2 and assimilation of this carbon to cellular material. This process also causes the in vivo evolution of H_2 and thus is analogous to the water-gas reaction carried out industrially. The oxidation of carbon monoxide by Rhodospirillum rubrum and other microorganisms plays a major role in the carbon monoxide cycle on earth. The carbon monoxide dehydrogenase enzyme has been isolated in its holo form containing iron, sulfur, nickel and zinc and in an apo form lacking nickel. The apo form can be activated in vivo and in vitro by the addition of nickel. An enzymatically incompetent form of the enzyme can be generated by substituting cobalt in place of nickel in the activation.

The enzyme is induced specifically by the presence of carbon monoxide in the medium and is specifically repressed by the presence of oxygen in the medium. The mechanism of these inductions and repressions are unknown and a goal of this research is to understand how dissolved gases can effect changes in gene expression in this microorganism. The in vitro activated 2enzyme will be investigated enzymologically and spectroscopically to determine the role of iron and nickel in this enzyme mechanism. Other metals such as cobalt will be substituted and these alternate forms of the enzymes will be investigated as well. The path of carbon monoxide assimilation

into the cell will be studied and the gene for carbon monoxide dehydrogenase and related activities will be isolated, mutagenized in vitro and used to generate mutants of these gene products in Rhodospirillum rubrum.

160. UNIVERSITY OF WISCONSIN - Madison, WI 53706

Starch Synthesis in the Maize Endosperm as Affected by Starch-Synthesizing Mutants
O.E. Nelson, Department of Genetics $78,650

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.

161. UNIVERSITY OF WISCONSIN - Madison, WI 53706

Carbon Isotope Fractionation in Plants
M.H. O'Leary $74,000

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 carboxylase in C_3 photosynthesis. We are studying the relative roles of diffusion and carboxylation in nocturnal CO_2 fixation in CAM plants. We are studying the use of isotopic content as an index of water-use efficiency in C_3 plants. We are developing new methods for studying carbon metabolism in plants.

162. UNIVERSITY OF WISCONSIN - Milwaukee, WI 53201

Mechanism of formation of the carboxyl of acetate by acetogenic bacteria

S.W. Ragsdale, Department of Chemistry $85,000

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

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

163. UNIVERSITY OF WISCONSIN - Madison, WI 53706

Gas Exchange Characteristics of leaves as Indicators of the Basic Limiting Factors in Photosynthesis
T.D. Sharkey, Department of Botany $69,827

The response of photosynthesis to light, CO_2 and O_2 is studied by measuring the exchange of gases (water vapor and CO_2) between the atmosphere and plant leaves. These measurements are combined with simultaneous measurements of fluorescence, fractionation of stable carbon isotopes, and measurements of metabolite levels and enzyme activities on leaf sections freeze-clamped during gas analysis measurements. The purpose of this research is to identify those physical and chemical factors which limit and regulate photosynthesis.

In the past year new fluorescence techniques (Developed by U. Schreiber, Wurzburg, FRG) have been used to investigate O_2 insensitive photosynthesis. The prediction that electron transport rates are reduced at high CO_2 when photosynthesis is O_2 insensitive was confirmed. The occurrence of O_2 insensitive photosynthesis was tested for and found under natural conditions. Plants were grown at high CO_2 to determine the adaptability of starch and sucrose synthesis. It was found that plants varied in their ability to adapt to increased availability of sugars produced by photosynthesis. We have also characterized a mutant plant deficient in one of the enzymes required for sucrose synthesis. In the coming year we will continue the biochemical investigations and take up investigations of the biophysical limitations on the rate of photosynthesis.

164. UNIVERSITY OF WISCONSIN - Madison, WI 53706

Molecular Mechanism of Energy Transduction by Plant Membrane Proteins
M.R. Sussman, Department of Horticulture $73,000

Proton pumps are membrane-bound proteins that act as biological energy transducers. The plasma membrane of plants and fungi contains an ATP-coupled proton pump (H^+-ATPase) that converts chemical into electrical energy. This enzyme is essential for the growth of plants and fungi and provides the driving force used to catalyze the uptake and accumulation of solutes. The plant/fungal plasma membrane H^+-ATPase contains a single polypeptide of Mr=100,000. It's simple polypeptide structure makes this enzyme an attractive candidate for studying the molecular basis of energy transduction. The aim of this project is to identify aspects of the enzyme's primary structure that are essential for converting chemical into electrical energy. Protein modification and sequencing techniques are used to study the structure of a hypothetical protein transport 'channel' as

well as to identify essential amino acids in the enzyme's other active sites. DNA cloning and sequencing techniques are used to obtain the complete amino acid sequence for ATPase structural genes present in higher plants. Based on chemical modification and computer-assisted sequence studies, amino acids that are predicted to be important in the catalytic mechanism are tested for essentiality through site-directed mutagenesis and expression of cloned genes in Saccharomyces cerevisiae. These studies provide data necessary for generating and testing hyptheses concerning the molecular mechanism of protein-mediated proton conduction and energy transduction.

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

Mechanism for the Selective Conjugation of Ubiquitin to Phytochrome
R.D. Vierstra $71,000

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

166. YALE UNIVERSITY - New Haven, CT 06511

Molecular cloning and structural characterization of the R locus of maize

Stephen L. Dellaporta, Department of Biology

$50,000 (eight months)

The R locus of maize has been implicated in the regulation of anthocyanin pigmentation patterns in the seed and plant. Many alleles of R are comprised of multiple tissue-specific genes, called components, that are highly related at the DNA sequence level. Our objectives have been to characterize the structure of the R locus of maize and to determine the basis of tissue-specific regulations of pigmentation patterns. During the last project period, we have completed the cloning of genomic fragments of several tissue-specific R components including the plant (P), seed (S), Navajo (Nj), and self-color (Sc) components. The (P) and (S) component comprise the standard allele of R-r. We are currently attempting chromosome walking techniques to obtain genomic clones representing the complete R-r allele to determine the structure and orientation of (P) and (S) compo- (Sc) component to determine the fine structure (promoter sequence, exons, introns, etc.) of a single R gene. By analysis of genomic sequences related to R, we have cloned the B locus of maize, a second locus implicated in anthocyanin regulation. The structural relationship between the R and B loci is currently under study.

167. YALE UNIVERSITY - New Haven, CT 06520

Control of Genes Encoding Catabolic Enzymes in Bradyrhizobium

D. Parke and L.N. Ornston, Department of Biology $77,000

The goal of this project is to understand energy metabolism and gene control in bacteria of the genus Bradyrhizobium. These bacteria are versatile purveyors of metabolic energy, being capable of fixing nitrogen in symbiosis with legumes and of surviving on low levels of nutrients. Although the organisms are relatively fastidious with respect to such growth substrates as carbohydrates they grow at the expense of a wide range of monocyclic phenolics which originate from lignin and plant root exudates. Diverse phenolic compounds are broken down in bacteria by metabolic pathways that converge on the beta-ketoadipate pathway. Inducible in all other microbes studied to date, enzymes of the beta-ketoadipate pathway are expressed constitutively in Bradyrhizobium. One enzyme in particular, beta-ketoadipate succinyl CoA transferase, a product of the pcaE gene, is expressed at high levels in saprophytic and symbiotic Bradyrhizobium. The properties of this enzyme will be studied in an effort to understand the physiological basis for its high constitutive expression. The investiga-

tion will also analyze whether unregulated enzyme synthesis is a feature of other peripheral catabolic pathways in Bradyrhizobium. The selective basis for the observed lack of regulation will be examined by a number of approaches, including isolation of mutant strains blocked in the catabolism of plant phenolics. The mutant strains will be used to determine whether the ability to metabolize phenolics is a factor in survival of Bradyrhizobium in the soil, in its proliferation in the rhizosphere, or in competition for nodulation.

168. **YALE UNIVERSITY** - New Haven, CT 06520

Mechanisms and Control of K^+ Transport in Plants and Fungi
C.L. Slayman, Department of Cellular and Molecular Physiology
$212,000 (two years)

The purpose of this proposal is a comprehensive study of potassium transport mechanisms in plants and fungi. It will have three distinct components:

I) Exploration of the mechanisms for K^+ transport in plant protoplasts, particularly those of Arabidopsis thaliana. This will be a descriptive, cell physiological study designed to map out the systems present (i.e., pumps, cotransporters, and channels (?)) in relatively undifferentiated cells, and describe their behavior under conditions of limited stress (e.g., K^+ starvation or overloading, hormone perfusion).

II) Cloning and sequencing of the gene for the high-affinity K^+-H^+ symporter in Neurospora. These molecular biological experiments will provide a crucial tool for future manipulation of transporter structure and function.

III) Continued functional analysis of the high-affinity K^+-H^+ cotransport system in Neurospora. This is mainly a biophysical study in which variations of cytoplasmic ion concentrations will be explored in relation to overall transport and electrical kinetics, in order to construct a comprehensive kinetic model of the cotransport mechanism.

The main goal of the experiments is to understand the mechanisms of potassium transport in a model fungus and a model plant, at both cellular and molecular levels. As the predominant cytoplasmic cation, potassium's status is necessarily an important feature of any physiological process involving cell volume or turgor, including the environmental stresses of high salt, dehydration, chemical assault, and extreme temperature. Therefore, the knowledge of both techniques and mechanisms emerging from the proposed experiments should in the future permit specific manipulation of the stress responses of plants and fungi.

169. YALE UNIVERSITY - New Haven, CT 06511

Transfer RNA Involvement in Chlorophyll Biosynthesis
Dieter Soll, Department of Molecular Biophysics & Biochemistry
$87,001

Chlorophyll is the major pigment in photosynthesis. One molecule of chlorophyll is synthesized from eight molecules of δ-aminolevulinic acid, the universal precursor of porphyrins. Chlorophyll synthesis is regulated at the synthesis of δ-aminolevulinic acid, which is formed in the stroma of greening plastids from glutamate. This pathway differs from δ-aminolevulinic acid formation in bacteria, fungi or mammals, where it is formed from glycine and succinyl-coenzyme A. The mechanism of δ-aminolevulinic acid synthesis is still poorly understood. A solid body of evidence has accumulated to show that at least in barley, Synechocystis 6803 and Chlamydomonas it involves the reduction of glutamate to glutamate-1-semialdehyde which is subsequently converted to δ-aminolevulinate. Studies on the in vitro synthesis of δ-aminolevulinate in barley chloroplast extracts showed a specific $tRNA^{Glu}$ to be involved in this process. Glutamate is attached to the tRNA via an aminoacyl bond by the regular glutamyl-tRNA synthetase. In the subsequent reduction of glutamate to glutamate semialdehyde the tRNA is required as a specific "cofactor". $Glu\text{-}tRNA^{Glu}$ species from other organisms or cytoplasmic ones do not efficiently substitute for the homologous RNA in this reaction. The goal of these studies is to clone and characterize the genes for the enzymes involved in this process and to investigate in detail the mechanism of action and regulation of these molecules. As experimental systems we shall use the cyanobacterium Synechocystis 6803 and barley. These studies should uncover novel principles regarding the role of tRNA as a cofactor in metabolic conversions.

INVESTIGATOR INDEX

SUBJECT INDEX

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

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