DOE/ER-0291

Annual Report and Summaries of FY 1986 Activities Supported by the Division of Biological Energy Research

September 1986

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

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Washington, D.C. 20545

PROGRAM OVERVIEW

The Biological Energy Research (BER)* program of the Office of Basic Energy Sciences is devoted to discovering and describing biological mechanisms that might be employed as the basis of future energy-related biotechnologies. Clearly, studies on green plants as producers of renewable resources are a key aspect of the BER program. Plant productivity encompasses a broad array of processes that are integrated to form the complex entity, a terrestrial or aquatic plant. The comprehension of these component processes and their regulation is one of the major objectives of the program. Another major aspect of the BER program examines that portion of the microbiological world involved with biochemical transformations that yield fuels and chemicals. These bioconversion processes can provide a substitute for fossil energy resources directly or through energy conserving measures in which biological mechanisms displace industrial energy inputs. In each instance, the research is aimed at understanding the fundamental mechanisms of conversion rather than a description or optimization of a process.

Since the BER broad program scope is much the same as last year, the list of research topic areas is largely unchanged. Topic areas covered in the **plant science** segment of the BER program include:

A. **Bioenergetic Systems**, including photosynthesis, the major solar energy conversion process and other energy transformation mechanisms.

B. **Control of Plant Growth and Development**, the series of processes that often determine how much solar energy is ultimately captured and converted into a chemical form (as fixed carbon) and how the fixed carbon will be utilized. The regulatory processes are analyzed in terms of biochemical and physiological mechanisms.

   1. *Metabolic Regulation*, the mechanisms by which the pathways are controlled directly by affecting the levels and/or activity of the enzymes involved, e.g., feed-back inhibition, and enzyme modification.

   2. *Genetic Regulation*, the mechanisms by which the genetic material controls the modulation of a pathway by turning it on or off, and other physiological genetic control mechanisms.

*The Biological Energy Research program has a Department of Energy budget designation of Energy Biosciences; there should be no confusion, however, because the activities under the two names are identical.

3. *Hormonal and Environmental Regulation*, the mechanisms by which plant growth substances and external signals (e.g., light) affect the control of activities in cells or organs and ultimately the whole plant.

C. **Stress Response Mechanisms**, to temporal and long-term exposure to natural suboptimal environmental conditions which affect solar energy conversion in plants and ultimately the yield of renewable resources.

D. **Genetic Transmission and Expression** in plants, knowledge that ultimately will allow the development of new and unique plant materials that represent improvements in the production of renewable resources.

E. **Plant-Microbial Interactions**, an area covering the basis of symbiotic and pathogenic relationships that bear on the efficiency of plant production, either positively or negatively.

F. **Plant Cell Wall Structure and Function**, which includes the understanding of the chemical makeup, synthesis and degradation of the most abundant reservoir of fixed carbon, and the emerging insights into the physiological activities of cell wall constituents.

The **microbiological** aspects of the BER program emphasize a number of research areas including:

G. **Lignocellulose Degradative Mechanisms**. Comprises studies on the genetic and biochemical control of the polysaccharide-degrading enzymes and the breakdown of lignin components. These areas are aimed at understanding and eventually being able to develop controlled microbial or enzymatic systems for the degradation of these abundant renewable materials.

H. **Mechanisms of Fermentations** probing the nature and metabolic regulation of the bioconversion of relatively abundant substrates into organic acids, fuels, and solvents.

I. **Genetics of Neglected Microorganisms** involved in bioconversions and other functions of interest. These studies involve attempting to define genetic systems in organisms that have been generally neglected in research. Examples are anaerobic microorganisms and those involved in plant-microbe interactions.

J. **Energetics and Membrane Phenomena** in response to conditions of stress (e.g., high temperature, high salinity, factors frequently encountered in bioreactors or in nature).

K. **Thermophily and Thermotolerance** are studied to determine the molecular basis for these phenomena.

L. **Microbial Ecology Associations**, includes microbe-microbe interactions involved in mixed culture fermentations and other consortia as well as some microbiological interactions with plants.

M. **One-Carbon Metabolism**, covering the probing of biotransformations such as **methanogenesis** and other key processes found in nature in which large quantities of one-carbon compounds (e.g., carbon dioxide, carbon monoxide), may yield fuels or chemicals of interest.

While the topic areas listed above portray the major thrusts of the BER program, other types of studies are also included.

The abstracts contained in the pages of this report which have been submitted by the investigators should give the reader an accurate impression of the scope and flavor of the program. Two representative illustrations of the kinds of advances occurring within the program follow:

- A key enzyme (1-aminocyclopropane-1-carboxylate synthase) in the pathway of synthesis of the plant growth regulator, ethylene, has recently been purified and isolated. Ethylene is involved with both normal development of plants and responses to physical and biological stresses making the control of its synthesis a crucial question in understanding plant growth. The enzyme which occurs as only one ten-thousandth of a percent of the total protein complement was successfully purified by chromatography and later isolated using monoclonal antibody techniques. This achievement opens the way now to applying molecular biological probing of the question of how the gene for this pathway is turned on and off by physiological factors.

- In recent years, investigations have begun to define the biologically mediated breakdown of lignin. An enzyme with "ligninase" activity has been isolated from a wood rotting fungus. Using current molecular biological techniques, it has been possible to genetically transfer the "ligninase" gene from the fungus to a bacterium where it is expressed in the production of new copies of the enzyme. This accomplishment not only permits a detailed structural analysis of the gene and the enzyme product, but also opens the way to do various modifications to enhance the level of ligninase activity as a prelude to potential developments on a commercial scale of utilizing this second most abundant renewable resource.

As an endeavor, science is a dynamic entity with ever changing horizons. The BER program, to stay abreast of the most current technical developments, uses workshops and other conferences for identifying critical research related to its mission as well as to encourage dissemination and discussion of results. During FY 1986 BER provided support for the following activities:

1. Partial support for organization of the "VII International Congress on Photosynthesis" August 10-15, 1986, at Brown University, Providence, Rhode Island. (Proceedings to be published.)

2. Partial support for the 9th Annual University of California-Riverside Symposium in Plant Physiology, January 1986, Riverside, California. (Proceedings to be published.)

3. Partial support for "Symposium on Current Topics Biochemistry and Physiology," April 1986, University of Missouri, Columbia. (Proceedings to be published.)

4. Partial support of the "Seventh International Symposium on Structure and Function of Plant Lipids", July/August 1986, University of California, Davis, California.

5. Partial support of Conference on "Extrachromosomal Elements in Lower Eukaryotes", June 1986, University of Illinois, Urbana, Illinois. (Proceedings to be published.)

6. Partial support of "Third International Symposium on Plant-Microbe Interactions", July 1986, Montreal, Canada. (Proceedings to be published.)

7. Partial support of the symposium "The Mitochondrion", June 1986, Johns Hopkins University, Baltimore, Maryland. (Proceedings to be published.)

8. Partial support of "International Workshop on Higher Plant Mitochondrial DNA", October 1986, Airlie House, Airlie, Virginia. (Proceedings to be published.)

9. Workshop on the need for and characteristics of a computerized data base for structural information on complex carbohydrates, August 1986, Auburn, New York. (Report to be issued.)

In December of 1985 a workshop was convened at the American Chemical Society's Belmont Conference Center as part of the continuing program effort for quality assurance and to ascertain whether the program is oriented toward appropriate scientific directions. Such research directions workshops are convened triennially and involve as participants highly respected researchers from a variety of fields who have no affiliation with the program. There was general agreement among the participants that the makeup of the program was of high quality and that the scope of the program was appropriate and should not change appreciably. The workshop participants singled out for positive comment the program's emphasis on innovative research and attention to "orphan" research areas, as for example, methanogenesis and plant cell wall structure and function that are generally not

covered by other agencies adequately or in some cases not at all. The participants also strongly approved of the use of workshops to stimulate research areas where a focus has not yet been achieved or there is limited appreciation of the importance of an area. There was a view expressed that the BER program has acted as a catalyst in many respects. The workshop members were also very positive in concurring with the program's efforts at extending the length of grants to five years where the quality of work and productivity of the investigator warranted such an action.

In another instance, in recognition of the technical difficulties in culturing fastidious anaerobic microorganisms and the relatively small research community dealing with some of the organisms of interest, the BER program has provided partial support of a research-training session at the Marine Biological Laboratory in Woods Hole, Massachusetts, in the summer of 1986. The session was keyed to problems associated with handling anaerobic microorganisms.

The evolution of the BER program is principally affected by the advances in understanding in the research areas of concern. For example, recent rapid progress in defining systems of genetic transformation in plants has given optimism that such transformations leading to high fidelity expression of genetic information will be standardized in a reasonable time span. These techniques, coupled with an enhanced ability to manipulate cell cultures to produce whole plants, have given rise to expectations for commercialization in genetic engineering of plants probably sooner than might have been predicted a few years ago. These developments have also suggested to many that additional emphasis must be given to research defining the target loci to be altered. This means a return with the newer tools of biological research to many unanswered questions about biochemical pathways and their control, as well as to the many long term problems about growth, development, adaptation, host-pathogen interactions as well as inheritance of characters controlled by multiple genes.

Thus the BER program perception continues to be that there is great need to achieve better understandings of the genetic basis of biochemical and physiological processes. Increasing emphasis on biochemical studies aimed at defining regulatory mechanisms in plants and microorganisms is going to be critical in the coming years, particularly if the potentials of biotechnology are to be realized. More preparation to deal with biochemical phenomena must be factored into training curricula. Of considerable importance also will be efforts to encourage the utilization of the many powerful analytical techniques of chemistry in approaching difficult biological problems (e.g., NMR spectroscopy, molecular structural studies using neutron and x-ray diffraction of crystalline biomolecules, and others). The implication is that much more truly interdisciplinary research activity is requisite in such approaches. All of this, of course, must proceed in parallel and interactively with the rapid progress in molecular genetics.

The BER program research is carried out in many institutions; however, most of the research is conducted in university laboratories (see below).

	Number of projects	FY 86 funding (in thousands of $)	Percent of total funds
University of Contracts and Grants	94	7066	60%
Michigan State University Plant Research Laboratory	11	1857	16%
National Laboratories Brookhaven National Lab Lawrence Berkeley Lab Oak Ridge National Lab Los Alamos National Lab	15	2279	19%
Solar Energy Research Institute	1	101	1%
Other research institutions (federal, state, industrial, nonprofit)	9	367	3%
Conferences & miscellaneous	11	136	1%
	141	11806	

Upon examining funding levels for projects within this report, some readers may observe a discrepancy between the levels of support of individual projects at universities and those at DOE laboratories (Brookhaven, Lawrence Berkeley Laboratory, Michigan State University). This is explicable by noting that in a number of cases at the latter institutions large proportions of salaries are included along with other costs not usually associated with individual university projects.

Although the operating funds available in FY 1986 were less than in FY 1985, the program continued to evolve with the addition of 13 new projects. This was accomplished through deletions of other projects. During FY 1986, congressionally mandated cuts were applied across the board according to an agency policy. This put considerable pressure on many projects that were already at conservative levels of funding. There were numerous meritorious proposals that could not be funded because of limited resources.

All projects, both new and on-going, are subjected to some form of peer review (mail reviewers, panel reviews, site visit reviews) on a regular basis.

It is a matter of considerable importance to the BER program to acknowledge the most valuable continuing cooperation and assistance of several hundred scientists, both in this country and abroad, who generously provided their time and expertise in the review of projects and proposals. It is fair to say that much of the strength and quality of the U.S. federal system of research support derives from such participation.

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

Dr. Robert Rabson
or
Dr. Gregory L. Dilworth
Division of Biological Energy Research
Office of Basic Energy Sciences, ER-17, GTN
U.S. Department of Energy
Washington, D. C. 20545
Phone: (301)353-2873

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SUMMARIES OF FY 1986 ACTIVITIES
SUPPORTED BY THE
DIVISION OF BIOLOGICAL ENERGY RESEARCH

ARIZONA STATE UNIVERSITY - Tempe, AZ 85287

1. Antenna Organization in Green Photosynthetic Bacteria

R.E. Blankenship, Department of Chemistry $44,650

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 2000 bacteriochlorophyll/reaction center. The majority of the antenna pigment molecules are contained in chlorosomes, ellipsoidal vesicles attached to the cytoplasmic side of the cell membrane. Additional antenna pigments and reaction centers are contained in integral membrane proteins. The project objective is to determine the molecular organization and the mechanism of excitation transfer in the antenna system of green photosynthetic bacteria. The principal strategy involves isolation of the antenna system, biochemical resolution into its constituent subassemblies, and characterization using absorption and fluorescence spectroscopies. Results have identified a sequence of at least four distinct pigment species that transfer energy from the chlorosome into the membrane and eventually to the reaction center. The 740 nm-absorbing bacteriochlorophyll *c* that is the main pigment in the chlorosome has an extremely short (<30ps) fluorescence lifetime. Fluorescence excitation spectra indicate that the excitation is efficiently transferred to the membrane. Recent evidence indicates that the pigments are probably organized by pigment-protein and direct pigment-pigment interactions into what are essentially pigment oligomers. Future work utilizing techniques with faster time resolution will test this model and will be aimed at understanding the factors that enable this system to avoid the rapid loss mechanism (presumably by electron transfer quenching) observed in chlorophyll aggregates *in vitro*.

BOYCE THOMPSON INSTITUTE - Ithaca, NY 14853

2. Carbon Metabolism in Legume Nodules

T.A. LaRue $43,730

Symbiotic nitrogen fixation in legume nodules consumes more photosynthate than can be accounted for by the known energy requirements for nitrogenase. The object of our research is to determine how the legume nodule metabolizes carbohydrate to provide energy and reductant for nitrogen fixation. Because the plant cells in the nodule contain very little free oxygen, we are investigating the implications that the microaerobic environment has on the metabolic pathways of the plant. We found that mitochondria isolated from the nodule differed from mitochondria from aerial tissue in two unusual ways: they were very low in malic enzyme activity and they lacked the alternative cyanide-insensitive respiratory pathway. When incubated under the low 0_2 concentrations typical of nodules, the mitochondria produce little ATP. This suggests that their function in the nodule is not energy production. Because malic acid is a major constituent of nodules, we are investigating how it is produced and metabolized in the nodule.

BRANDEIS UNIVERSITY - Waltham, MA 02254

3. Carbon and Hydrogen Metabolism of Green Algae in Light and Dark

M. Gibbs, Institute for Photobiology of Cells & Organelles $58,000

The focus of this project is the elucidation of anaerobic metabolism in eucaryotic green algae. Isolated intact chloroplasts of *Chlamydomonas* catalyze the photoreduction of CO_2 at a rate of one third that detected in cells and protoplasts when adapted under H_2 demonstrating the association of hydrogenase and anaerobic adaptation with these plastids. The plastids catalyzed photoevolution of H_2 but not the oxyhydrogen reaction coupled to CO_2 assimilation. Photoreduction had a lower osmoticum relative to aerobically maintained chloroplasts (50 mM vs. 120 mM mannitol). Photoreduction but not photosynthesis was inhibited in chloroplasts and cellular oxyhydrogen reaction was inhibited by rotenone indicating an involvement of a NAD(P)H plastoquinone oxidoreductase in the hydrogen utilizing pathways. Salicylhydroxamic acid (SHAM) inhibited both photoreduction and the oxyhydrogen reaction and photosynthesis to a smaller extent. Photoreduction and the oxyhydrogen reaction may involve some common components of thylakoidal electron transport pathways in *Chlamydomonas* including NAD(P)-plastoquinone reductase and the plastoquinone pool. Under N_2 *Chlamydomonas* evolve 1.7 mole H_2 and 0.8 moles CO_2 per mole acetate consumed. Stoichiometry inhibitors and isotopic data support the occurrence of anaerobic and light-dependent citric acid and glyoxylate cycles that affect the conversion of acetate to CO_2 and H_2 prior to its conversion to cellular material. Extracts catalyzed the reductive carboxylation by ferredoxin of acetyl-CoA to pyruvate and the CO_2, pyruvate exchange indicating the presence of the reductive tricarboxylic acid cycle.

BROOKHAVEN NATIONAL LABORATORY - Upton, NY 11973

4. Chlorophyll-Complexes: Photoregulation of Transcription, Stability, and Phosphorylation

J. Bennett, Biology Department $220,000

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 to be studied include reaction center proteins of photosystems I and II and the light-harvesting chlorophyll *a/h* protein (LHCP). Structural studies involve: (1) gene cloning and sequencing, (2) *in situ* proteolysis and chemical labeling, (3) antibody binding, and (4) binding of chlorophyll to proteins synthesized from cloned genes in *E. coli*. Functional and biosynthetic studies will center on mechanisms by which light controls the organization and abundance of chlorophyll-protein complexes. Three areas of adaptive control are being explored: (1) regulation of mRNA levels, (2) regulation of protein turnover, and (3) protein phosphorylation. Studies on mRNA levels concern the role of phytochrome in regulating the expression of chloroplast and nuclear genes encoding photosystem II proteins and, for comparison, the large and small subunits of ribulose-1,5-bisphosphate carboxylase/oxygenase. Protein turnover is an important determinant of LHCP levels; studies deal with the identity of the photoreceptor(s) involved in stabilizing LHCP against breakdown and with characterizing the protease responsible for breakdown. Analysis of mRNA levels and protein turnover will reveal the ways plants adapt the composition of their photosynthetic membranes to changes in the intensity. spectral quality, and daily duration of light. The ability of LHCP to donate excitation energy to the two photosystems is controlled by protein phosphorylation. Purification of the LHCP kinase will permit the study of its substrate specificity, redox control, and location within thylakoids.

5. Plant Molecular Genetics

B. Burr and F.A. Burr, Biology Department $240,000

Molecular genetics can have a positive impact on plant improvement in many interrelated areas including: the development of analytical techniques and the elucidation of biological phenomena that limit crop productivity. Host traits that influence yield are controlled by many genes acting in concert. Little progress has been made in mapping or characterizing the genetic factors involved in quantitative inheritance in higher plants. Polymorphisms at the DNA level are very abundant in maize as shown by the use of restriction endonucleases and hybridization probes specific for unique sequences. These polymorphs can be mapped like other genetically controlled traits. Two families of recombinant inbred lines have been constructed and are being used to make a molecular genetic map of of the maize genome. Recombinant inbreds provide a

permanent segregating population, and since all mapping information obtained for a family is cumulative, provide a valuable resource for any investigator interested in mapping a trait for which differences can be found among the inbreds. This, of course, includes quantitative traits influencing flowering time, plant morphology, combining ability, and resistance to viral and fungal pathogens. It is expected that a few of the quantitative trait loci might eventually be isolated and characterized after tagging by transposable elements. Two regulatory genes, *C1*, controlling structural genes involved in seed pigmentation, and *Opaque2*, regulating the level of transcription of storage protein genes, are currently under study in this laboratory. The former gene has been cloned by Dr. Karen Cone who used an *Spm* probe to isolate an *Spm* induced mutant allele of this gene. Point mutations occur in maize plants regenerated from tissue culture ten times more frequently than spontaneous mutations. Evidence points to the induction of an error prone replication system in these plants. Mutations from tissue culture derived plants are being characterized at the molecular level.

6. Mechanisms of Energy Conversion in Photosynthesis

G. Hind, Biology Department $285,000

The project goal is to discover how energy is transformed in photosynthetic systems. Cyclic electron transport is of prime concern and is studied in intact chloroplasts of the C-3 plant, *Spinacia oleracea* and the C-4 plant, *Zea mays*, and in heterocysts of the filamentous bacterium, *Anabaena 7120*. It is mediated by combined activities of the cytochrome *b/f* and photosystem 1 complexes, which conduct electrons across the membrane. These interact at the stromal surface through ferredoxin, which also supplies electrons to $NADP^+$, hence the generation of reductant competes with the ATP-generating function of cyclic electron flow. Ferredoxin:$NADP^+$ reductase probably determines the electron's fate as determined by stromal ATP and NADPH demand; the mode of attachment of this enzyme to the membrane and its regulation by membrane energization is 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 effected through activity of a membrane bound protein kinase. The isolation and characterization of this enzyme and its substrates is in progress.

These investigations will provide knowledge of the factors limiting photosynthetic reduction of CO_2 and N_2 and are also of relevance to the design of biomimetic energy conversion devices.

7. Plant Gene Regulation and Expression

D. Sciaky, Biology Department $120,000

In order to understand plant gene regulation and expression, we are inserting modified genes into the genome of *Nicotiana plumbaginifolia*. The genes of choice include: (1) the neomycin phosphotransferase II gene (NPT II) from Tn5 flanked by the nos promoter on the 5' end and the nos polyA addition site on the 3' end, and (2) a gene from the bacteriophage T7 encoding the synthesis of T7 RNA polymerase (gene 1) also flanked by the nos regulatory sequences. We have introduced specific defined mutations into the NPT II gene and after transformation into plant cells will determine how these modifications affect the expression of kanamycin resistance in plants and compare this resistance expression to what is found in bacteria. We will, therefore, determine whether the rules that govern gene expression in bacteria are similar in plants. We are also using gene 1 to determine what signals are necessary for the transport of cytoplasmically produced proteins into the nucleus.

8. The Physiology and Biochemistry of Cyanobacteria

H.W. Siegelman, Biology Department $175,000

Cyanobacteria are cosmopolitan organisms of soils, marine and freshwaters. They frequently are responsible for water blooms and can cause serious water management problems in rivers, lakes and reservoirs. The molecular structure and composition of their photosynthetic energy collection system, which consists of an assembly of biliproteins called phycobilisomes, are being characterized. Phycobilisome composition is highly photoregulated, and we can assess the effects of steady-state light intensities and light qualities. Light intensity overrides the influence of light quality in photoadaptation studies. Antibodies are raised to the biliproteins to examine the control mechanisms regulating biliprotein synthesis. The DNA of *Synechocystis* 6701 is heavily methylated and is cleaved only by restriction enzymes sensitive to adenine and cytosine methylation. Amino acid sequences of at least a portion of the biliproteins are being determined to permit synthesis of an oligonucleotide probe to isolate the biliprotein genes by complimentary binding. Several heptapeptide toxins are being isolated from the cyanobacterium *Microcystis aeruginosa* by chromatography on a hydroxy-vinyl polymer followed by separation on octadecyl silica. The mean lethal dose of the toxins is 0.1 ug/g body weight in mice. Pathophysiological studies show that agents, such as hydrocortisone and partially hydrolyzed shellac which can block these toxins also inhibit the action of the mushroom toxin, phalloidin. Localization of the target cells for the toxins is being sought by fluorescent labelling of the toxin combined with mammalian cell tissue cultures.

UNIVERSITY OF CALIFORNIA - Berkeley, CA 94720

9. Transcription Control Elements and Manipulation of Chloroplast Genes

W. Gruissem, Department of Botany $67,340

A prerequisite for the manipulation of crop plants by introducing desirable genes into plant cells, or by transformation of chloroplasts, is our understanding of regulatory elements that are involved in the expression of genes. This project focuses on the molecular mechanism of chloroplast gene expression. The DNA sequences in the 5' flanking regions of several chloroplast genes have been investigated for their promoter function in vitro, utilizing a recently developed chloroplast transcription system. The genes for which promoter and regulatory DNA regions have been examined include several tRNA genes (trnM2, trnR1 trnS1, trnH1 and trnI1), and the genes for the 32 kilodalton polypeptide of photosystem II (psbA), the beta-subunit of the ATPase complex (atpB), and the large subunit of ribulose-1.5-bisphosphate carboxylase (rbcL). While the promoter regions for most of these genes have considerable structural and DNA sequence homology with the prokaryotic consensus promoter, some of the tRNA genes (trnR1 and trnS1), do not contain 5' upstream promoter regions. The promoter regions for the protein-coding genes (psbA, rbcL and atpB), will be tested for their function in vivo in transformed chloroplasts, while a mutational analysis of the coding region of the tRNA genes has been initiated to evaluate any possible promoter function. We will attempt to further characterize the different RNA polymerase activities involved in the expression of ribosomal RNA, tRNA and protein-coding genes. Our understanding of gene regulatory processes in chloroplast should be helpful for evaluating future attempts to manipulate and optimize the photosynthetic apparatus.

10. The Regulation of Enzyme Synthesis and Secretion in Plant Cells by Calcium

R.L. Jones, Department of Botany $84,660

The cereal aleurone synthesizes and secretes several hydrolytic enzymes in response to treatment with gibberellic acid (GA) and CA^{++}. We will focus our research on the roles of GA and Ca^{++} in the synthesis of specific alpha-amylase isoenzymes and on the intracellular transport and modification of these isozymes. The roles of GA and Ca^{++} in the synthesis of alpha-amylase isoenzymes will be studied with cDNA clones complementary to mRNAs for both low and high isoelectric point (pI) alpha-amylase isoenzymes. We have established that wall-less aleurone protoplasts respond to GA and Ca^{++} as do aleurone layers. We propose to use protoplasts to measure cytoplasmic free Ca^{++}. We have already shown that the Ca^{++}-sensitive dye FURA-2 is taken up and hydrolyzed by aleurone protoplasts. Fluorescence from FURA-2 shows that cytoplasmic Ca^{++} is present at about 0.5 uM. Aleurone protoplasts can also be used to measure Ca^{++} concentration with Ca^{++}-sensitive microelectrodes since the aleurone cell,

unlike other plant cells, does not contain a large central vacuole. Intracellular alpha-amylase transport is being studied in aleurone layers with the aid of the ionophore monensin. Monensin inhibits the secretion of alpha-amylase isoenzymes having high pIs without affecting their synthesis. We propose to study the intracellular accumulation of the alpha-amylase isoenzymes accumulate with the aim of understanding how the various isoenzymes of alpha-amylase are compartmentalized.

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

S.E. Lindow, Department of Plant Pathology $62,833

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. While descriptive studies of some epiphytes bacteria have been made, almost nothing is known of the phenotypes of these bacteria that confer the ability to grow or survive in the hostile leaf surface environment. The objectives of this study are to identify genes and/or phenotypes of strains of *Pseudomonas syringae* and *Erwinia herbicola* that are necessary for epiphytic growth and survival on bean leaves. The generality of such traits in conferring epiphytic survival on other strains of these species as well as other species common on leaf surfaces will be determined. The necessity of these traits determined to be required for epiphytic survival on bean will be determined on other plants for both *P. syringae* and *E. herbicola* strains. Factors important in epiphytic colonization of plants will be determined by an analysis of the growth and/or survival of 10,000 individual Tn5 induced insertion mutants of both *P. syringae* and *E. herbicola* on bean leaves in plant growth chamber conditions simulating the natural leaf surface environment. Tn5 mutants having reduced growth rates, lower maximum population sizes, or high death rates on leaf surfaces will be determined by estimating population size with a rapid leaf freezing assay due to a correlation of leaf freezing temperature and ice nucleus concentration. Thus an unbiased selection of mutants with a genetic tag and effected in epiphytic survival will be made. The phenotype of at least some Tn5 induced mutants will be determined *in vitro* and will be associated with alterations in growth or survival on leaf surfaces and other environments.

UNIVERSITY OF CALIFORNIA - Davis, CA 95616

12. Fluorescence Photobleaching Measurements of Plant Membrane Viscosity: Effects of Environmental Stress

R.W. Breidenbach, D.W. Rains,
M.J. Saxton, Plant Growth Laboratory

$15,000
(6 months)

Since temperature and salinity are among the most important factors limiting plant productivity, an understanding of the molecular basis of resistance to chilling and salinity will be useful in developing new plant varieties for the production of food, fuel, and chemicals. This project will examine the role of the plasma membrane and tonoplast in resistance to these stresses by means of fluorescence photobleaching recovery measurements of lateral diffusion rates of membrane proteins and lipids. The membrane viscosities for chilling-sensitive and chilling-resistant plants will be determined at various temperatures to determine whether the viscosity in chilling-sensitive plants increases abruptly at the critical temperature for chilling injury. Similarly, salt-tolerant and salt-intolerant lines of alfalfa and barley will be compared. The use of photobleaching measurements on labeled proteins is particularly appropriate, since many cellular physiological mechanisms are believed to be directly dependent on lateral motion of membrane proteins. Further experiments will study the effect of the cell wall and the cytoskeleton on lateral diffusion, and search for domain structure in the membrane. We also will test whether a lateral diffusion mechanism is the trigger for the production of defensive compounds by plants attached by pathogenic fungi.

13. Restriction of Virus Infections by Plants

G. Bruening, Department of Plant Pathology

$73,210

The productivity of a particular cultivar, in terms of biomass, food and/or fiber, often is limited by the action of plant pathogens. A direct and generally ecologically sound approach to limiting the deleterious effects of a pathogen is to develop a cultivar that is resistant or immune to it. Although plant genetic sources of resistance to viruses frequently have been used by plant breeders, often such sources are not available for the most economically important viruses. The objective of this proposal is to identify the mechanisms by which certain cowpeas resist cowpea mosaic virus (CPMV). The long term goal is to develop the necessary background information to make the engineering of resistance TO CPMV and other, more economically important viruses possible. Previous work identified a cowpea line, Arlington, from which CPMV-resistant protoplasts were recovered. Analyses showed that Arlington protoplasts, unlike protoplasts from susceptible Blackeye 5 cowpeas, interfere with the production of CPMV proteins. Extracts of Arlington cowpea protoplasts, but not Blackeye 5 cowpea protoplasts, interfered with an *in vitro* assay of a proteinase that

cleaves a CPMV polyprotein. This proteinase is essential to CPMV replication, so the proteinase inhibitor may explain the resistance of Arlington protoplasts to CPMV. Attempts to isolate the inhibitor from intact plants revealed two additional activities, an inhibitor of translation and a proteinase. We are investigating the relationship of all of these activities to the immunity that Arlington cowpea seedlings exhibit against CPMV.

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

J.L. Ingraham, Department of Bacteriology $57,720

Denitrification is the biological process by which certain bacteria reduced fixed nitrogen in the form of nitrate or nitrite ions to the gaseous species N_2O and/or N_2 and thereby deplete the available nitrogen in terrestrial or aquatic environments. Many of the aspects of this process, including certain of the proteins that catalyze it and the mechanism by which expression of denitrification genes is controlled, remain to be elucidated. We will study these aspects using the techniques of physiological genetics, employing as a test organism, *Pseudomonas stutzeri*, an active denitrifier capable of natural genetic transformation by DNA in solution. We have isolated a set of mutant strains generated by frame shift mutagenesis that are blocked in the step between N_2O and N_2O and another set blocked in the step between nitrate ion and N_2O. By transforming these strains to prototrophic anaerobic growth and to tetracycline-resistance using a pool of soluble DNA from *P. stutzeri* in which Tn10 elements (encoding tetracycline-resistance) had been randomly inserted, we have selected strains in which Tn10 elements are located adjacent to denitrification. Using tetracycline-resistance we will clone the denitrification genes into a broad host range vector transformed into *E. coli*. Our studies will focus principally on these clones. Using standard techniques we will determine the physical relationship among these genes, determine which denitrification functions they encode, fuse them to beta-galactosidase and return them *P. stutzeri* in order to study expression of these genes in that organism.

UNIVERSITY OF CALIFORNIA - Irvine, CA 92717

15. Bioenergetics of Salt Tolerance

J.K. Lanyi, Department of Physiology and Biophysics and
L. Packer, University of California, Berkeley, CA
Department of Physiology and Anatomy $142,582

The bioenergetic aspects of salt tolerance at the cellular level include ion transport across membranes, redirection of metabolic pathways for the increased synthesis of osmoregulatory compounds, replacement of some salt-sensitive cellular

components with salt-resistant ones, modification of the photosynthetic apparatus for increased efficiency, and the signals which turn these processes on and off. We are studying these phenomena in halobacteria and cyanobacteria, systems which are uniquely suited to answer specific questions on molecular and physiological mechanisms. We are characterizing the mechanism of chloride ion transport in the halobacteria via halorhodopsin during illumination, and exploring the possibility of a so-far undefined chloride transport system in the dark. We are describing aspects of sodium ion transport and their relationship to respiration, photosynthesis, as well as the biosynthesis of osmoregulatory compounds, in the cyanobacteria. Using this broad approach, we intend to extend and refine the conceptual basis we have developed during the past few years for the physiology of cellular adaptation to high salinities.

CALIFORNIA, UNIVERSITY OF - Los Angeles, CA 90024

16. Energy Capture and Use in Plants and Bacteria

P.D. Boyer, Molecular Biology Institute $90,000

This project focuses on how plants use energy from light, and bacteria the energy from oxidations, to make ATP, the "currency" of the cell. We are using multiple approaches to unravel the function of the subunits of the complex, membrane-spanning ATP synthase. Emphasis is on the further assessment of the binding change mechanism, on the postulated participation of three catalytic sites in cooperative sequence, and on the possible rotational change in position of the catalytic subunits relative to a noncatalytic core. Approaches being used are as follows: The photolabeling of active sites with ATP analogs and 1/3 site reagents to assess affect of catalytic turnover on subunit labeling patterns; the attachment of fluorescent labels to catalytic and noncatalytic subunits to measure effects of catalysis on energy transfer between the probes; correlation of subunit cross-linking with cleavable cross-linkers to catalytic activity loss and regain; design and synthesis of specific cross-linkers to assess possible rotational movements of subunits; development of procedures that will allow separation and replacement of catalytic beta subunits to aid assessment of subunit positional interchange accompanying catalysis; and site-directed mutagenesis of specific residues of the catalytic subunit of the *E. coli* enzyme that have been implicated in catalytic or control events.

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

R.P. Gunsalus, Department of Microbiology $61,984

Methanogenic acetate utilization is a primary rate limiting step in anaerobic decomposition of biomass. However, little is known about the enzymes responsible for acetate metabolism or the regulation of their synthesis. A recently described acetate-utilizing species, *Methanosarcina acetivorans*, is uniquely suited for biochemical and genetic studies because unlike previously described acetotrophic methanogens that have a thick heteropolysaccharide cell wall, this species has a protein cell wall that can be gently disrupted to obtain protoplasts or lysed to yield intact DNA. Our laboratory is conducting basic experiments to examine the regulation and synthesis of enzymes involved in the acetate utilization pathway. Genomic libraries will be constructed from *M. acetivorans* DNA and the gene(s) for a regulated enzyme, carbon monoxide dehydrogenase, will be cloned and characterized. This enzyme is associated with the acetotrophic pathway and appears to be regulated in response to the availability of alternative substrates for methanogenesis. In other experiments we will attempt to develop a gene transfer system for this bacterium. Methods will be developed for the efficient plating of cells on defined media, for chemical mutagenesis, and for the isolation of mutants. *M. acetivorans* and a number of related isolates will be screened for the presence of the plasmid DNA so that a plasmid shuttle vector can be constructed. Experiments to develop a transformation protocol based on protoplast generation will then be performed. The availability of such a plasmid shuttle vector will facilitate the study of the genes and enzymes of the aceticlastic pathway *in vivo*. The goal of these studies is to understand how acetate is utilized by *M. acetivorans* at the molecular level. We anticipate that *M. acetivorans* will serve as a useful model for examining mechanisms of gene regulation in other acetotrophic methanogens.

UNIVERSITY OF CALIFORNIA - Riverside, CA 92521

18. Catalytic Mechanism of Hydrogenase from Aerobic N_2-Fixing Microorganisms

D.J. Arp, Department of Biochemistry $45,280

This project aims at elucidating the catalytic mechanism of hydrogenase from aerobic N_2-fixing microorganisms. This enzyme efficiently recycles the H_2 evolved by nitrogenase. Several properties of these hydrogenases make them ideal to function in an

environment in which all of the available substrate is generated in situ (e.g., a very low rate of the back reaction, hydrogen evolution, and a low K_m for H_2). We are particularly interested in the enzymes from *Rhizobium*-induced root nodules and the soil microorganism, *Azotobacter vinelandii*, because of their role in improving the efficiency of biological N_2 fixation. Both enzymes are Ni- and Fe-containing dimers composed of subunits with molecular weights of 65,000 and 35,000. We are interested in determining the role of the metals and each of the subunits in catalysis. The experimental approaches we are using include determination of the effect of subunit dissociation, metal extraction, or protein modification (e.g. modification of sulfhydryls) on the various activities of these enzymes (H_2 oxidation, isotope exchange, H_2 evolution). The results of this project will provide insight into the catalytic mechanism of H_2 oxidation by *Rhizobium* and *Azotobacter* hydrogenases that, in turn, will lead to a better understanding of H_2 cycling in these organisms. This information is important to genetic engineering of new strains and may lead to improved techniques for selecting the best strains of *Rhizobium* for inoculation of legumes.

19. Mechanisms of Flooding Tolerance in Roots and Leaves of Maize, Studied using in vivo NMR spectroscopy

J.K.M. Roberts, Department of Biochemistry, UC Riverside
J. Callis, Dept. Biological Sciences, Stanford University $40,000

We are interested in defining the roles of inducible enzymes in flooding tolerance of maize. The inducible enzymes under investigation are alcohol dehydrogenase (ADH) and enzymes involved in nitrate metabolism. Experiments consist of three components: (1) varying the concentration of the particular enzymes by genetic or environmental means; (2) quantitation of flooding tolerance; and (3) measurement of intracellular pH during flooding stress. We have shown that cytoplasmic acidosis can decrease tolerance of hypoxia in plant tissues, and that levels of ADH and nitrate metabolism can influence cytoplasmic pH and flooding tolerance. Using mutants differing in levels of ADH we are determining the range over which ADH levels can influence tolerance of hypoxia. In root tips of a mutant exhibiting about 50% of wild-type ADH activity, no significant loss of tolerance of hypoxia was apparent. Mutant root tips with about 1% of wild-type activity are much less tolerant of hypoxia. These results indicate that tolerance of our air-grown, wild-type roots to extreme hypoxia is not limited by ADH. We will examine other mutants to more precisely define the dependence of flooding tolerance on ADH levels. We will also quantitate flooding tolerance in roots in which proteins synthesized under hypoxia, such as ADH, are more abundant; and quantitate flooding tolerance in leaves, which normally have low ADH levels, of maize plants fed different nitrogen sources. These experiments will allow us to determine if induction of these enzymes can play a significant role in flooding tolerance.

UNIVERSITY OF CALIFORNIA/SAN DIEGO - La Jolla, CA 92093

20. Characterization and Biosynthesis of Complex Protein-Bound Carbohydrates

M.J. Chrispeels, Department of Biology $72,000

When glycoproteins are transported from their site of synthesis, the rough endoplasmic reticulum, to their destinations in the cell, many of the asparagine linked high-mannose oligosaccharides are modified by Golgi-localized enzymes. The objective of our study is to determine why certain oligosaccharides are modified, whereas others are not when both are present on the same protein. We use as a model system the biosynthesis and modification of phytohemagglutinin, a glycoprotein lectin with a high-mannose and modified oligosaccharide sidechain on each polypeptide. We have probed radioactively-labelled phytohemagglutinin with various glycosidases and found that the sidechain which remains in the high-mannose form is not accessible to these glycosidases unless the protein is first denatured. Precursor of mature phytohemagglutinin obtained from the endoplasmic reticulum has a high-mannose chain which is susceptible to glycosidases, and this chain is in the same position on the protein (i.e., attached to the same asparagine residue) as the modified sidechain on mature phytohemagglutinin. We, therefore, conclude that availability of sidechains to glycosidases (and glycosyltransferases) is of primary importance in the control of sidechain modification. Our next objective is to determine the sequence of events in the formation of these modified sidechains, and the characteristics of the Golgi localized enzymes. To reach this objective, we are characterizing the alpha-mannosidase and the glycosyl-transferases with transfer xylose, fucose and N-acetylglucosamine to glycoproteins.

21. Identification and Manipulation of Rhizobium Phytohormone Genes

G. Ditta, Department of Biology $71,190

Nodule development during *Rhizobium*-legume symbiosis involves specific changes in the growth pattern of root cortical cells. It has been known for many years that *Rhizobium* species can produce both auxin (indoleacetic acid; IAA) and cytokinin during vegetative (asymbiotic) growth, but it is not known whether phytohormones produced by the invading bacteria play a role in this process. The enzymatic functions of primary importance for auxin and cytokinin biosynthesis by *Rhizobium* are also unknown. We have begun investigating these questions by obtaining *Rhizobium*

mutants defective in auxin production. Some are uncharacterized mutants deficient in the ability to make IAA from tryptophan during normal vegetative growth, while others are specific aminotransferase mutants. Physiological and biochemical studies have indicated that the production of indolepyruvic acid from tryptophan through aminotransferases is a major route for IAA biosynthesis in *R. meliloti*. Such data also suggest that many of the mutants currently under investigation may be exhibiting pleiotropic effects on aminotransferase activity *in vivo*. The symbiotic properties of the various mutants, such as nodulation, nodule development, and nitrogen fixation capability are being examined.

An alternative experimental strategy involves the creation of strains of *R. meliloti* that overproduce auxin and cytokinin. For this purpose we have constructed plasmids that place auxin and cytokinin biosynthetic genes derived from other organisms under the control of known *R. meliloti* symbiotic promoters. Changes in the pattern of symbiosis should reveal aspects of development that are hormone-regulated.

UNIVERSITY OF CALIFORNIA - Santa Cruz, CA 95064

22. Tonoplast Transport and Salt Tolerance in Plants

L. Taiz, Department of Biology $62,620

One approach to extending the range of agriculture into marginally arable lands is to develop salt tolerant crops. Towards that end, we are studying the mechanism of salt tolerance in plants, focusing on the mechanism of salt accumulation in the vacuole. The primary driving force is the transtonoplast pH and electrical gradient provided by two proton pumps: an H^+-ATPase and an H^+-pyrophosphatase. Our studies on the structure of the maize tonoplast ATPase indicate that it is a large multimeric protein, 400 kDa MW, consisting of at least three subunits: 72, 62 and 16 kDa. Based on inhibitor binding and immunological studies, the 72 kDa subunit contains the catalytic site. Treatment of maize plants with excess salinity causes a marked increase in the activity of this proton pump in the roots. We have recently found that maize roots, unlike those of beets, lack a Na^+/H^+ antiporter on the tonoplast, but appear to have a sodium channel. In the presence of excess salt, sodium accumulates in the stelar parenchyma of the mature region of the root. We have correlated this accumulation with the presence of a tonoplast sodium channel and a decline in the tonoplast ATPase activity. Our present hypothesis is that maize, a glycophyte, normally excludes salt from the vacuole by the action of the ATP- and PPi-driven electrogenic proton pumps. In the older region of the root, sodium accumulates in the vacuole because of the decline in pump activity and the development of passive sodium channels.

CARNEGIE INSTITUTION OF WASHINGTON - Washington, DC 20008

23. Isotope Fractionation During Oxygen Production and Consumption by Plants

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

$56,000
(two years)

The $^{18}O/^{16}O$ of atmospheric oxygen is 1.023 times that of seawater (i.e. the atmosphere is enriched in ^{18}O by +23 ‰). Known as the Dole effect, this difference is thought to result from isotopic discrimination during photosynthetic oxygen production and respiratory oxygen consumption. A few experiments conducted with microorganisms seem to confirm this explanation. Knowledge of the fractionation of oxygen isotopes in the important reactions of the oxygen cycle, however, is incomplete. Studies of isotopic fractionation by plants in reactions which produce or consume oxygen are being conducted under this project. Our studies show that illuminated spinach thylakoids supplied with $K_3(FeCN)_6$ or *Anacystis nidulans* cells provided with CO_2 produce oxygen that is very similar to that of the source water ($\Delta = 0.13 \pm 0.27$ ‰) when rigorous precautions are taken to avoid simultaneous oxygen uptake reactions. In contrast when *Asparagus sprengeri* mesophyll cells are illuminated in a closed vessel at their O_2 and CO_2 compensation point, the oxygen in the vessel comes to a steady-state composition of about +22 ‰ relative to the source water. Substantial fractionation occurs in the reactions of photorespiration. The glycolate oxidase reaction (conducted with or without catalase) had a fractionation value (α) of 21.9‰. Further experiments are in progress to measure simultaneously the oxygen and carbon isotope fractionation during O_2 uptake and CO_2 fixation, respectively, by Rubisco. Corresponding studies of isotope fractionation by intact leaves in a gas-exchange system will also be conducted under different CO_2 and O_2 concentrations and at different temperatures to extend the understanding of the environmental regulation of these processes. Studies with respiration indicate a large variation among organisms. Yeast has a fractionation factor of $\alpha = 16.0$, whereas whole alfalfa sprouts had an α of 21.5‰ and *Asparagus* cells had an α of 20.8‰. Preliminary results show that respiration in the presence of cyanide results in greater fractionation indicating a possible difference in the mechanism of oxygen uptake. These studies have significance in an improved understanding of the oxygen cycle and may lead to further possible applications of stable isotope methods to plant biology.

UNIVERSITY OF CHICAGO - Chicago, IL 60637

24. Organization and Regulation of the Genes for Nitrogen Fixation in *Rhodopseudomonas capsulata*

R. Haselkorn, Department of Molecular Genetics & Cell Biology $75,000

We have already cloned a number of fragments of DNA containing genes necessary for nitrogen fixation from the photosynthetic bacterium *Rhodopseudomonas capsulata.* The *nif* genes are locally clustered but the clusters are on non-neighboring DNA restriction fragments; there are at least five unlinked *nif* gene regions. We propose to determine the physical linkage arrangement of these fragments, to determine their relationship, if any, with the corresponding *nif* genes of *Klebsiella*, and to determine the nucleotide sequence of some of the fragments. So far we have identified five regulatory genes among these, using a *nifH::lac* fusion. *Lac* fusions to the regulatory genes themselves will be used to define the regulatory circuit in *R. capsulata.* The regulatory proteins will be produced from expression vectors and used in *in vitro* transcription experiments to define the molecular mechanisms governing *nif* gene expression in *R. capsulata.* These experiments will be focussed on, among other variables, the state of supercoiling of the DNA templates. We have recently found that inhibitors of DNA gyrase specifically prevent the transcription of *nif* genes, as well as of genes required for photosynthesis. We believe that the requirement for anaerobiosis in order to express the *nif* genes is related to the activity of DNA gyrase; this connection will be pursued vigorously.

COLUMBIA UNIVERSITY - New York, NY 10023

25. Regulation and Genetic Organization of Hydrogenase

A.I. Krasna, Department of Biochemistry & Molecular Biophysics College of Physicians and Surgeons $68,400

Hydrogenase is an enzyme of unique biochemical interest because of the nature of its substrates, H^+ and H_2, which are the simplest stable molecules. The enzyme plays an important role in the anaerobic metabolism of many bacteria and algae; in fermentative reactions, photosynthesis, and nitrogen fixation. The objectives of the research are to elucidate the regulation and genetic organization of hydrogenase in *E. coli.* This laboratory has isolated a number of mutant strains of *E. coli* with altered hydrogenase activity. By the use of a hydrogenase-negative mutant, a plasmid has now been isolated containing a 2.1 kb chromosomal insert from wild-type *E. coli* DNA which restores the hydrogenase activity in the mutant. To characterize the

cloned gene, its protein product will be identified by various criteria to establish whether it is a structural gene or a regulatory gene. The base sequence will be determined which will lead to the amino acid sequence of the protein. In a similar manner, the hydrogenase gene from *Chromatium* and *Proteus vulgaris* will be cloned in the hydrogenase-negative mutant strain of *E. coli*. The gene(s) required for growth of *E. coli* on fumarate plus H_2 has also been cloned as a 6.5 kb insert and its protein's products will be characterized and its role elucidated. Other mutant strains of *E. coli*, which are hydrogenase-positive, express different levels of hydrogenase activity under various growth conditions and the regulatory mechanisms responsible for the control of their expression will be examined. Some of the hydrogenase-positive strains differ from the parental strain in reactivity with exogenous dyes as substrates. It will be established whether this is due to a requirement for other factors or to mutations in hydrogenase.

CORNELL UNIVERSITY - Ithaca, NY 14853

26. Anaerobic Metabolism of Aromatic Compounds by Phototrophic Bacteria

C.S. Harwood, Department of Microbiology, and
J. Gibson, Section of Biochemistry,
Molecular and Cell Biology

$47,847

Vast quantities of aromatic compounds in the form of lignin, lignin derivatives, and aromatic pollutants are continually being introduced into the biosphere and much of this material accumulates in anaerobic environments. The objectives of this project are to elucidate anaerobic routes of benzoate and 4-hydroxybenzoate metabolism by the phototrophic bacterium, *Rhodopseudomonas palustris*. These two compounds are degraded through distinct metabolic pathways and so may be taken as models for two modes of anaerobic attack. *R. palustris* is particularly well suited for these studies because its ability to separate carbon metabolism from energy generating mechanisms frees it from the thermodynamic constraints that restrict the anaerobic metabolism of aromatics by pure cultures of fermentative bacteria. Information obtained in these studies can then be used to develop models which can be tested with nonphototrophic anaerobic cultures and consortia. Studies include identification of the number and specificity of enzymes involved in benzoate and 4-hydroxybenzoate metabolism, identification of cofactors and electron carriers involved in each pathway, and a determination of the precise nature of the products formed. Mutants that are blocked in aromatic metabolism are being isolated and will be used to corroborate the biochemical data. These mutants will also be used, together with physiological approaches, to identify compounds (inducers and repressors) that regulate the expression of genes for aromatic degradation. This work will provide basic information about the biochemistry, regulation, and genetics of anaerobic metabolism of aromatics. At present very little is known about mechanisms responsible for the degradation of this large and quantitatively important group of compounds in anaerobic environments.

27. Mechanisms of Inhibition of Viral Replication in Plants

P. Palukaitis, M. Zaitlin, Department of Plant Pathology $70,000

Measures designed to increase plant cultivation or biomass, for the purpose of conversion to fuels and chemicals, must take into account the problems of crop losses caused by pathogens such as viruses, and incorporate control measures for such pathogens into the program. This project is concerned with analyzing the molecular mechanisms of inhibition of viral replication and movement in plants, by inserting viral genome segments into the plant genome (via a Ti-plasmid vector) and analyzing such plants for the ability to (1) interfere or interact with plant genes involved in restricting viral movement, and (2) inhibit the replication of related plant viruses by the process called crossprotection. In the former situation, plant viral genes that potentiate the cell-to-cell movement of the virus will be inserted into the plant genome. Such plants will be tested for the ability of the inserted and expressed viral movement genes to potentiate the cell-to-cell movement of unrelated viruses. Protoplasts from such plants will be analyzed for the molecular interactions that occur when such viral movement genes interface with plant genes involved in inhibiting the cell-to-cell movement of plant viruses. In the latter case, various segments of a plant virus genome will be inserted into a plant genome, in both orientations, permitting expression of both the (+) and (-) viral RNAs, and these will be tested to determine their effectiveness in crossprotection, at the level of both the whole plant and in protoplasts.

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

P.L. Steponkus, Department of Agronomy $65,420

The objective of this project is to characterize the cellular and molecular aspects of freezing injury and cold acclimation with special emphasis on the plasma membrane. With isolated protoplasts, destabilization of the plasma membrane may be effected in at least three different ways: 1) when cooled to relatively warm subzero temperatures (-3 to -5°C), freeze-induced osmotic contraction results in the irreversible endocytotic vesiculation of the plasma membrane so that lysis occurs during subsequent osmotic expansion; 2) when cooled to lower subzero temperatures (-10 to -20°C), freeze-induced dehydration results in lamellar-to-hexagonal$_{II}$ phase transitions in the plasma membrane and subtending lamellae; and 3) when cooled at rapid rates (>3°C/min) to temperatures below -15 to -20°C, mechanical failure of the plasma membrane results in intracellular ice formation - possibly as a consequence of freeze-induced electrical transients. Following cold acclimation, the behavior of the plasma membrane during a freeze-thaw cycle is dramatically altered: 1) freeze-

induced osmotic contraction results in exocytotic extrusion of the plasma membrane which is readily reversible; 2) freeze-induced dehydration does not result in lamellar-to-hexagonal$_{II}$ phase transitions, and 3) intracellular ice formation occurs at much lower temperatures (-42°C). These differences in the stability of the plasma membrane are associated with pronounced changes in its lipid composition, e.g., significant changes in the phospholipid molecular species, decreases in the glycolipid content (steryl glycosides and acylated steryl glycosides), and changes in the proportions of free sterols. Current studies are directed to establishing a causal relationship between these changes in the composition of the plasma membrane and its stability to the various freeze-induced stresses.

29. Importance of Phytoalexin Tolerance and Detoxification for Pathogenicity

H.D. VanEtten, D.E. Matthews, Department of Plant Pathology **67,340**

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 pea phytoalexin pisatin is effective only against isolates of *Nectria haematococca* which cannot metabolize pisatin, or cannot do so rapidly. This fungus possesses at least four genes for pisatin demethylation, some of which are distinguishable by the rate of demethylation they confer. Only the more rapid of these phenotypes are associated with virulence towards pea. We are examining the relationship between these genes and the enzyme pisatin demethylase, which is a substrate-inducible microsomal monooxygenase with two protein components, NADPH-cytochrome c reductase and cytochrome P-450. Antibodies to the reductase cross-react with reductases from isolates of *N. haematococca* and other fungi that do not demethylate pisatin, and reductases from sources as distantly related as *Saccharomyces cerevisiae* are competent to support pisatin demethylation. The content of reductase in *N. haematococca* is not strongly regulated, and thus does not account for the inducibility of pisatin demethylase activity. Qualitative and quantitative differences in pisatin demethylation among *N. haematococca* isolates therefore appear to be mediated by cytochrome P-450 genes or their regulation. The cytochrome from a virulent isolate has been partially purified, and DNA containing the corresponding gene has been cloned by expression of demethylase activity in *Aspergillus nidulans*. This protein and its gene will be compared with their counterparts in *N. haematococca* isolates possessing low demethylase activity and low virulence.

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

D.B. Wilson, Department of Biochemistry, Molecular and Cell Biology $34,030

Thermomonospora fusca is a thermophilic, bacterium which produces active, stable cellulase and xylanase activities. The project objectives are (1) to purify the *T. fusca* cellulases and xylanases, characterize each enzyme and determine its role in the hydrolysis of crystalline cellulose; (2) to clone all the *T. fusca* cellulase genes and determine the mechanisms that regulate cellulase and xylanase synthesis. We have purified five different cellulases from *T. fusca* culture supernatant using hydroxyl apatite chromatography, DEAE chromatography and preparative gel electrophoresis. All five are endocellulases, but they differ in molecular weights, substrate specificities, and immunologically. Mixtures of enzyme E_3 and any of the other enzymes show synergism in degrading native cellulose. We have cloned two cellulase genes into *E. coli*. Both of them appear to code for endocellulases and one of them codes for enzyme-E_5 while the other codes for an unidentified enzyme. The sequence of 1 kb of the E_5 gene has been determined as has the sequence of the N-terminus of E_5 identifying the start of the coding region for E_5. Efforts continue: (1) to fractionate the *T. fusca* culture supernatant to isolate any additional cellulases that are present; (2) to characterize the enzyme produced by the unidentified cloned gene and determine the role of each enzyme in the hydrolysis of crystalline cellulose; (3) to construct a *T. fusca* DNA library of *Streptomyces lividans* and screen the clones to look for the uncloned cellulase genes. *S. lividans* has been shown to express and excrete the E_5 cellulase.

31. Microbial Ecology of Thermophilic Anaerobic Digestion

S. H. Zinder, Department of Microbiology $76,960

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 to methane. Recent results include: (1) the isolation of a thermophilic *Methanothrix* which grows

much more rapidly (Td=24 h) than do mesophilic strains and the development of a defined medium for its growth; (2) the demonstration that the *Methanothrix* has high levels of carbon monoxide dehydrogenase but little, if any, hydrogenase; (3) the isolation in axenic culture, using ethylene glycol as a growth substrate, of the acetate-oxidizing member of a thermophilic two-membered coculture which converts acetate to methane using interspecies hydrogen transfer; (4) the finding that levels of hydrogen in the acetate-oxidizing coculture were about tenfold higher than predicted by standard thermodynamic calculations; (5) 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 of the acetate-oxidizer and its interactions with hydrogen-consuming methanogens, and on extending studies on degradation of ^{14}C-radiotracers by bioreactor populations.

DESERT RESEARCH INSTITUTE - Reno, Nevada 89506

32. Gas Exchange Characteristics of Leaves as Indicators of the Biochemical Reactions Limiting Photosynthesis

T. D. Sharkey, Biological Sciences Center $60,224

The response of photosynthesis to light, CO_2 and O_2 is studied by measuring the exchange of gases (water vapor and CO_2) between the atmosphere and the leaf. These measurements are combined with measurements of metabolite levels and enzyme activities which can disclose the molecular events which limit or regulate the rate of photosynthesis. The purpose of this research is to identify the underlying molecular events which give rise to easily measured photosynthetic characteristics so that those processes which are most important in regulating or limiting photosynthesis in leaves can be identified.

A significant result of this research has been the identification of the cause of O_2 insensitive photosynthesis in C_3 plants. It has been determined that anomalous behavior is caused by deactivation of the primary carboxylating enzyme, RuBP carboxylase, in low O_2. It is believed that this deactivation occurs because the plant is unable to use all of the products of photosynthesis that could be made if deactivation did not occur. Current efforts are directed toward understanding how this deactivation may be advantageous to the plant and how rapidly it can occur.

FLORIDA STATE UNIVERSITY - Tallahassee, FL 32306

33. Guard Cell Biochemistry: Response to Environmental Stimuli Causing Changes in Gas Exchange

W.H. Outlaw, Jr., Department of Biological Sciences $94,280

The aperture size of stomatal guard cells in leaves is regulated to effect a compromise between the opposing priorities of avoiding water loss and of admitting CO_2. Pore enlargement is brought about by swelling of the subtending guard cell pair, resulting from accumulation of solutes (K^+ and, to a lesser extent, Cl^-) from the apoplast and synthesis of low MW substances (e.g., malate) from osmotically inert substances (e.g., starch). The specialized metabolism that these cells have evolved to fulfill their crucial role in the plant's physiology extends to more subtle aspects, which this project studies. Despite the presence of green plastids, which conduct linear electron transport, guard cells lack the ability to photosynthetically reduce significant quantities of CO_2. To clarify its role in guard cells the organization of PS II has been investigated by studying the kinetics of the Chl a fluorescence rise in single cell pairs upon illumination. (These results are being correlated with ultrastructure of the guard cell plastids determined in other studies. Presumably, ABA is an endogenous signal that mediates stomatal closure, but little is known regarding the presence of ABA or of any enzyme specific for its metabolism in guard cells *in situ*. Our sensitive immunological approach hopefully will replace the current conjectures with a data base. PEP Carboxylase, which catalyzes a reaction at a regulated metabolic point in anion biosynthesis, exists as various isoforms, even in C_3 leaves. Because of its central role in guard cell biochemistry, we are studying the properties of this enzyme using kinetic analysis and techniques for physical separation. This latter includes microelectrophoretic analysis of pure cell extracts and the construction of a microdensitometer. To study the control of anion synthesis further, we have completed an initial investigation of cellular compartmentation in tissues of a CAM plant. Advances in these three areas will improve our understanding (and possibly control) of water loss and CO_2 uptake by leaves.

UNIVERSITY OF FLORIDA - Gainesville, FL 32611

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

L.O. Ingram, Department of Microbiology and Cell Science $92,000 (two years)

Zymomonas mobilis is the only obligately fermentative microorganism which utilizes an Entner-Doudoroff pathway for glycolysis. This pathway produces a single net ATP per glucose consumed. In *Z. mobilis*, ethanol and carbon dioxide are produced as the

principal waste products during cofactor regeneration. The first half of the Entner-Doudoroff pathway differs from that of the more common Embden-Meyerhof pathway with pyruvate and glyceraldehyde-3-phosphate as initial products of hexose cleavage. Glyceraldehyde-3-phosphate is further metabolized to pyruvate utilizing the same series of enzymes found in the Embden-Meyerhof pathway. Since the conversion of glucose to ethanol and carbon dioxide in *Z. mobilis* proceeds with 98% efficiency, glycolytic flux can be conveniently measured by respirometry. Investigations into the regulation of glycolysis in *Z. mobilis* should provide a novel evolutionary comparison to the extensive studies with yeast, plant and mammalian systems. In this initial study, we propose to focus on the unique enzymes involved in alcohol production which are common to all organisms producing ethanol as the major fermentation product, pyruvate decarboxylase and alcohol dehydrogenase. During growth, these two enzymes constitute over 5% of the total cellular protein.

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

R.A. Jensen, Department of Microbiology and Cell Science **$94,280**

The shikimate pathway of higher plants is of crucial importance: (i) because of the diversity of endproducts produced (amino acids, vitamins, growth regulators, and a multitude of secondary metabolites of pharmacological and agricultural interest), and (ii) because of the massive quantitative output of this biochemical network. We take the approach of doing in-depth work with *Nicotiana silvestris* (as a system for continuing development of biochemical genetics) in combination with comparative enzymological studies of other higher plants (to gain insight into what features can be generalized). The enzymic construction of the aromatic pathway, its allosteric and transcriptional regulation, and its subcellular location are current focal points of emphasis. We have shown that *L*-arogenate is the precursor of both *L*-phenylalanine and *L*-tyrosine in chloroplasts and proplastids. We have shown that an intact aromatic pathway that is subject to a stringent and novel pattern of allosteric control exists within plastids. We are pursuing the hypothesis, supported by evidence based upon several key enzymes, that a second, intact pathway exists within the cytosol. This essentially unregulated pathway in the cytosol ney supply precursors for secondary metabolism via a simple overflow mechanism.

UNIVERSITY OF GEORGIA - Athens, Georgia 30602

36. Structural Studies of Complex Carbohydrates in Plant Cell Walls

P. Albersheim, Complex Carbohydrate Research Center $175,000

The cell walls of a plant determine the plant's structure and morphology. 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 include detailed analyses of two pectic cell-wall polysaccharides, rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II), as well as the hemicellulosic cell-wall polysaccharide xyloglucan (XG). 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), and KDO (3-deoxy-D-manno-2-octulosonic acid). Although, RG-I contains only five glycosyl residues, it still exhibits tremendous structural complexity. RG-I is composed of a backbone of alternating rhamnosyl and galacturonosyl residues with side chains attached to 50% of the rhamnosyl residues. At least 30 structurally different side chains consisting predominantly of arabinosyl and galactosyl residues have already been identified. We are investigating the possibility that discreet families of side chains are attached to the RG-I backbone. We are studying the cell walls of monocotyledons and gymnosperms as well as dicotyledons. So far, our studies of the polysaccharides in these cell walls show that, although the quantities of the polysaccharides within the cell walls vary greatly, every cell wall contains the same array of polysaccharides.

37. Development of Methods to Structurally Characterize Complex Carbohydrates

P. Albersheim, A. Darvill, H. van Halbeek, W. York, Complex Carbohydrate Research Center $110,000

This research focuses on the development of methods to structurally characterize complex carbohydrates isolated from microorganisms, plants, and animals. The founding of the Complex Carbohydrate Research Center at the University of Georgia has provided access to a high-field FAB mass spectrometer and to a 500-MHz NMR spectrometer. This equipment will allow us to develop new methods to study complex carbohydrates from a variety of perspectives, an approach that is necessary in order to obtain eventually a complete three-dimensional picture of the molecules. We believe that it will be possible to establish structures and solution confirmations for oligosaccharides (from plant cell walls) composed of up to 16 glycosyl residues and for oligosaccharides (from glycoproteins and glycans) similar or slightly larger size. Other methods we are studying that will aid in our structural investigations include reactions for specifically cleaving complex carbohydrates and techniques for labeling oligosaccharides to allow simple but highly sensitive detection during purification. The labeling procedures should retain if possible any biological activity of the complex carbohydrate being studied.

38. Studies on Oligosaccharins: Carbohydrates Possessing Biological Regulatory Activities

A. Darvill, Complex Carbohydrate Research Center $145,000

This project is concerned with the isolation and characterization of oligosaccharins, which are naturally occurring complex carbohydrates that possess biological regulatory activities. We have hypothesized that oligosaccharins, when released from the complex carbohydrates of plant cell walls, regulate various biological functions within the plant. We are studying the following oligosaccharins: (1) Oligosaccharins isolated from plant cell walls that elicit phytoalexin (antibiotic) accumulation in plant tissues. Elicitors of phytoalexins from dicotyledon and monocotyledon cell walls are being studied; bioassay systems using both monocotyledon and dicotyledon plant tissues will be used in the studies. (2) An oligosaccharin that may be a trigger for the hypersensitive-resistant response in plants. We are studying an enzyme secreted by *Pyricularia oryzae* that releases this oligosaccharin from plant cell walls. (3) Oligosaccharins that can induce flowers, roots, vegetative buds, and callus in isolated tobacco epidermal strips. To aid in the purification of oligosaccharins that induce specific physiological events in the epidermal strips, we are attempting to identify specific molecular markers for flowering, root development, vegetative bud development, and callus development in these tissues. (4) Oligosaccharins capable of determining the sex of flowers in the dioecious plant, *Mercurialis annua*.

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

39. Genetics and Biochemistry of Surfactant Synthesis in Arthrobacter Species H-13A

W.R. Finnerty, Department of Microbiology $80,810

The biosynthesis of an extracellular biosurfactant by *Arthrobacter* species H-13A is under study with respect to the biochemistry and mechanisms of genetic transfer in this genus. Genetic transfer mechanisms have not been described for the genus. A plasmid transformation system for *Arthrobacter* sp. H-13A was developed using an *Escherichia coli* - *Arthrobacter* shuttle vector. *Arthrobacter* H-13A harbors 3 cryptic plasmids - pMVS100, pMVS200, pMVS300 - with molecular weights of 45,18.3 and 14.7 Kb, respectively. A 3.7 Kb restriction fragment derived from pMVS300 was cloned into pIJ30, a 3.6 Kb plasmid containing an ampicillin resistance gene and a thiostrepton resistance gene derived from *Streptomyces*. A 10.0 Kb recombinant plasmid, pMVS301,

was isolated from an ampicillin resistant transformant of E. coli DH1. This recombinant plasmid (pMVS301) was transferred into AS-50, a cured strain of H-13A lacking pMVS300, by polyethylene glycol assisted transformation of AS-50 protoplasts and screening for thiostrepton resistance. A 10.0 Kb plasmid, identical to pMVS301 by restriction analysis, was isolated from 2B1, a thiostrepton resistance transformant of AS-50. The cloned 3.7 Kb fragment, derived from pMVS300, contains an *Arthrobacter* origin of replication. The bifunctional plasmid, pMVS301, encodes ampicillin resistance in *E. coli* and thiostrepton and ampicillin resistance in *Arthrobacter* sp. H-13A and is capable of interspecies transfer and replication. This research represents the first report of plasmid transformation and of foreign gene expression in *Arthrobacter* species, allowing for the delineation of specific genes involved in the biosynthesis and regulation of biosurfactant synthesis in *Arthrobacter* sp. H-13A.

40. Environmental Stress-Mediated Changes in Transcriptional Regulation of Protein Synthesis in Crop Plants

J.L. Key

$85,618

The influence of high temperature stress and other environmental stress agents (e.g. heavy metals, water, etc.) on gene expression is under study in soybean and other major crop plants. The major focus is on heat shock (HS) and the analysis of HS genes, HS proteins, and the physiological significance of the HS response. A detailed analysis of the low molecular HS proteins of some 10 crop species has been made. All species tested synthesize from 12 (wheat) up to 27 (soybean) HS proteins of 15 to 25 kD; all of these accumulate during HS to become major abundant proteins (i.e. stainable by Coomassie blue or silver) in all species studied. One or more of these HS-induced proteins is present at stainable levels in control non-HS seedling tissues. A much less complex but similar pattern of high molecular weight HS proteins is synthesized in these species during HS, but their level of accumulation is generally not significantly greater under HS than non-HS conditions. The physiological significance of the HS proteins is also under investigation. Most of them differentially localize during HS, but most become soluble cytoplasmic proteins during recovery from HS (except those localized in chloroplasts and mitochondria); the HS proteins are stable for many hours and relocalize during a subsequent HS. The HS proteins appear to be involved in thermalprotection to otherwise lethal temperatures. In addition, cytoplasmic structure is maintained, though altered somewhat, during a tolerant (40°C for soybean) HS based on EM analyses. A lethal 45° HS totally disrupts the membrane structures of most cells; however, if the 45° HS is preceded by a 40° HS for 2 hr or more, the membrane structures are stabilized to damage at 45° as is seedling viability.

An analysis of the expression and structure of a number of HS genes has been made. Cadmium and arsenite induce a rather typical "HS" response, although the kinetics of HS mRNA and HS protein accumulation is somewhat slower than the response to HS. Most other common environmental stresses do not cause activation of the HS system to significant levels, although they often induce some minor changes in gene expression/protein accumulation.

41. Soybean Ribulose Bisphosphate Carboxylase Small Subunit Gene Family: Gene Structure and Regulation of Gene Expression

R.B. Meagher, Department of Genetics $73,000

The RuBPCss gene families have been characterized in a number of plant species. We have made quantitative sequence comparisons of ribulose bisphosphate carboxylase small subunit (RuBPCss) genes from monocots, dicots, algae and cyanobacteria. Our data suggest that, unlike globin and actin genes, RuBPCss genes accumulate replacement substitutions quickly in most of the coding region and very slowly in a minor portion. The RuBPCss gene family members within a species are highly homologous, while comparison of closely related plant species shows substantial divergence of the gene family members. This finding suggests that the RuBPCss gene family in higher plants may have undergone expansion and contraction in ancestral plants, resulting in concerted evolution of the genes within a species.

The RuBPCss gene family in soybean contains at least six members. We have shown that two of these genes, SRS1 and SRS4, are strongly light regulated at the level of transcription. These two genes account for 2-3% of the transcription in soybean leaf nuclei and 80% of the small subunit mRNA in leaves. Furthermore, the transcription of both genes is negatively controlled by darkness and far red light, proving that a phytochrome-linked response is involved. Experiments in transgenic plants indicate most of the information for this regulation is contained in the 5' portion of the gene. This region of DNA is being examined for the ability to specifically bind potential regulatory molecules in the light and in darkness. We hope to elucidate the molecular components of this transcriptional control system. An understanding of these DNA sequences and specific regulatory molecules should be useful in controlling the synthesis of foreign gene products in plant cells.

42. Microbiology and Physiology of Anaerobic Fermentations of Cellulose

H.D. Peck, Jr., L.G. Ljungdahl, L. Mortenson, J.K.W. Wiegel
Departments of Biochemistry and Microbiology $299,333

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

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

43. Phytochrome in Photosynthetically Competent Plants: Characterization by Monoclonal Antibodies

L. H. Pratt, Department of Botany $52,736

Green, photosynthetically competent plants respond to incident radiant energy in ways that influence their photosynthetic productivity and the partitioning within them of fixed carbon. Phytochrome is the chromoprotein responsible for mediating most of these photomorphogenic responses. Little is known, however, about phytochrome in green plants. Together with the observation that there is little of it in green plants, the recent discovery that it is immunochemically distinct from the phytochrome that is present in etiolated shoots of the same species and cultivar creates serious methodological difficulties. While it is not yet possible to determine whether the antigenic differences between phytochrome from green and etiolated shoots reflect differential posttranslational modification of the same gene product or the existence of two gene products, accumulating data tend to favor the latter possibility. Consequently, efforts are being initiated to test the hypothesis that a plant has at least two differentially regulated genes for phytochrome, one of which

is expressed abundantly only in total darkness. Because of the importance attached to obtaining antibodies specific to the immunochemically distinct phytochrome that is obtained from green plants, a major emphasis is being placed on production and selection of monoclonal antibodies with this property. Concurrently, those few monoclonal antibodies that recognize phytochrome from both green and etiolated plants are being used to begin physicochemical characterization of phytochrome from green shoots. Further characterization of this newly discovered phytochrome in green plant tissues will contribute to understanding how, at a primary molecular level, photomorphogenesis is regulated in photosynthetically competent plants.

44. Nitrogen Control of Photosynthetic Protein Synthesis

G. W. Schmidt, Department of Botany **$68,302**

Plant growth is severely affected by impaired photosynthesis resulting from nitrogen deficiency. The molecular basis of this effect is being studied in the green alga *Chlamydomonas* grown in continuous culture systems. Photosynthetic membranes of nitrogen-limited cells are dramatically depleted in chlorophylls, xanthophylls and proteins of the light-harvesting complexes. In contrast, enzymes of the reductive pentose phosphate cycle and electron transport chain complexes are reduced only 40-65% on a per cell basis comparison with nitrogen-sufficient cultures. From analyses of mRNA levels by in vitro translation and hybridization analyses with cloned DNA sequences for photosynthetic proteins, we have found there are rather minor effects of nitrogen deficiency on nuclear or chloroplast gene transcription. Moreover, the cells are not engaged in a generalized stress response as determined by direct comparisons of RNA and protein synthesis in heat-shocked cells. However, maturation of a transcript of the nuclear-encoded small subunit of ribulose 1,5-bisphosphate carboxylase is inhibited in nitrogen-deficient cells and causes accumulation of large amounts of mRNA precursors. Most of the effects of nitrogen deficiency on photosynthetic proteins appears to result from post-transcriptional regulatory processes: light-harvesting protein synthesis is sustained but their integration into photosynthetic membranes is impaired. Nitrogen-deficient cells lack violaxanthin. This pigment appears to be an essential component in the structure, function and biogenesis of the antenna complexes. Finally, the accumulation of massive amounts of starch and triglycerides in nitrogen-limited cells is being studied with regard to nitrogen-dependent changes in gene expression.

45. Transcriptional Analysis of the R Locus of Maize

S.R. Wessler, Department of Botany $100,000 (18 months)

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

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

UNIVERSITY OF GEORGIA - Tifton, GA 31793

46. 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 backgrounds, ploidy levels, and chromosome numbers crossed and backcrossed with different genotypes of diploid and tetraploid pearl millet, *P. americanum*, to produce partially fertile interspecific hybrids and derivatives. We have shown how valuable germplasm can be masked on certain genomes by other genomes and stored in a perennial or vegetatively propagated wild species such as *P. purpureum*. We have been able to transfer this hidden or stored germplasm to cultivated pearl millet and are in the process of evaluating it.

Partially fertile and obligate apomictic derivatives have been produced between pearl millet and *P. squamulatum* and show potential for transferring apomixis from the wild to cultivated species to fix hybrid vigor. Both cytogenetic and cell culture techniques are being tested to transfer the genes controlling apomixis. Diverse cytoplasms from the wild species have been transferred to pearl millet and are in the initial stages of evaluation. The overall impact is on increased, more efficient, and more reliable production of food, fiber, and forage.

HARVARD UNIVERSITY - Cambridge, Massachusetts 02139

47. Unravelling Photosystems

L. Bogorad, Department of Cellular and Developmental Biology

$175,000
(two years FY 85 funds)

The objective of this project is to identify and characterize essential protein components of the energy-transducing reaction centers in photosynthetic membranes and to understand how these components are arranged in the membrane. This information is essential for understanding how the photosynthetic apparatus converts light energy into electrical potential energy. In one of the two lines of research on which we have been concentrating, we have been determining the conformation, *in situ*, of the herbicide-binding protein of photosystem II: this protein normally binds quinone and is very likely at the reaction center of photosystem II. Synthetic peptides corresponding to stretches of this protein that have been predicted to be hydrophilic have been used to elicit antibodies. We have determined, for each antibody, whether it reacts with right-side out or with inverted vesicles made of photosynthetic membranes. From these data, we have come to a radically different conclusion regarding the arrangement of the polypeptide chain in the membrane than had been proposed previously: the earlier model was based on a mathematical treatment of amino acid sequence data derived from DNA sequences taking into consideration the nature of the residues. Our solution to the specific problems of this protein is important for advancing our understanding of the photosystem II reaction center and, furthermore, this general approach should be useful in further specifying the organization of proteins in photosynthetic membranes. In the second line of work, we have been developing a system for studying the functions of photosynthetic membrane proteins by isolating genes for these proteins from cyanobacteria, then modifying them, and reintroducing them. These comparatively simple organisms carry out the same type of oxygen-evolving photosynthesis as chloroplasts of higher green plants. Cyanobacterial genes corresponding to a few chloroplast genes for photosynthetic membrane proteins have been isolated from cyanobacteria and are being studied at the present time.

HARVARD UNIVERSITY - Petersham, Massachusetts 01366

48. Structure and Function of *Frankia* Vesicles in Dinitrogen Fixing Actinorhizal Plants

J.G. Torrey, Cabot Foundation, Harvard Forest $72,612

Frankia, a filamentous bacterium which induces N_2-fixing root nodules on the roots of a wide range of woody dicotyledonous plants, is the first known actinomycete which fixes dinitrogen when growing in free-living culture. The nitrogenase enzyme is induced in many strains of this organism by with-holding fixed nitrogen compounds from its nutrient medium. Terminal swellings of the bacterial filaments develop rapidly and acetylene reduction activity (= nitrogenase) increases in proportion to the number of terminal vesicles formed. The induction of vesicles and establishment of acetylene reduction occurs under aerobic conditions and the evidence is accumulating which demonstrates the existence of a multilaminate vesicle envelope which serves as a physical barrier protecting the oxygen-labile nitrogenase from denaturation. Our studies are concerned with the physiology, biochemistry and structural development of the N_2-fixing apparatus in *Frankia* grown *in vitro* and in root nodules of host plants. Diverse strains of *Frankia* are under study isolated and cultured from different host plants. Two strains have been studied especially HFPArI3, an isolate from nodules of the red alder *Alnus rubra* and HFPCcI3 isolated from root nodules of the tropical tree *Casuarina cunninghamiani*. The goal is to understand the structure and function which leads to optimum effectiveness for dinitrogen fixation.

During the past year, our studies have been extended to new strains of *Frankia*, HPFA11I1 from *Allocasuarina lehmanniani* and HFPGpI1 from *Gymnostoma papuanum*. These strains form vesicles in culture when induced by withdrawal of combined nitrogen from the medium but form no vesicles in symbiosis although they show acetylene reduction. The factors controlling these expressions are under study.

UNIVERSITY OF IDAHO - Moscow, ID 83843

49. Genetics and Chemistry of Lignin Degradation by *Streptomyces*

D.L. Crawford, Department of Bacteriology and Biochemistry $74,070

Current project objectives are (1) to utilize chemical mutagenesis to generate *Streptomyces* mutants which are either negative for or enhanced for production of enzymes involved in lignin solubilization, and in cellulose and hemicellulose degradation; (2) to generate *Streptomyces* mutants which are deregulated and constitutive for the production of extracellular lignin solubilizing enzymes, and (3) to clone the *Streptomyces viridosporus* gene(s) coding for lignin depolymerization into a nonlignolytic *Streptomyces, S. lividans*. Stable enhanced lignin-degrading and cellulose-degrading mutants of *S. viridosporus* have each been isolated and characterized. We

find that lignin degradation and cellulose degradation are apparently regulated independently. Using electrophoretic and other techniques we are now studying selected lignin degradation mutants in an attempt to isolate their extracellular lignin depolymerases. Methodologies for isolating lignin degradation deregulated mutants are being developed, and we hope to soon obtain mutants which produce ligninolytic enzymes in the absence of lignin inducer. We have refined our plasmid vector cloning system and are now using plasmid pIJ702 as a cloning vector. The system functions well, and we now have the capability to clone genes between *S. viridosporus* and *S. lividans*. Methods for the selection of *S. lividans* clones expressing genes coding for lignin depolymerization are being developed. Our ultimate goals are to isolate the genes coding for lignin degradation in *S. viridosporus*, identify the enzymes coded by those genes, and determine the role of each in lignin degradation.

UNIVERSITY OF ILLINOIS - Urbana, IL 61801

50. Fatty and Aromatic Acid Catabolizing Bacteria in Methanogenic Ecosystems

M.P. Bryant, Department of Animal Sciences $68,250

The objectives are to isolate and to determine the systematics, physiology, and catabolic biochemistry of syntrophic obligate acetate- and H_2-forming anaerobic bacteria that require coculture with a H_2-using methanogen or other hydrogenotroph to grow and catabolize saturated fatty acids and mono-aromatics. Anaerobic bacteria not requiring syntrophy are also of interest. We previously described *Syntrophobacter* that decarboxylates propionate to acetate and H_2, *Syntrophomonas* that *Beta* oxidizes C_4 and C_8 fatty acids to acetate and propionate (odd-numbered C fatty acids) and H_2, another *Syntrophomonas* that *Beta* oxidizes C_4-C_{18} fatty acids, and *Syntrophus* that catabolizes benzoate to acetate and H_2. A more versatile syntrophic bacterium, PA-1, catabolized several aromatics, glucose, aspartate, fumarate and pyruvate to acetate, CO_2 and H_2 but later lost its ability to catabolize the aromatics. It grows in pure culture by decarboxylating succinate to propionate or, on other non-aromatic compounds, when H_2 is removed by gasing the culture tubes. *Eubacterium oxidoreducens* catabolizes gallate (3,4,5-trihydroxybenzoate) via a decarboxylase and a hydroxyl shifting enzyme to phloroglucinol, an NADPH-linked reductase to dihydrophloroglucinol, a hydrolase to 3-hydroxy-t-oxohexanoate and hence to acetate and butyrate via CoA transferase, thiolase, enoyl-CoA hydrase, acyl-CoA dehydrogenase, phosphotransacetylase and acetate kinase activities. Exogenous formate or H_2 is required to generate the required NADPH. *Syntrophococcus* gen. nov. uses sugars as electron donors and H_2-using methanogen, methoxyaromatics or formate as electron acceptor systems and produces acetate and the corresponding hydroxyaromatic as the only organic products. It requires fairly polar lipid as a growth factor. Studies on the catabolic biochemistry continues.

51. Photosynthesis in Intact Plants

A.R. Crofts, Department of Physiology & Biophysics $96,200

The objectives of the project are to study photosynthesis in intact plants, either in the laboratory or under field conditions, with a view to determining whether limitations on photosynthesis under normal or stressed conditions of growth are attributable to inhibition of electron transfer or coupled energy transfer processes. This work will be complemented by studies on *in vitro* systems designed to aid in the understanding of the *in vivo* work. We have constructed several laboratory based instruments, and have also been developing portable instrumentation for use in field studies; a field flash-fluorescence photometer, and a fluorescence induction photometer have been built, and a field flash-spectrophotometer is currently under construction. 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. In our preliminary work, we will use the field spectrophotometer to study the electrochromic changes, which provide a built-in membrane voltmeter, and which allow reactions leading to the formation and decay of the proton gradient to be assayed. We will compare the electrochromic changes with those of the electron transfer components, and compare the results observed *in vivo* with those from *in vitro* experiments. 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 with the development of methodologies for optical resolution of the components of the water-splitting reaction. We will continue our studies of the two-electron gate in *Anycystis* strains showing natural herbicide resistance, or with the D_1 protein modified by site-directed mutagenesis.

52. Mechanism of Proton Pumping in Bacteriorhodopsin

T.G. Ebrey $53,150

The purple membrane of *Halobacterium halobium* probably represents the simplest biological solar energy conversion system. Light absorbed by bacteriorhodopsin, a small protein whose chromophore is retinal, directly leads to the transport of protons across the cell membrane. The resulting chemiosmotic potential can be used to make ATP. An additional feature of the purple membrane is its ability to pump protons over a wide variety of salt concentrations including extreme saline environments. This project investigates the relationship between the transport of protons across the membrane and structure and conformation of bacteriorhodopsin. We have made an especially intriguing discovery, that removal of the cations drastically alters both the color and the photochemistry of bacteriorhodopsin. The color can be returned to purple by any cation we have tried so far, but many aspects of the photochemistry depend on the type of cation.

53. Studies on the E. coli Respiratory Chain

R.B. Gennis, Dept. Chemistry & Dept. Biochemistry $82,732

The aerobic respiratory chain of *E. coli* is responsible for providing the energy required by the cell for oxidative phosphorylation and for driving active transport. The respiratory chain essentially directs electron flow from the oxidation or organic substrates (e.g. succinate) to oxygen, which is reduced to water. The electron flow is coupled to proton translocation across the cytoplasmic membrane, thus generating a proton motive force. This project will identify all the biochemical components of this system and explore the mechanism of proton translocation at the molecular level. Two major components have been identified as ubiquinol oxidases: the cytochrome *o* complex and cytochrome *d* complex. They carry out the same reaction and are redundant in the cell. Our research effort focuses on the cytochrome *o* complex. The enzyme is now purified, and contains four polypeptides by SDS-PAGE analysis. The redox centers appear to be iron-protoporphyrin IX and, probably, copper. Both monoclonal and polyclonal antibodies have been raised against the enzyme, but they appear to be non-inhibiting. The pure enzyme can be reconstituted into single-walled phospholipid vesicles and appears to insert uni-directionally. Electron transfer from quinol to oxygen in the reconstituted system proceeds rapidly and generates a transmembrane potential. Mutants deficient in cytochrome *o* have been obtained and mapped, and the gene coding for the enzyme has been cloned, resulting in considerable overproduction. The *cyo* gene has been localized on a 5.5 Kbasepair DNA fragment which has been cloned into pBR322. *In vitro* transcription/translation experiments with this plasmid show that it encodes the cytochrome *o* subunits. DNA sequencing of *cyo* is currently in progress.

54. Genetics of the Methanogenic Bacterium *Methanococcus voltae*

J. Konisky, Department of Microbiology $55,123

The overall goal of this project is to develop genetic systems and understand mechanisms of gene expression in methanogenic bacteria. We emphasize studies on *Methanococcus voltae*, a marine archaebacterium. One facet of the project involves isolation and characterization of methanogen plasmids from newly isolated methanococcal strains. We intend to exploit such DNA for both the construction of shuttle vectors for use in *Mc. voltae-E. coli* gene transfer and in studies on gene expression. The physiological role of such plasmids in methanogen metabolism is being explored. In collaboration with Dr. W. Whitman of the University of Georgia we are searching for methanogen viruses with the hope that they can be utilized to mediate gene transfer between methanogen strains. The ability to move methanogen genes between organisms would be very useful in the construction of appropriate strains for physiological and biochemical studies. Bromoethanesulfonate (BES) is a toxic analogue of methyl-CoM, a cofactor in methanogenesis. We are continuing our characterization of BES-resistant mutants and are currently focusing our attention on a characterization of the cell membranes prepared from mutant strains. It is anticipated that such studies will lead to new information concerning methanogen membrane structure and function.

55. 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 $173,207 (2 yrs, FY85 funds)

The project deals with underlying mechanisms for cytoplasmic male sterility (CMS) in maize, and with the molecular-genetic bases for both cytoplasmic and nuclear reversion to male fertility in the *cms-S* sterility system. Studies involve genetic and molecular characterizations of nuclear and mitochondrial DNA (mtDNA) of spontaneously occurring cytoplasmic and nuclear reversions of *cms-S* to male fertility. When cytoplasmic reversion occurs, the S1 and S2 mtDNA episomes may or may not be lost in the revertants, depending on the inbred line (nuclear) genotype. In two backgrounds examined, the episomes are no longer replicated in free state in the revertants, and in one, that of WF9, they are retained. Cytoplasmic reversion to fertility (forty cases analyzed) is invariably associated, regardless of nuclear genotype, with rearrangement of the integrated S1 or S2 episomal sequences in the main mitochondrial genome, and often with rearrangement of cytochrome oxidase (COI) gene sequences as well. Integrated S1 or S2 episomal sequences are disrupted in cytoplasmic revertants occurring in inbred lines M825 and 38-11, whereas integrated S1 and S2 sequences are intact in WF9 revertants. It appears that replication of the episomes depends on gene products encoded by intact integrated S1 and S2 sequences. Recent evidence indicates that, even without reversion, substitution of one nuclear genotype by another leads to reorganization of the mtDNA genome. These studies are being extended. In experiments under separate auspices we are attempting to isolate and characterize nuclear restorer genes (*Rf*) and to develop *Rf* probes that can be used to characterize the spontaneous nuclear revertants. These experiments now take advantage of the transposable properties of several of the *cms-S* spontaneous restorer genes in genetic protocols that have high selective efficiency for mutations involving insertions into already characterized wild-type genes such as $Adh1^+$, Sh^+, Wx^+ and Bz^+. We are also (DOE) analyzing strains that carry the newly-transposed *Rf* element in the absence of the original-site *Rf*, to determine whether (1) there are preferred integration sites, (2) transposition is to both linked and unlinked sites, (3) genetic characteristics of the transposed *Rfs* are altered in transit, (4) transposition is replicated or nonreplicative, and (5) the standard *Rf3* in chromosome 2L undergoes transposition as well.

56. Acetoclastic Methanogenesis

R.S. Wolfe, Department of Microbiology $85,212

The microbial formation of methane is carried out by a unique group of bacteria known as methanogens. These strict anaerobes are widespread in nature, found in diverse habitats (the rumen and caecum of herbivores, the intestine of mammals, the digestive tract of termites, sludge digesters, sanitary landfills, marine and freshwater aquatic sediments, marshes, and bogs) wherever active anaerobic biodegradation of organic matter occurs. Of the substrates used by methanogens, acetate, a major metabolic product by fermentative bacteria in these habitats, accounts for about 60%

of the methane formed. Our investigations focus on (1) the interaction of methanogens that use acetate (acetophilic methanogens) with organisms that produce it and (2) the biochemistry of methanogenesis. We have studied the microbial components that carry out the conversion of phenol and various derivatives of phenol to methane and carbon dioxide. The project objectives are to define the microbial interactions in the recovery of energy (methane) from anaerobic treatment of industrial wastes. Of the newly discovered coenzymes (methanofuran and tetrahydromethanopterin) of methanogens, we are studying the presence and activity of these cofactors in acetate-grown methanogens. Methanofuran was found in about the same amount (2 nmol/mg dry cells) in *Methanosarcina* grown on hydrogen and carbon dioxide or on acetate. Similar results were obtained for tetrahydromethanopterin. At the molecular level we have found that methanofuran isolated from *Methanosarcina* has properties that differ from the molecule isolated from *Methanobacterium*. We are determining the structural differences.

THE INSTITUTE OF PAPER CHEMISTRY - Appleton, WI 54912

57. Raman Microprobe Investigation of Molecular Structure and Organization in the Native State of Woody Tissue

R.H. Atalla, Chemical Sciences Division $48,696

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 aromatic rings of the phenyl propane units of lignin are oriented relative to the plane of the cell wall surface, and suggest the existence of nodes in the organization of lignin not unlike those known to occur in many celluloses. With respect to the carbohydrate components of the cell walls, the key findings are that cellulose is at least as highly organized in wood cell walls as in seed hairs and bast fibers, and that a high level of architectural complexity prevails in woody tissue. Plans for future work include more comprehensive mappings of molecular organization and compositional variation in cells from a wide range of morphological features. The effort will be greatly facilitated by assembly, this summer, of a Raman Microprobe system, with multichannel detection, optimized for our application, jointly funded by the DOE University Research Instrumentation Program, and The Institute of Paper Chemistry.

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.

UNIVERSITY OF KENTUCKY - Lexington, Kentucky 40546-0091

58. Photoinhibition of PSII Traps; Photoactivation and Reconstitution of the PSII Trap/Water Oxidizing Complex

G.M. Cheniae, Department of Agronomy $84,100

The goal of this project is to determine the minimum components (pigments, polypeptides, metals, quinones) required for efficient conversion of light into chemical energy and the oxidation of water to molecular oxygen. Ultimately, we want to understanding the topography/organization/folding patterns of polypeptides which impart water oxidizing activity to a tetra-Mn-complex yet shield the complex from reactions with reduced redox reagents causing inactivation of the Mn-complex and the dissociation of the Mn atoms. This work currently employs specific disassembly/-reassembly of components along with analyses of coupling of PSII traps to the water oxidizing complex, charge recombination reactions of PSII traps, and determination of number, the specific ligands, and the affinity constants of the Mn and Ca required in the PSII trap/water oxidizing complex. Using PSII membranes, we also are trying to put the kinetically well defined process of photoactivation of O_2 evolution on a chemical basis. This is a multi-quantum process which results in photoligation of Mn^{2+} into the tetra-Mn-complex which occurs in the absence of all PSII extrinsic polypeptides; however, reconstitution of membranes minimally with the 33 kDa polypeptide is required for expression of catalytic activity (O_2 evolution) by the tetra-Mn-complex. In the absence of water oxidation, PSII traps are susceptible to photoinhibition even at very weak photon flux densities. Such photoinhibition invokes a requirement of light dependent synthesis/assembly of the chloroplast encoded 34 kDa intrinsic polypeptide (D_2) for recovery from photoinhibition. We thus believe this photoinhibition somehow is inactivating the secondary donor, Z, to $P680^+$: we want to know the chemistry of the process.

LAWRENCE BERKELEY LABORATORY - Berkeley, CA 94720

Applied Science Division

59. Photochemical Conversion of Solar Energy

L. Packer, R. Mehlhorn and I. Fry $120,300

This project seeks to understand the mechanisms of energy conversion by photosynthetic cyanobacteria and by bacteriorhodopsin (BR), found in halobacteria. Photooxidative damage, which limits the efficiency of cyanobacteria, is also being studied. The studies of cyanobacteria include measurements of electrochemical membrane potentials in intact cells, analyses of the roles of photosynthesis and respiration in overall energy conversion, and assays of photooxidative damage, which seek to identify sources and targets of active oxygen molecules and the protective systems that guard against them. The interaction of these protective systems with

cellular energy pools is also being elucidated. An ESR oximetry method developed during the preceding year has provided detailed information about intracellular O_2 concentrations in cyanobacteria due to respiration and photosynthesis. Low temperature analyses of Cu-EPR spectra, characteristic of cytochrome oxidase, show that respiration plays a major role in enabling cyanobacteria to survive under stress. BR is of great interest as a model for synthetic photoelectrical devices because of its stability and simplicity. The approach being used for understanding its function is primarily chemical modification for amino acid side chains, to identify those that are involved in the different aspects of energy conversion. Optical and paramagnetic reporter groups on derivatized amino acids provide supportive structural data. Laser light scattering studies of larger BR arrays, including arrays of "white membranes", which lack the retinal chromophore, allow aggregation states to be determined and have shown that aggregation exerts marked effects on proton release.

Biology and Medicine Division

60. Resonance Studies in Photosynthesis

A. Bearden $62,600

Recently, mutants have become important adjuncts to photosynthetic research. These mutants have been characterized on a biochemical level, but there has been little or no characterization on a biophysical level; i.e, detecting mutant-affected specific electron carriers. This work, making use of quantitative electron paramagnetic resonance (EPR) and optical spectroscopic assays of photosynthetic activities, evaluates known mutants and also uses new mutants that will provide new data on structure-function relations in photosynthetic membranes. Algal mutants of *Chlamydomonas* and *Scenedesmus* currently available are claimed to have minimal Photosystem I (PSI) activity as measured by the quinol-plastiquinone oxidoreductase activity associated with the cytochrome b_6 - f system. An important question is whether a single component or a whole array of proteins are missing in these mutants? It is possible to generate new mutants that are sensitive to the electron transport inhibitor DCMU. In addition, we plan to isolate mutants with impaired function with additional specific inhibitors (antimycin A, DBMIB, DNP-INT, etc.). Other planned studies with cyanobacteria will utilize concomitant research using cloned genes for the various cytochrome complexes that will ultimately lead to experiments on site-directed mutagenesis. Another approach to be utilized is to generate mutants through chemical or radiation means as employed for yeast, but using an initial screening process based on antibody reactions and fluorescence. This screening would be followed by biochemical assays carried out on agar plates and then followed by more definite spectroscopic biophysical characterizations with individual strains.

Chemical Biodynamics Division

61. Light-Regulated Expression of Nuclear and Chloroplast Gene Expression

J.C. Bartholomew $58,000

The project objective is to gain information about how photosynthetic cells regulate the expression of the genes they have in their nuclear and chloroplast genomes. We are studying the factors that regulate the expression of the genes encoding the photosynthetic components of *Euglena gracilis*. We are testing the hypothesis that the organization of DNA 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 genes are replicated late. It is not known whether the transcriptional activity of the gene drives the replication order, or vice versa. We are studying the light and cell cycle regulated expression of genes coding for photosynthetic components of *Euglena gracilis*. We have studied the growth of wild-type and bleached mutants of Euglena in the dark and in the light to compare their cell cycle properties. We are cloning the genes for light-harvesting chlorophyll proteins (LHCP) from the genomes of these cells to determine their relative positions in the genome and to have probes for studying the expression and replication of the various members of this presumed multi-gene family. Once the cloning is complete, we will synchronize the cells at the beginning of their DNA synthesis period in the cell cycle by light-dark training and measure the time in this period that each member of the LHCP gene family is replicated. We want to determine if the order of replication and transcription is altered in dark grown cells relative to light grown cells. If expression drives the time in the DNA synthetic period that a gene is replicated, then mutants not expressing LHCP should replicate these genes late in S; whereas wild-type Euglena may even replicate the expressed copies of LHCP early and the silent copies late.

62. Regulation of Plant Gene Expression

J.A. Bassham $103,000

Mechanisms of regulation of genetic expression of photosynthetic and biosynthetic carbon paths in green plants are elucidated. Expression of enzymes as a function of species, development, and environment is evaluated through measurements of mRNA, polypeptides, enzyme activity, and so forth. Current investigations focus on expression of enzymes of the C_4 carbon metabolism shuttle mechanism, believed to be responsible for the high productivity of C_4 plants such as corn and sugar cane. Regulation of expression of these enzymes is followed in C_4 (especially maize) and in C_3 (especially wheat) plant tissues, in plant cells in tissue culture, and in isolated protoplasts and organelles. The timing of expression of key enzymes and the

transition from heterotrophic to autotrophic type in relation to developmental morphology is followed. The enzymes required for the C_4 carbon shuttle are expressed to some degree in the C_3 plant (wheat), although the amounts in most cases are much less. The leaf form of pyruvate, Pi dikinase (PPDK), which is nuclear-encoded, cytoplasmically synthesized, and processed after transfer through the chloroplast membrane into the chloroplast to give mature protein, is present in wheat leaves at about 1 to 2% of the level found in maize. There may be a small amount of intracellular carbon transport in wheat, which lacks Kranz anatomy and thus does not have an intracellular transport like C_4 plant leaves. Techniques of somatic fusion (including electrofusion, sorting of fusion products by flow cytometry, culturing of hybrids, and plant regeneration) will be used to investigate control mechanisms between chloroplasts and nucleus. Results are expected to provide information needed for future genetic engineering to improve productivity.

63. Plant Hydrocarbon Biosynthesis

M. Calvin, J.W. Otvos $77,000

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. The relative quantities of end products are controlled by enzymes at branch points in the subsequent conversion of IPP to larger molecules. Our present work is principally with *Euphorbia lathyris*, and focuses on (1) the characterization of hydroxymethylglutaryl CoA reductase (HMGR), which is assumed to catalyze the rate-limiting step between acetyl-CoA and IPP; (2) the nature of squalene cyclization to make sterols in this species; and (3) the characterization of subcellular organelles involved in terpenoid biosynthesis. These studies will provide information essential to future investigation of control of gene expression regulating the quantity and quality of plant hydrocarbon production.

64. Mapping Photosynthetic Genes in Prokaryotic and Eukaryotic Cells

J. Hearst

$208,500

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

Recently it has been shown that lowering O_2 concentration in cultures of *R. capsulata* and *R. sphaeroids* increases the level of mRNA for LH-I, RC and LH-II complexes, while the amount of RNA for pigment biosynthetic enzymes shows only a small increase when the O_2 concentration is reduced. According to one set of experiments, the genes for Crt biosynthesis are not directly regulated by O_2. More recently a sequential and independent expression of LH-I and LH-II genes has been shown in *R. capsulata* during ICM development.

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

65. Mechanism of Water Splitting and Oxygen Evolution in Photosynthesis

M.P. Klein $145,000

Oxygen evolution in photosystem II of green plants is thought to involve reactions through a cycle of four states by which electrons are removed from H_2O and donated to the oxidized $P680^+$ reaction center. The most successful interpretation of the data regarding this cycle is Kok's S-state scheme, which postulates a series of five (S_0-S_4) states through which electrons are cycled during oxygen evolution. Manganese is thought to play a central role in these reactions. X-ray absorption spectroscopy using synchrotron radiation is used to determine the structural and electronic state(s) of the manganese sites. In Photosystem II particles of both spinach and the cyanobacterium *Synechococcus*, we have determine that the manganese occur as a binuclear complex with Mn-Mn separation of about 2.7 A. We have also observed an absorption edge energy shift of manganese upon the transition from S1 to S2, which implies an oxidation state change of manganese. These studies provide the first direct evidence that manganese is directly affected by the light reactions. Both X-ray absorption and high resolution EPR spectroscopy indicate that chloride, essential for oxygen evolution, is not a ligand of manganese. We are beginning to employ ENDOR and electron spin echo spectroscopy to determine the types and numbers of atoms that are ligands to manganese, the locus of halide binding, the sites of water entry into the complex, the modifications to the complex as the 16, 24 and 33 kDA polypeptides are removed and the topology of the manganese complex relative to other redox sites in the photosynthetic apparatus.

66. Tissue Specific Gene Regulation

F. Leach, J.C. Bartholomew, J.E. Hearst $58,000

Our goal in this research project is to identify cellular factors which regulate the tissue specific expression of plant genes. There is some evidence that the expression efficiency of genes on the T-DNA of Ri plasmids is dependent upon the plant tissue in which the DNA resides. It is our hypothesis that it is factors within the different tissues which regulate the efficiency of expression of different genes on the T-DNA. Our approach to testing this hypothesis is to use a short term transfection system to introduce into protoplasts from the roots and leaves of *Nicotiana tobacum* the Ri T-DNA, and then to measure the transcription efficiency of the different open reading frames. From the known DNA sequence of the Ri T-DNA we will identify promoters which are controlling the transcription of the tissue specific regulated genes. These promoters will be linked to the gene coding for the chloramphenicol acetyltransferase (CAT) enzyme. The activity of this enzyme can easily be measured from cell extracts. By transfecting these tissue specific CAT

plasmids into protoplasts in which the promoter is normally inactive followed by harvesting the transcription complexes from these cells, and adding extracts from tissues in which the promoters are active, we will develop an assay for tissue specific promoter activators. Using this assay we will begin purifying the various factors from cells that confer tissue specificity on the promoters. These studies will give a better understanding of the mechanism of tissue specific regulation of gene activity, and may allow the genetic modification of plants to allow for tissue specific production of gene products.

67. Chemistry of Phycobiliproteins and Phytochrome

H. Rapoport $63,000

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.

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

68. Photosynthetic Membrane Structure and Photosynthetic Light Reactions

K. Sauer $181,500

Through measurements of the nanosecond decay kinetics of fluorescence from photosynthetic membranes, we are able to monitor the dynamics of excitation transfer among the antenna pigments and of trapping in the reaction centers. A model is emerging that describes how the light-harvesting pigments are organized and how this organization changes as a consequence of the metabolic control processes. To simplify the analysis, we are examining the behavior of isolated sub-complexes associated with photosystems I and II of higher plants as well as for the cyanobacterium *Synechococcus*. Particularly for PS II we are studying the association of particular functions (quinone binding, chlorophyll binding, manganese binding, reaction center activity) with specific proteins in the complex. To further physical measurements (e.g., optical absorption, EPR, and x-ray spectroscopies), we are preparing complexes highly enriched in the components under investigations, and we are monitoring the

relevant activities to insure the integrity of the complexes. After initial identifications, we are determining N-terminal amino acid sequences. Based on the sequence information, we will construct probes to locate the genes that code for these proteins and ultimately will clone and sequence the genes. In parallel we will attempt crystallography of the important membrane sub-complexes to determine their structure by x-ray diffraction analysis. A similar study is underway for the reaction center complex of the photosynthetic bacterium *Rhodopseudomonas capsulata*.

LEHIGH UNIVERSITY - Bethlehem, PA 18015

69. A Genetic Approach to Secretion and Hyperproduction of Cellulase by *Trichoderma*

B.S. Montenecourt, J. Sands, Department of Biology $79,750

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 cellobiohydrolase in the endoplasmic reticulum, Golgi and secretory vesicle regions, confirming that this general pathway, which has been firmly identified in yeast and mammalian cells, also exists in *Trichoderma*. Temperature sensitive secretion mutants have been isolated and one mutant, LU-ts 1, was shown to be ts for cellulase and not for protease, amylase and xylanase. At the non-permissive temperature heterogeneous protein bands are exhibited by this mutant which are inactive. This characteristic of ts for secretion is reversible. At the permissive temperature normal cellulase enzymes are secreted.

LOS ALAMOS NATIONAL LABORATORY - Los Alamos, NM 87544

70. Study of Plant Cell Wall Biosynthesis and Structure

J.W. Heyser, L.O. Sillerud $96,000

This project focuses on the application of multinuclear magnetic resonance (NMR) methods to the study of basic mechanisms in plant growth and metabolism. The non-invasive and real-time nature of high resolution NMR techniques allow *in vivo* monitoring of metabolism of natural abundance and ^{13}C-enriched compound and ^{31}P high energy compounds. Cell wall biosynthesis by millet tissue cultures (a Monocotyledon) is being studied by NMR. Cell walls isolated from cell suspensions and protoplasts fed (1-^{13}C)-glucose, (1-^{13}C) or (2-^{13}C) labeled arabinose, or other ^{13}C labeled sugars have been fractionated into major polysaccharides including cellulose, pectins, hemicelluloses, etc., and further characterized by *in vitro* ^{13}C-NMR and gas chromatography/mass spectroscopy (GC/MS) methods. The proso millet *(Panicum miliaceum)* suspension culture constitutes a reasonable model for the study of cell wall biogenesis in embryonal cells of a graminaceous species. Their cell wall composition is remarkably consistent throughout the culture cycle. Covalent linkages were determined by corresponding chemical shifts *in vitro* ^{13}C NMR spectra. The energy state of cells will be determined by ^{31}P NMR. A long term aeration system will be developed so that the incorporation of ^{13}C labeled sugars into the cell walls of intact cells can be monitored for periods of 24 to 48 hours. An arabinogalactan glycoprotein, secreted into the culture medium and reaching about 20% for the total dry weight of the cells, is being characterized by GC/MS and NMR analysis. Its relationship as a soluble secretory product to similar hemicelluloses found in primary cell walls is being determined. The new Bruker 400 NMR spectrometer located in the Life Sciences Division is one of the highest resolution spectrometers that is presently available for basic studies of plant metabolism and has been in operation for one year. Its use will greatly facilitate our studies and our rate of progress for these NMR studies.

MARTIN MARIETTA LABORATORIES - Baltimore, MD 21227

71. Studies of Photosystem II Using Artificial Donors

R.J. Radmer, Martek Corp., Columbia, MD 21046 $55,000 (8 months)

The objective of this project is to study the mechanism of photosynthetic water oxidation by the use of artificial donors. The approach uses specialized mass spectrometry and flash-kinetic spectrophotometry techniques developed in-house.

Experiments done in collaboration with Dr. G. M. Cheniae and colleagues (Univ. of Kentucky) used O_2, N_2, and TMPD-oxidation flash yield measurements to characterize PS II preparations modified by the removal of specific polypeptides. These experiments

showed that, for example, removal of the 17 and 23 kD proteins modified specific PS II reactions (i.e., it increased the charge-loss reactions and the miss parameter alpha) but did not abolish the overall water-oxidation process. We proposed a $Q^-/'C'/Z^+(S_2)$ cycle to rationalize the results of these studies. These and other data suggest that the 17 and 23 kD proteins do not have a direct role in the O_2 evolution pathway, but rather serve as buffers, modifying the effects of Cl^-, Ca^{++}, etc. on membrane components.

A mass spectrometer system was constructed which allowed the chloroplast milieu to be rapidly changed before isotopic analysis of O_2 flash yields. Experiments in which chloroplasts in $H_2{}^{18}O$ were preilluminated with one or two flashes before being washed in unlabeled H_2O showed that the higher oxidation states of the O_2-evolving system contained no appreciable bound, non-exchangeable H_2O. This suggests that H_2O oxidation takes place via a rapid, concerted, all-or-none mechanism, rather than by a mechanism involving stable, partially oxidized H_2O-derived intermediates.

UNIVERSITY OF MARYLAND - College Park, MD 20742

72. Energy-Dependent Calcium Transport Mechanisms in Plant Membranes

H. Sze, Department of Botany **$63,411**

Though changes in cytoplasmic calcium (Ca^{2+}) levels in cells modulate several functions important for plant growth and development, the mechanisms of active and passive Ca^{2+} transport are poorly understood. The objectives of this project are to identify and characterize active Ca^{2+} transport systems using isolated organelles or purified membrane vesicles from oat roots *(Avena sativa)* and carrot suspension cells *(Daucus carota)*. A Ca^{2+}/H^+ antiport has been identified in tonoplast vesicles prepared from isolated vacuoles. ATP-dependent Ca^{2+} transport in tonoplast vesicles was dependent on a pH gradient and was vanadate-insensitive and nitrate-sensitive. Thus Ca^{2+} accumulation into vacuoles depends on a proton motive force generated by the tonoplast H^--pumping ATPase. Using an artificially-imposed pH gradient, the properties of the Ca^{2+}/H^+ antiport has been directly studied. Another Ca^{2+} pump, dependent directly on the Ca-ATPase, has been localized on the endoplasmic reticulum. The relative importance of these Ca^{2+} porters are being evaluated. To understand how Ca^{2+} fluxes are regulated, we are studying the effect of modulators, phytohormones and toxins on various Ca^{2+} transport systems. The toxin of *Helminthosporium maydis* T decreases active Ca^{2+} uptake into mitochondria of susceptible corn but not resistant corn. The toxin induces an increase in membrane permeability to Ca^{2+} and H^+. The specific mode of toxin action is being investigated. These studies will help us understand how Ca^{2+} transport and its regulation modulate plant growth and development.

MASSACHUSETTS INSTITUTE OF TECHNOLOGY - Cambridge, MA 02139

73. Antibody Analysis of the Rhizobium meliloti Surface

E.R. Signer, Department of Biology $82,732

The formation by rhizobia of nitrogen-fixing nodules on the roots of leguminous plants presumably involves communication between bacterial and plant cells, and is thus likely to depend on interactions between the surfaces of the two symbiotic partners. We are using monoclonal antibodies to probe the surface of the alfalfa symbiont *Rhizobium meliloti* SU47, in order to identify surface antigens involved in nodulation and fixation, and to isolate mutants with various symbiotic defects. Mutants of one class, deficient in exopolysaccharide, form nodules that fail to fix nitrogen and lack the root-hair curling and infection threads characteristic of wild-type nodules; the mutants invade directly through the root epidermis, and within the nodule are found only between cells in superficial root tissue. Analysis of the mutant loci has revealed a previously uncharacterized megaplasmid, distinct from a megaplasmid of similar size already known to carry other symbiotic loci. Coinoculation with exopolysaccharide-producing bacteria allows the mutants to invade intracellularly, but they still fail to fix nitrogen or to differentiate into bacteroids, suggesting exopolysaccharide is involved in a complex way. Mutants of a second class have a somewhat similar phenotype, but appear to be altered in lipopolysaccharide instead. These studies should eventually reveal the role of these defined molecules in symbiosis, which in turn should ultimately help in the genetic manipulation of rhizobia for improved efficiency of nodulation and/or nitrogen fixation in the field.

UNIVERSITY OF MASSACHUSETTS - Amherst, MA 01003

74. Conversion of cellulose to ethanol by mesophilic bacteria

E. Canale-Parola, C.E. Dowell, Jr., S.B. Leschine $75,036

The project objective is to study the genetics of mesophilic anaerobic bacteria that ferment cellulose to ethanol. Bacteria used in this investigation are strains of *Clostridium* that (1) convert to ethanol not only cellulose, but also components of the hemicellulosic portion of biomass and (2) are relatively ethanol tolerant. Studies include plasmid analysis and fine-structure mapping of genes involved in the breakdown of cellulose to soluble sugars. These genes code for cellulase system enzymes such as endo-1,4-beta-glucanases, exo-1,4-beta-glucanases, beta-glucosidases,

and cellobiose phosphorylase. The research includes characterization of cellulase system enzymes present in ethanol-producing mesophilic clostridia as well as enzymes used by these bacteria in the breakdown of hemicellulosic components. This work will provide fundamental information on the genetics of cellulose-fermenting, ethanol-producing clostridia. This information will be valuable for understanding metabolic processes used by anaerobic bacteria to degrade cellulose, and for developing clostridial strains that can be used in the industrial conversion of biomass to ethanol. Finally, the research will elucidate basic aspects of the genetics of clostridia. At present, very little is known about the genetic systems of this large group of anaerobic bacteria.

MEHARRY MEDICAL COLLEGE - Nashville, TN 37208

75. Respiratory Enzymes of *Thiobacillus ferrooxidans*

R.C. Blake, II, Biochemistry Department $70,515

Certain chemolithotropic bacteria inhabit ore-bearing geological formations exposed to the atmosphere and obtain all of their energy for growth from the dissolution and oxidation of minerals within the ore. Despite the environmental and economic importance of these organisms, very little basic information is available concerning the identity and disposition of the respiratory enzymes responsible for these activities. The aim of this research is to initiate the systematic isolation and characterization of the respiratory enzymes expressed by these chemolithotropic bacteria when grown on both reduced metal substrates and reduced inorganic sulfur compounds. Our current focus is on the iron-oxidation system of *Thiobacillus ferrooxidans*. Rusticyanin, a soluble blue copper protein alleged to be the initial electron acceptor from ferrous ion, has been purified to electrophoretic homogeneity. Stopped-flow spectrophotometric studies indicate that a soluble, uncharged $Fe\text{-}SO_4$ complex is the actual reductant of purified rusticyanin, but that the rate constant for reduction is several orders of magnitude too low to explain the facile reduction of cytochromes by ferrous ion in the intact organism. We have identified and are currently purifying an acid-stable cytochrome *c* which is reduced very rapidly by ferrous ion. The project will eventually contribute to a basic understanding of biological energy transduction. It can also provide useful information toward manipulating *T. ferrooxidans* and related organisms for commercial use.

MICHIGAN STATE UNIVERSITY - East Lansing, MI 48824

76. Molecular cloning of the Genes Encoding Certain Key Enzymes Involved in Lignin Biodegradation by *Phanerochaete chrysosporium*

C.A. Reddy, Department of Microbiology & Public Health $56,760

Lignin is the most abundant renewable aromatic polymer in the biosphere. Lignin catabolism and its potential utilization as a source of aromatic chemical feedstock are of considerable interest. The objective of this project is to clone and characterize selected genes involved in lignin degradation by a white-rot fungus *P. chrysosporium*. We have focused on isolation, identification, and characterization of ligninase cDNA clones. Several cDNA clones have been isolated and were shown to be ligninase cDNA clones. Detailed restriction maps will be determined for selected clones. Genomic fragments with homology to the clones of interest will be identified. Appropriate Northern blotting experiments will be performed to demonstrate that the clones are unique to 6-day mRNA. Complete nucleotide sequencing will be performed on the most desirable clone(s). cDNA insert from such a clone would be subcloned into an appropriate yeast expression vector to obtain expression of an active ligninase protein. Physical and catalytic properties of this protein would be compared to the enzyme produced by wildtype *P. chrysosporium*.

77. One Carbon Metabolism in Anaerobic Bacteria: Organic Acid and Methane Production

J. G. Zeikus, Department of Biochemistry $86,580

Our project deals with understanding of one- and multi-carbon metabolism in acetogenic and methanogenic bacteria. We study the biochemistry of acetate catabolism in *Methanosarcina barkeri* in relation to various component enzymes, co-factors, and intermediates. Biochemistry of CO conversion to hydrogen is examined by studying CO dehydrogenase and hydrogenase including cloning, their genes and analysis of enzyme structural functional features. Isotope tracer studies and other biochemical-genetic approaches are also used in these studies. In our studies with the acetogen, *Butyribacterium methylotrophicum*, we focus on the regulation of carbon and electron flow in relation to fermentation of multicarbon compounds, catabolic enzyme activities and hydrogen cycling during fermentation of various compounds. The relationship between one- and multi-carbon metabolism is examined by studying catabolite repression mutants of *B. methylotrophicum*. We also conduct studies of membrane bound electron carriers and other components coupling electron transport of the proton motive force (PMF) in *B. methylotrophicum*.

MICHIGAN STATE UNIVERSITY/DOE PLANT RESEARCH LABORATORY - East Lansing, MI 48824

78. Differential Gene Expression in *Bradyrhizobium*

B.K. Chelm $163,000

The interaction of bacteria of the genera *Rhizobium* and *Bradyrhizobium* with a legume host to establish a symbiotic, nitrogen-fixing relationship requires a series of developmental steps in both the bacterium and the host plant. The project objective is to identify the underlying molecular mechanisms by which the expression of the bacterial genome is regulated during this process. We have focused on the *B. japonicum*-soybean system. Specific genes whose expression is regulated during nodule development or by other physiological changes and genes involved in the regulation of these expression changes have been isolated. These genes have been isolated by hybridization screening procedures using analogous genes from other organisms as hybridization probes, by complementation of genetically-defective bacterial strains by cloned *B. japonicum* DNA, or by protein purification and sequencing followed by oligonucleotide synthesis. The genes isolated thus far include those encoding the nitrogenase subunits, glutamine synthetases I and II, the large subunit of ribulose bisphosphate carboxylase, deltaaminolevulinate synthetase, adenylate cyclase, and several nif regulatory genes termed nifA-like genes. The expression patterns and structures of these genes are being further characterized. Promoter regions are being localized by S1 protection analysis, and the nucleotide sequences of these regions are being determined. Current research is aimed at understanding the molecular mechanisms that act during the nodulation process to regulate the activities of these genes. We are also investigating the biosynthesis of heme in nodules.

79. Resistance of Crop Plants to Environmental Stress

A.D. Hanson $158,000

Plants have biochemical or metabolic adaptations to environmental stresses, as well as adaptations expressed at higher levels of organization. Biochemical and genetic understanding of metabolic adaptations to stress would allow them to be used in crop improvement, via conventional or recombinant DNA technologies. The project objectives are (1) to identify adaptive metabolic responses of plants to stress, (2) to find the enzymes and genes involved, and (3) to explore the effect on the whole plant of genetically modifying metabolic adaptations. We are investigating two metabolic responses to stress: betaine accumulation and lactate glycolysis. During water- and salt-stress, certain plants accumulate betaine. Indirect evidence indicates that betaine acts as a non-toxic cytoplasmic osmoticum during stress. We have shown that betaine is synthesized in the chloroplast by a two-step oxidation (choline → betaine aldehyde → betaine) and that the second step is catalyzed by a stomal, NAD-linked dehydrogenase. We are isolating this enzyme and studying its regulation

by salinity stress. Lactate dehydrogenase (LDH) activity is induced by hypoxia in cereal roots and catalyzes lactate glycolysis under hypoxic conditions. Lactate glycolysis plays a key role in the regulation of cytoplasmic pH during hypoxia. We have purified barley LDH and have used antibodies against it to demonstrate that enzyme induction involves an increase in LDH protein synthesis. We are now proceeding to cDNA cloning of barley LDH.

80. Action and Synthesis of Plant Hormones

H. Kende $197,000

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-adenosylmethionine. The first enzyme in this pathway, 1-aminocyclopropanel-carboxylate (ACC) synthase, is usually the limiting enzyme in ethylene synthesis. It can be induced by a variety of chemicals and conditions, including stress. We have purified this very important enzyme in plant development and have produced antibodies against it, enabling us to study its regulation at the molecular level. We are also investigating the enzyme responsible for ethylene formation from ACC. We have characterized some of its properties in isolated vacuoles and found that it requires membrane integrity and probably a transmembrane ion gradient. We are attempting to reconstitute it after breakage of membranes. The role of stress ethylene is being investigated in deepwater rice where low-oxygen stress occurring during submergence induces ethylene biosynthesis. Ethylene, in turn, mediates the growth response of submerged plants. We are investigating the effect of ethylene on a number of biochemical processes that are related to the growth response. We are attempting to localize the site of action of cytokinins using a cytokinin photoaffinity probe. We have developed several methods to synthesize such a probe, namely 8-azido-benzyladenine ($8N_3BA$). Identification of cytokinin receptors will be attempted in cultured tobacco cells and in protonemata of mosses, both of which respond to cytokinins.

81. Cell Wall Proteins

D.T.A. Lamport $122,000

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 anticlinal (radial) orientation *in muro*. Thus the long axes of cellulose

(warp) and extensin (weft) may be in perpendicular planes. The presence of the cross-linked amino acid isodityrosine in covalently bound extensin implies that cross-linkage of extensin monomers occurs *in muro*. We suggest that extensin networks of defined porosity form around microfibrils, thereby mechanically coupling the load-bearing polymers. Current work deals with the two extensin precursors P1 and P2 (i.e., their primary structure, EM visualization, and immunochemistry). P1, P2, and their HF-deglycosylated polypeptides dP1 and dP2 were antigenic, eliciting four sets of rabbit polyclonal antibodies that cross-react specifically with the glycosylated and non-glycosylated epitopes whose primary structure was further elucidated. After HF-deglycosylation, and tryptic degradation of dP1 and dP2, a relatively few major peptides dominate each peptide map: (1) P1/H5 Ser-Hyp-Hyp-Hyp-Hyp-Thr-Hyp-Val-Tyr-Lys; (2) P1/H20 Ser-Hyp-Hyp-Hyp-Hyp-Val-Lys-Pro-Tyr-His-Pro-Thr-Hyp-Val-Tyr-Lys; and (3) P2/H3 Tyr-Lys, P2/H4 Ser-Hyp-Hyp-Hyp-Hyp-Val-Tyr-Lys. Tryptic degradation was essentially complete; these major peptides represent extensively repeated units, hence a highly periodic polypeptide. The hexapeptide Val-Lys-Pro-Tyr-His-Pro domain of the P1 hexadecapeptide H20 is the prime candidate for intermolecular cross-linkage. If so the average intermolecular cross-link frequency corresponds remarkably to that predicted for a network penetrated by cellulose microfibrils.

82. Regulation of Flowering

A. Lang $77,000

This project studies promotive and inhibitory factors in flower formation, particularly graft-transmissible, hormone-like materials (florigen and antiflorigen). The evidence for florigen and antiflorigen is so far physiological, based primarily on grafting experiments between plants maintained in flowering and nonflowering conditions. The next obvious step would be to isolate the responsible chemical compounds and determine their structures. For florigen, this has been repeatedly attempted but so far without success. Our major effort is directed at isolation and identification of antiflorigen; solution of this problem may also aid in the search for florigen. The work is being done using extraction and diffusion techniques, and tissue explants capable of regeneration of flowers as bioassay. In a related, physiological project it has been established that a short-day plant, the Maryland Mammoth cultivar of tobacco, does not seem to form antiflorigen when grown on long days in contrast to two related long-day plants, *Nicotiana silvestris* and *Hyoscyamus niger*, which under short days produce potent antiflorigen. In a third project it has been found that recently synthesized, powerful inhibitors of gibberellin synthesis (A-Rest, Tetcyclasis) inhibit stem growth, but have no effect on flower formation in a long-day, a short-day and a day-neutral tobacco. In previous work with the long-short-day plant *Bryophyllum* it had been found that gibberellin does participate in the endogenous regulation of flower formation. The new results indicate that this does not hold for plants in general.

83. Interaction of Nuclear and Organelle Genomes

L. McIntosh $163,000

The biogenesis of energy transducing mechanisms in oxygenic-photosynthetic organisms requires the regulation of single genes, or gene "families", and the stepwise assembly of soluble and membrane-bound complexes capable of photosynthetic and respiratory functions. In higher plants the biogenesis of organelles requires the coordinate expression of nuclear, mitochondrial, and chloroplast genomes. Oxygenic photosynthesis in cyanobacteria is essentially identical to that of higher plants. However, a key difference is that there are no organellar compartments, thus, making the genetic determinants more available for molecular/genetic modification.

We are pursuing gene-directed modification of photosynthesis using two approaches. First, we are studying the genes encoding photosystem II (PS II) polypeptides in the photoheterotrophic transformable cyanobacterium *Synechocystis* 6803. Several families of genes involved in PS II are being characterized including *psbA, psbD* and the gene(s) encoding the extrinsic 33 kilodalton polypeptide. Gene destruction with mutant recovery on 5 mM glucose has been employed for the *psbA* gene family in order to determine more specifically its electron transport functions. Secondly, we are investigating the molecular mechanisms of assembly and catalysis for ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco). We have expressed an active Rubisco, L_8-S_8 form, holoenzyme in *E. coli* and are employing *in vitro* mutagenesis to study the function of the small subunit in assembly and catalysis.

84. Sensory Transduction in Plants

K.L. Poff $122,000

The primary objective of this project is to understand the mechanisms of environmental information *via* light reception. We are studying the blue light photoreceptor pigment system(s), which control(s) numerous light responses such as phototropism in flowering plants, and are using specific inhibitors and mutants as probes to dissect the initial steps in the transduction sequence. A screening procedure using a pulsed light stimulus has been devised and used to isolate 35 mutants of *Arabidopsis* with an altered phototropic response. Geotropism for these photo-minus strains falls into three classes: normal, impaired, and lacking. The photo-minus, geo-normal phenotype should represent an alteration early in the phototropism pathway and could arise from an altered photoreceptor pigment. We are continuing to study the convergent pathways for phototropism and geotropism through mutant isolation, and genetic, physiological, and biophysical characterization. This genetic approach should permit positive identification of the photoreceptor pigment, access into the transduction sequence, and eventual understanding at the molecular level of the events from photoreception to the bending response.

85. Physiological and Molecular Genetics of *Arabidopsis*

C.R. Somerville $168,000

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 mutational analysis of carbohydrate and lipid metabolism, and with improving the suitability of *Arabidopsis thaliana* as a facile system for molecular genetics. In this latter respect, since it would be very useful to be able to isolate genes which have been identified by transposon tagging, we have initiated a series of experiments to introduce a transposable element into *Arabidopsis*. We are also exploring the utility of genetic selection schemes which may permit the isolation of an endogenous transposon. Our physiological studies with *Arabidopsis* are directed towards determining the functional significance of several enzymes involved in photorespiratory metabolism by the isolation of appropriate mutants. We are also examining the relationship between membrane lipid composition and chilling resistance by the isolation and biochemical characterization of single gene mutations which alter the leaf membrane fatty acid composition of *Arabidopsis*. We are investigating the basis for the observation that alteration of source/sink ratios are frequently accompanied by a change in the photosynthetic capacity of the plant by the physiological analysis of mutants which are unable to synthesize leaf starch and, therefore, have genetically altered source/sink ratios.

86. Molecular Basis of Disease Resistance in Barley

S.C. Somerville $133,000

Plant diseases are considered a major limitation to crop yields. However little is known of the molecular basis of disease development or host resistance mechanisms. The long term goal of this project is to develop a description of the biochemical events of the infection process in compatible and incompatible combinations of barley lines and *Erysiphe graminis* f.sp. *hordei* races. *E. graminis* is the causal agent of powdery mildew disease. Our approach is to identify a host resistance gene product, the *Reg* 1 gene product, using monoclonal antibodies as probes for antigenic differences between resistant and susceptible congenic barley lines. Since *E. graminis* infection is confined to the epidermis, we have included a preliminary screen for epidermal-specific monoclonal antibodies.

In addition we have established a collection of mutants of *Arabidopsis thaliana* defective in anthocyanin biosynthesis. Anthocyanins accumulate in the epidermal tissues in response to a variety of environmental stresses including high light, drought, mineral deficiencies, and growth on adenine, auxins or +/- cinnamic acid. Among the collection of mutants we hope to identify regulatory genes whose products mediate cellular responses like anthocyanin biosynthesis to environmental signals.

87. Developmental Biology of Nitrogen-Fixing Algae

C.P. Wolk $163,000

Certain cyanobacteria (blue-green algae) trap solar energy via photosynthesis and use the resulting chemical-reducing power to fix atmospheric nitrogen gas (N_2). They thereby produce ammonia, which is used as the nitrogen source for cellular growth. The initial steps of N_2 assimilation take place in specialized cells called heterocysts. The project objective is to understand the supply of energy and electrons to, and the oxygen protection of, nitrogen fixation within heterocysts. Our approach makes use of our recently developed methodology for introducing cloned genes into nitrogen-fixing cyanobacteria by conjugation from *Escherichia coli*. Derivatives of *Anabaena* 7120 and *Nostoc* 29150 unable to fix nitrogen under aerobic conditions were isolated. The heterocysts of certain of these mutants lack a single envelope glycolipid, whereas others appear defective in their envelope polysaccharides. These mutants are probably defective specifically in protection of nitrogenase from oxygen. Attempted complementation of the mutants is in progress. Upon transfer to strains of *Anabaena* of *lux* genes from *Vibrio fischeri* and *Vibrio harveyi*, the cyanobacteria become capable of light production. Insertion of strong cyanobacterial promoters upstream from these genes can lead to a hundred-fold increase in luminescence; *lux* genes therefore have potential for transcriptional reporting from cyanobacteria. Site-specific, insertional mutagenesis is being attempted using antibiotic resistance genes transcribed from such strong promoters. 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.

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

J.A.D. Zeevaart $135,000

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 is to characterize the hormones involved, how their synthesis and catabolism are regulated by the environment, and how these hormones act. We are investigating stem growth and flowering in rosette plants, and have found with a cell-free system from spinach leaves that two enzymes involved in the synthesis of active gibberellin (GA) are under photoperiodic control. The activities of these enzymes increase in plants under long days, while their activities decrease rapidly in darkness. Thus, under long days the GA biosynthetic pathway is turned on, which causes an increase in GA production, and this results in stem growth. Further work is aimed at elucidating how light affects the GA enzyme activities. Single-gene dwarf mutants of tomato are used to study the role of GA in flower development.

Although flower primordia are initiated in these GA-deficient mutants, abortion occurs at an early stage. If GA is applied to the young flower bud cluster, normal flower development and fruit set occur. We are now studying the stage at which flower bud development is arrested in the absence of GA. In our studies on the biosynthesis of abscisic acid (ABA) we are comparing stress-induced accumulation of ABA in leaves and roots, since these two organs differ greatly in their contents of xanthophyll, the putative precursor of ABA. After rehydration of wilted leaves, stress-induced ABA is rapidly converted to phaseic acid. We have found that his conversion is blocked by recently developed growth retardants, such as paclobutrazol and tetcyclacis, suggesting that ABA-hydroxylase is a cytochrome P-450 oxidase. Current work is aimed at isolating this enzyme and then to study how its activity is regulated by the water status of the tissue.

UNIVERSITY OF MINNESOTA - Minneapolis, MN 55455

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

P. Rogers, Department of Microbiology **$72,631**

The objective of this research is to elucidate the molecular mechanisms used by the obligate anaerobe, *C. acetobutylicum* to regulate the synthesis and activity of the key enzymes catalyzing reactions in this fermentation pathway. The four enzymes involved in the two pathways branching from butyryl-CoA, producing butyric acid and butanol, will be purified. The kinetic constants and allosteric modifiers will be studied to determine the molecular mechanism of switching from acidogenic to solventogenic fermentation. The solventogenic pathway enzymes appear to be induced simultaneously during later linear growth. We propose to search for regulatory mutants that either turn off or super induce solvent pathways using four different screening procedures. The transformation and fusion of *C. acetobutylicum* protoplasts will be studied in detail in order to develop an efficient genetic system to define and classify regulatory mutants into complimentation groups. Transformation studies are designed to study gram-positive plasmids already developed with antibiotic markers and shuttle properties. These will be tested for transformation, replication, and expression in *C. acetobutylicum.* It is proposed to develop a new vector using cryptic *C. acetobutylicum* plasmids, if necessary. Physiologic experiments will be used to classify and examine the regulatory mutants for temporal solvent and enzyme formation and their response to conditions that block or promote solventogenesis in *C. acetobutylicum.* These studies will connect the genetic and biochemical finds with the growth phase shifts or commitment to a sporulation program by this organism.

UNIVERSITY OF MINNESOTA - Navarre, MN 55392

90. Genetics of Bacteria that Utilize Carbon Compounds

R.S. Hanson, Gray Freshwater Biological Institute $61,000

Bacteria that grow on methane and methanol are considered useful for the fermentative production of amino acids and other products because they grow on simple, inexpensive substrates. In some bacteria that grow on methane, methane monooxygenase (MMO) represents over 20% of the total cellular protein. In some bacteria that grow on methanol, methanol dehydrogenase (MDH) represents 10 to 15% of the cellular protein. Both types of bacteria assimilate formaldehyde for the synthesis of cell material. Facultative methylotrophs can also grow on non-C_1 substrates. In *Methylobacterium organophilum* the enzymes responsible for the 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 five regions of the genome separated by 50 kilobase pairs or more. Three sites separated by five kilobase pairs that control the synthesis of MDH have been identified. The promoter required for the synthesis MDH has been fused to a beta galactosidase gene and high-level expression of beta galactosidase has been achieved in *E. coli*. The nucleotide sequence of this promoter will be compared to the sequence of the trpE promoter of *M. organophilum* that gives low-level expression of the trpE gene product. The function of the other two genes in MDH expression is being analyzed. Genes encoding five protein subunits of MMO from *Methylococcus capsulatus* (BATH) 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.

UNIVERSITY OF MINNESOTA - St. Paul, MN 55108

91. Molecular Approaches to Genomic Organization

I. Rubenstein, Dept. Genetics & Cell Biology
R.L. Phillips, Dept. Agronomy & Plant Genetics $160,287
(2 yrs, FY85 funds)

The goal is to gain a fuller understanding of the macro-organization of the maize eukaryotic genome. The approach seeks to determine the chromosomal location of physical markers. Hybridization probes will be used that consist of cDNAs and genomic clones of known function (e.g. zein), genomic clones of highly repeated sequences, and cDNA clones prepared from the mRNAs of a maize suspension cell culture. The chromosomal location of each of the unique probes will be determined by restriction fragment length polymorphism using DNAs from monosomic plants. This technique has been successfully used to locate the 27 kd water-soluble reduced glutelin zeins to chromosome 4. The chromosomal locations of a number of highly repeated sequences will be determined by *in situ* hybridization. By this means we are attempting to chromosomally locate a repeated sequence isolated from a zein genomic clone. The genomic patterns of a number of repeated sequences will be determined. We hope to identify clones that will enable us to identify specific sets of chromosomes.

UNIVERSITY OF MISSOURI - Columbia, MO 65211

92. Photosynthesis, Cloning, and Bioconversion of Solar Energy in Cyanobacteria

L.A. Sherman, Biological Sciences Division $67,340

This project identifies and clones the genes coding for membrane proteins involved in cyanobacterial photosynthesis. Cyanobacteria perform an aerobic photosynthesis nearly identical to green plants, and are suitable for genetic, biochemical, and biophysical studies in photosynthesis. The strains used for these studies, *Anacystis nidulans* R2 and *Aphanocapsa* sp., are both transformable; *Aphanocapsa* can be grown heterotrophically and photoheterotrophically, and is thus ideal for the isolation of photosynthesis mutants. This year we investigate some novel membrane proteins. We have identified a carotenoprotein with a molecular mass of 45 kDa; this protein is a major component of the plasmalemma in *Aphanocapsa* when cells are grown under autotrophic but not when grown under heterotrophic conditions. We will purify this protein, produce antibody, and clone the gene from a lambda gt11 library. We will then analyze the regulation of this gene in the shift between heterotrophic to autotrophic growth. We have also cloned the *A. nidulans* R2 gene coding for a 34 kDa chlorophyll-binding protein. Many sub-clones of this gene are now available and the gene will be sequenced. We will insert the transposon Tn5 into a 1.4 kb clone of this gene, transform into wild-type *A. nidulans*, and select mutants that have the transposon in the gene. This will enable us to study the functional significance of the gene in PSII photochemistry. Similar experiments will be performed in both *A. nidulans* and *Aphanocapsa*. Once the nucleotide sequence has been obtained, we will perform site-directed mutagenesis to determine the importance of specific amino acids in the function of this gene.

MOUNT SINAI SCHOOL OF MEDICINE - New York, NY 10029

93. The Respiratory Chain of Alkalophilic Bacteria

T.A. Krulwich, Department of Biochemistry $76,960

The respiratory chain of the extremely alkalophilic, non-marine bacilli is present in very high concentration in the cytoplasmic membranes of these organisms. The respiratory chain of the obligately alkalophilic bacilli, which grow optimally at pH 10.5 and fail to grow at near neutral pH values, appears to pump protons (and only protons) optimally at high pH values. The overall goals of the study are: to understand the special structural/functional adaptations that these bacteria may possess; to elucidate the basis for obligate vs facultative alkalophily; and to clarify factors

involved in pH-dependent regulation of cellular levels of cytochromes on the alkalophiles. Studies of individual respiratory chain complexes will continue during the next project period. In addition, newly isolated facultatively alkalophilic strains will be increasingly utilized to examine the patterns of cytochrome synthesis during changes in extracellular pH: recent data indicate that a shift from pH 7.5 to pH 10.5 may be associated with rather specific changes in the ratios of the cytochrome components of the respiratory chain. Further studies of this phenomenon will be complemented by an examination of the membrane lipids under the same experimental conditions.

NATIONAL INSTITUTES OF HEALTH - Bethesda, MD 20205

94. Partial Support of GenBank: The Genetic Sequence Data Bank

J. Cassatt, National Institute of General Medical Sciences $38,480

GenBank, the Genetic Sequence Data Bank, is an internationally available repository of all reported nucleotide sequences greater than 50 nucleotides in length, annotated for sites of biological interest, and checked for accuracy. As of December 31, 1985, GenBank contained 5.7 million bases, comprising 6,044 sequences. The data bank is operated under contract to Bolt Beranek and Newman Inc. (BBN) of Cambridge, MA. Data collection, verification, entry, and annotation is performed under the direction of Dr. Walter Goad at Los Alamos National Laboratory; while distribution, user support services, and overall data bank management are performed by BBN. This resource, administered by the National Institute of General Medical Sciences, and co-sponsored by five institutes of NIH, two divisions of DOE, the U. S. Department of Agriculture, the National Science Foundation, and the Department of Defense, is of particular interest to geneticists and molecular biologists. A copy of the data base is available for a modest fee on computer-readable magnetic tape to anyone requesting it, and is now available on IBM compatible floppy disks. Dial-up on-line access is also available, but only a limited number of users can be accommodated at any one time. A software clearinghouse is provided on-line to supply information only on sequence analysis software available worldwide. The second yearly hard copy edition of the data base was available in April 1985, as a four-volume supplement to *Nucleic Acids Research*.

UNIVERSITY OF NEBRASKA - Lincoln, NE 68588

95. Developmental Regulation of Plant Plasma Membrane Antigens

D.W. Galbraith, School of Biological Sciences **$71,188**

An understanding of the molecular architecture and developmental behavior of the plant plasma membrane is central to an understanding of plant growth and development and of the interactions of plants with the environment. We are characterizing the proteins and glycoproteins that are specifically located at the *Nicotiana* plasma membrane, using monoclonal libraries prepared with plant plasma membranes and protoplasts as immunogens. We are focusing on an analysis of a subset of antigens located on the plasma membrane that are expressed under heterotrophic but not photoautotrophic conditions, and are using immunorecipitation and gel electrophoresis under denaturing conditions following either *in vivo* or *in vitro* labeling for the molecular identification of these antigens. We are comparing these antigens to those plasma membrane glycoproteins and proteins that can be identified through conventional surface labeling and cell fractionation techniques. We have constructed cDNA libraries in phase expression vectors corresponding to the different protoplast developmental stages, and have also constructed complete genomic libraries in phage vectors. These are currently being screened in order to permit a characterization of the controls of gene expression involved in this developmental system, and to compare possible congruencies to controlling systems found in the plasma membranes of heterologous organisms. Finally, we are continuing the development of techniques of *in vivo* flow cytometry and cell sorting of protoplasts for the isolation of mutant protoplasts deficient in this control. In this way, we hope to provide the basis for an improved understanding of the mechanisms that control plant cell growth and division.

96. Viruses of Eukaryotic Green Algae

J.L. Van Etten, Department of Plant Pathology **$69,259**

PBCV-1 is a large, dsDNA (ca. 300 kbp) containing plaque forming virus which replicates synchronously in a unicellular *Chlorella*-like green alga, strain NC64A. The PBCV-1 *Chlorella* system is the first example of a virus infecting a eukaryotic, photosynthetic organism which can utilize procedures directly adapted from those used to study bacteriophage. *In vivo* and *in vitro* studies have established that PBCV-1 encodes an enzyme with restriction endonuclease-like activity and an enzyme(s) with methyltransferase activity. The PBCV-1 encoded methyltransferase transfers methyl groups to adenine in the sequence GATC. The PBCV-1 encoded restriction enzyme recognizes and cleaves the sequence GATC but will not cleave $G^{m}ATC$. Host DNA contains GATC sequences but PBCV-1 DNA contains $G^{m}ATC$. Thus PBCV-1 apparently encodes a restriction and modification system which may be analogous to similar systems in bacteria.

The objectives of this proposal are to isolate, characterize, and clone the PBCV-1 encoded restriction modification enzymes and to compare them to bacterial enzymes which recognize the same sequences. In addition, we will examine numerous new and distinct viruses, which we have isolated that infect the same host, for restriction-modification systems.

NEW YORK STATE DEPARTMENT OF HEALTH - Albany, NY 12201

97. Methane-Producing Bacteria: Immunologic Characterization

E. Conway de Macario, A.J.L. Macario, Laboratory of Immunology
M.J. Wolin, Laboratory of Environmental Biology & Field Services
Wadsworth Center for Laboratories and Research $57,720

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

NORTHWESTERN UNIVERSITY - Evanston, IL 60201

98. Genetics of Thermophilic Bacteria

N.E. Welker $58,680

The ability to genetically manipulate thermophilic bacilli will profoundly affect investigations of the biochemical mechanisms of thermophily, and facilitate studies of other thermophiles that lack a reliable genetic exchange system or possess growth characteristics that make them unsuitable for research purposes. Efficient and

reliable protoplasting, regeneration, and fusion techniques were established for the prototrophic strain *Bacillus stearothermophilus* NUB36. Auxotrophic mutants were isolated, and protoplast fusion was used to construct isogenic mutant strains and for chromosomal mapping. Markers were mapped using two-, three-, and four-factor crosses. The order of the markers was [hom-1-thr-1-his-1-(gly-1 or gly-2)-pur-1-pur-2]. These markers may be analogous to [hom, thrA, hisA, glyC], and [purA] markers on the *Bacillus subtilis* chromosome. No analogous [pur-1] marker has been reported in *B. subtilis.* The relative order of three of the markers [hom-1-thr-1-gly-1] was independently confirmed by transduction, with generalized transducing phages TP-42, TP-43, and TP-46. Plasmid pKML(Km^rTc^r) was introduced into *B. stearothermophilics* NUB 36148 Rif^r Res^- Mod^- Rec^- Spo^- by protoplast transformation. Kananycin-resistant transformants had lost cryptic plasmid pNUI (60-63 Kilobases). This system can be used for both the genetic analysis of this organism and for molecular cloning. The development of a host-vector system for cloning in *B. stearothermophilus* will make it possible to clone genes encoding cellulose hydrolysis, alcohol formation, or methane production. In addition, information gained from the genetic analysis of thermophilic bacilli may be of value in developing genetic systems in thermophilic eubacteria and archaebacteria.

OAK RIDGE NATIONAL LABORATORY - Oak Ridge, TN 37831

99. Energy and Nutrient Utilization Efficiency in Intensive Forest Biomass Production

S.B. McLaughlin, S.W. Johnson, N.T. Edwards, R.J. Luxmoore, R.F. Walker, Environmental Sciences Division **$96,000**

This study addresses plant physiological and soil-plant nutrient processes that are important considerations in using marginal land for silvicultural energy production. The objectives are (1) to provide physiological criteria for quantifying yield potential of biomass candidate species and (2) to determine the role of soil-plant nutrient dynamics on productive potential. Yellow poplar (Liriodendron tulipfera), loblolly pine (Pinus taeda), and black locust (Robinea pseudoacacia) are being studied in a 20 ha managed population. Treatments examined include mycorrhizal preconditioning, variable rate and timing of nitrogen supply, and mulching. Parameters measured include whole-tree allocation of energy between metabolic pools, plant water status, leaf photosynthetic rate, photosynthate allocation by foliage, and the kinetics of nitrogen mobilization and movement through the soil-plant nutrient cycles. Results of studies on nitrogen utilization by poplar and pine indicate that total nitrogen uptake and within-plant allocation are influenced by the timing of fertilizer application. Quarterly application of 25 Kg N Ha^{-1} y^{-1} resulted in greater utilization of N by nitrifying bacteria, greater nitrate nitrogen losses by leaching, and less efficient uptake by trees than when a single 100 Kg N Ha^{-1} application was supplied annually. Analyses of samples collected to characterize seasonal cycles of tissue biochemistry, differences in photosynthetic capacity of foliage, and patterns of photosynthate allocation are nearly complete and synthesis of relationships of whole-tree energy allocation patterns to nutrient utilization regimes will begin this summer.

OHIO STATE UNIVERSITY - Columbus, OH 43210

100. Basis of Competitiveness of Rhizobia

W.D. Bauer, W.R. Evans $76,960

Rhizobia are soil bacteria that symbiotically infect and nodulate leguminous plants to fix atmospheric nitrogen for their host. The agricultural benefit of this symbiosis depends on having the best available strains of rhizobia form most of the nodules on roots of host plants in the field. It has not been possible to achieve this consistently because rhizobia inoculated onto the seed at planting do not colonize and infect seedling roots well enough to compete against indigenous rhizobia. The project objective is to determine that factors contribute importantly to the competitive ability of inoculated rhizobia. Recent studies have shown that the relative competitiveness of two strains of rhizobia can depend markedly on what medium the host roots are grown in and what medium the bacteria are grown in. Complete reversals in relative competitiveness have been observed when roots were grown in growth pouches, sand/vermiculite, and montmorillonite clay and when rhizobia were cultured on media with different nitrogen sources. The molecular and cellular basis for these effects are being investigated. Studies of the effects of inoculum dose on root infection indicate that there are dose thresholds for two regulatory control phenomena in the host that inhibit nodule formation. Transposon mutants with altered root attachment capabilities have been isolated and are being analyzed in terms of competitiveness. Attempts are being made to determine the molecular and cellular bases for inhibition of root attachment of one strain by another.

101. Development of Genetic Exchange Systems for *Methanococcus vannielii*

J.N. Reeve and J.I. Frea, Department of Microbiology $69,745

The goal of this project is to determine the structure and mechanism(s) of regulation of genes in the methane producing archaebacteria known as methanogens. We have cloned and sequenced several methanogen genes from a range of methanogens including mesophilic species *(M. vannielii, M. smithii)* and thermophilic species *(M. thermoautotrophicum, M. thermolithotrophicus)*. The DNA sequences so far obtained strongly suggest that methanogen genes may be organized into multi-gene transcriptional units (operons) and that ribosome binding sequences are needed for initiation of protein synthesis. Our current experiments are designed to determine if these conclusions, based on DNA sequence data, are valid. We are using S1 nuclease protection experiments and Northern blot procedures to identify and characterize mRNAs synthesized *in vivo* in methanogens. We have purified DNA-dependent RNA-polymerase and ribosomes from methanogens to develop *in vitro* transcription and translation systems. RNA polymerase binding to cloned methanogen DNAs is being used to identify methanogen promoter sequences. We are also attempting to develop a procedure to introduce and obtain replication of DNA molecules in methanogens. We have constructed plasmids

which replicate in yeast by virtue of cloned *M. vannielii* autonomously replicating sequences (ARSs). Neomycin resistance (neo^r) has been shown to be a potentially useful selectable trait in methanogens and neo^r genes have been incorporated into the constructed plasmids. Current experiments are designed to obtain transcription of the neo^r genes using DNA sequences thought to act as promoters in *M. vannielii* and to determine if these plasmids can be used successfully as donor DNAs in transformation or mobilized into *M. vannielii* by conjugation.

OKLAHOMA STATE UNIVERSITY - Stillwater, OK 74078

102. The Structure of Pectins and Their Possible Role in Resistance of Cotton to Cotton Blight

A. Mort, Department of Biochemistry $76,000

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 an degradation of uronic acids with lithium in ethylenediamine) we will characterize, as fully as possible, two major regions of the pectic polymers of cotton suspension culture cell walls. (1) We will determine the size distribution of the homogalacturonan regions and distribution of methyl esterification and acetylation sites within it. None of these characteristics has been determined yet for a primary cell wall pectin. (2) We will characterize the region which (at least in sycamore cells) is a repeating disaccharide of rhamnose and galacturonic acid with sidechains on approximately half of the rhamnose residues. For this region we will characterize the backbone of the polymer and the sidechains with respect to their primary sugar sequence and linkages. We will also look for linkages to other polymers through the sidechains and, where possible, for acyl substituents. There are no published reports on the isolation and characterization of the sidechains, very few describing the backbone of the region, and none describing the acylation of the region.

UNIVERSITY OF OKLAHOMA - Norman, OK 73019

103. Metabolism and Bioenergetics of *Syntrophomonas wolfei*

M.J. McInerney, Department of Botany and Microbiology $65,000

Fatty acids, which are key intermediates in the conversion of organic matter to methane and carbon dioxide, are degraded by hydrogen-producing syntrophic bacteria. These bacteria are difficult to study because of their slow growth rate and the fact that they can only be grown in coculture with hydrogen-using bacteria such as methanogens. We have developed procedures to physically separate cells of the anaerobic, fatty acid degrader, *Syntrophomonas wolfei*, from cells of the methanogen, *Methanospirillum hungatei*, using isopycnic centrifugation as well as to obtain cell-free extracts of *S. wolfei* free from contamination by cellular components of the methanogen by lysozyme treatment. We have obtained pure cultures of *S. wolfei* by growing the organism with crotonate as the energy source. Pure cultures of *S. wolfei* produce acetate, butyrate, caproate and some hydrogen from crotonate. Activation of butyrate appears to occur by coenzyme A transferase activity rather than by acyl-coenzyme A synthetase activity. In addition to the beta-oxidation enzymes, *S. wolfei* also contains phosphotranacetylase and acetate kinase activities suggesting that energy is conserved by substrate-level phosphorylation. High concentrations of acetate inhibit butyrate degradation by *S. wolfei-M. hungatei* cocultures, but not by tricultures containing *S. wolfei, M. hungatei* and the acetate-using methanogen, *Methanosarcina barkeri*. These data suggest that acetate turnover is required for butyrate degradation. A method to detect nitrous oxide-producing bacteria in solid and liquid medium by following the oxidation state of resazurin has been developed.

OREGON GRADUATE CENTER - Beaverton, OR 97006-1999

104. Enzymic Components of the *Phanerochaete chrysosporium* Lignin Degradative System

M.H. Gold, Department of Chemical, Biological and Environmental Sciences $60,000

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

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

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

2. Enzymic degradation of polymeric dyes and lignin: Using culture transfer techniques, crude cell fractionation and HPLC separation of all of the soluble extracellular proteins from mutant and wild type strains of *P. chrysosporium*, we intend to resolve all of the components of the lignin degradative system. Lignin model compounds, polymeric dyes and radiolabeled lignin will be used to determine which enzymes are responsible for the cleavage of specific bonds and what is the minimal complement of enzymes required for the efficient depolymerization or transformation of lignin and lignocellulose.

OREGON STATE UNIVERSITY - Corvallis, OR 97331

105. Genomic Variation in Maize

C. Rivin, Department of Botany & Plant Pathology **$44,080**

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

PENNSYLVANIA STATE UNIVERSITY - University Park, PA 16802

106. The Role of Turgor Pressure in Plant Growth

D. Cosgrove, Department of Biology $76,000

We are investigating the nature of plant cell growth by defining the physical factors that control growth, in particular when water is plentiful and when it is lacking. Expansion of plant cells during growth requires a positive turgor pressure to drive wall extension. Water stress is commonly thought to inhibit growth by reducing cell turgor. Water stress may also make the cell walls less extensible, or alter uptake of solutes necessary for growth. We have characterized stem growth of young pea seedlings (*Pisum sativum* L.) as a function of water stress, imposed either by lack of soil moisture or by salinity. Direct measurements of turgor pressure in individual growing cells were made using the pressure microprobe technique. Our results indicate that salinity and low soil moisture may inhibit growth by different physical mechanisms. Using video and computer techniques, we have also constructed an advanced pressure microprobe which automatically carries out pressure relaxation, pressure clamp, and elastic modulus measurements. Cell solute concentrations are determined by nanoliter osmometry. To measure the wall properties relevant for growth (wall extensibility and wall yield threshold), we are using a novel *in vivo* stress relaxation method. These new techniques are enabling us to identify the physical factors that restrict plant cell growth and to elucidate how these factors are altered by internal and external stimuli that modify growth.

UNIVERSITY OF PENNSYLVANIA - Philadelphia, PA 19104

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

P.L. Dutton, Department of Biochemistry & Biophysics $83,000

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

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

ROCKEFELLER UNIVERSITY - New York, NY 10021

108. DNA Sequences Encoding Chlorophyll a/b Binding Polypeptides

A.R. Cashmore, Laboratory of Cell Biology $105,000

Major products of leaf nuclear gene expression are polypeptide components of the light-harvesting chlorophyll a/b (CAB) protein complex. These CAB polypeptides are made on cytosolic ribosomes as precursors which are subsequently imported into chloroplasts. The members of the small family of nuclear genes encoding the CAB polypeptide demonstrate both tissue specific and photoregulated expression. There are a total of 5 CAB loci in the tomato genome. We have characterized DNA sequences from 3 of these loci and we propose to characterize sequences corresponding to the other loci. Polypeptides encoded by the different loci show interesting differences including in some cases pronounced divergence within the amino termini of the mature polypeptides. The functional significance of these differences remains to be determined. Of particular interest to us is to characterize the nucleotide sequences that determine the specific expression characteristics of the individual CAB genes. We have demonstrated that photoregulated gene expression is mediated by enhancer sequences and we propose to define in detail the nature of those sequences. For these studies we will use *Agrobacterium*-mediated transformation.

RUTGERS UNIVERSITY - New Brunswick, NJ 08903

109. Cellulase - A Key Enzyme in Fermentation

D.E. Eveleigh, J.D. Macmillan, Dept. of Biochemistry and Microbiology $63,973

Cellulosic biomass represents a potential source of chemical feedstock materials. It is recycled in nature via an enzyme complex, cellulase, but the nature of the synergistic interaction of the enzyme's multi-components is poorly understood. To aid in elucidating the roles of the individual enzymes, monoclonal antibodies towards cellulases are being used as specific probes. A monoclonal antibody to *Trichoderma reesei* cellobiohydrolase I has been obtained, which reacts specifically with this enzyme without interference by endoglucanases. This monoclonal antibody has been immobilized and used for the single step purification of cellobiohydrolase I. Furthermore, there is no direct assay available for this enzyme, but the monoclonal antibody has now allowed the development of a direct ELISA assay for it that is both sensitive and rapid. The approach is being extended to other cellulase systems.

RUTGERS UNIVERSITY - Piscataway, NJ 08854-0759

110. Corn Storage Protein: A Molecular Genetic Model

J. Messing, Waksman Institute of Microbiology $89,470

Storage proteins are the main protein components of seeds. Since some of our staple food sources are seeds, in particular those from cereals, the amino acid composition of these storage proteins is directly related to the nutritional quality of the diet used in animal feed. Consequently, substantial effort has been spent in generating new varieties with improved nutritional value. The previous efforts are now expanded by novel approaches such as the use of Molecular Biology to understand the basic genetic mechanism that underlies the control of gene action. In the past years, we developed new tools for gene sequencing and genetic engineering that are applicable to genetic manipulations for all areas of biology. In addition, we have focused on the gene structure of storage proteins in maize as a model system for plant genes. These efforts have led to the understanding of the organization of these genes in the maize genome and of the primary structure of their gene products. In particular, we have learned that storage proteins have three domains, unique amino- and carboxy-terminal regions, and a central region that contain repeated blocks of amino acids. This repeat contains the amino acid residues that are highly amplified, such as glutamine which is an important nitrogen acceptor. This architecture turns out to be fundamental for all storage proteins studied so far.

We are now developing the mechanism by which the methionine content of maize kernels is controlled by the 10 kd zein protein.

SMITHSONIAN INSTITUTION - Rockville, MD 20852-1773

111. A Primary Light-Harvesting System: The Relationship of Phycobilisomes to Photosystem I and II

E. Gantt, Environmental Research Center $60,901
(15 mos, FY85 funds)

The organization and composition of the major light harvesting components in cyanobacteria and red algae is different than in green plants. To understand and account for the functional competence of phycobilisome-containing plants the architectural framework in which major components of light gathering complexes exist is being studied. Effects of nitrogen limitation, commonly observed in nature, are being explored in laboratory cultures of the unicellular red alga *Porphyridium cruentum*. Nitrogen limitation in these cultures leads to cessation of growth with respiration exceeding photosynthesis, and reduction of chlorophyll and phycoerythrin. The variation in the photosystem I antennae (Chl/P700) is relatively small compared to the

total loss of the major photosystem II antennae, the phycobilisomes. It appears that photosystem I is significantly less affected than is photosystem II, and that net photosynthesis and growth are largely dependent on the restoration of the photosystem II antennae. Apoproteins of the photosystem I reaction centers have been purified for polyclonal antisera production, and purification of photosystem II reaction center components are in progress. Antisera will be used as *in situ* probes, and to follow the changes with varying environmental conditions. Studies on the link between the phycobilisomes and photosystem II are continuing. Two independent terminal fluorescence emitters (685 and 683 nm, -196°C) were resolved by second derivative spectra in phycobilisomes of *Nostoc* sp. Energy flow from allophycocyanin seems to occur independently to each pigment. The long wavelength pigment (F685 nm) has been isolated and identified as the 94 kD putative anchor polypeptide. An integrated approach at the structural and functional level has significant implications for understanding of photosynthetic membrane structure, regulation of energy transfer, and evolutionary development of oxygen-evolving plants.

SOLAR ENERGY RESEARCH INSTITUTE - Golden, CO 80401

112. The Water-Splitting Apparatus of Photosynthesis

M. Seibert, Photoconversion Research Branch $101,000

The water-oxidizing (O_2-evolving) supramolecular complex of photosystem II (PS II) supplies the reductant ultimately used by algae and green plants to fix carbon during photosynthesis. We are using biochemical, genetic, and surface-probe techniques to identify the components of the complex, to determine how the components are related structurally, and to understand the mechanism of O_2 evolution. Immunological studies have demonstrated that the 34-kDa intrinsic protein, shown to have oxidizing-side function from analyses of the LF-1 mutant of *Scenedesmus*, is the product of the *psb* A gene. This protein, also called the D1 or Q_B protein, is known to have reducing-side function. Homology between the protein in higher plants and *Scenedesmus* was demonstrated, and evidence showing that it is required for binding the 17- and 23-kDa extrinsic proteins associated with O_2 evolution was obtained. We conclude that the 34-kDa/D1 protein affects function on both sides of the PS II reaction center. Freeze-etch electron microscopy has related tetrameric protrusions on the lumenal surface of PS II membranes to the three extrinsic proteins (17, 23, and 33 kDa) associated with O_2 evolution. Surface-enhanced Raman scattering (SERS) spectroscopy can be used to monitor the loosely bound pool of Mn in spinach and *Scenedesmus* PS II membranes (this Mn represents about half the total amount and is required for O_2 evolution). New SERS evidence suggests that the loosely bound Mn is liganded to Cl in an S-state greater than S_2 and that the tightly bound Mn pool is also required for O_2 evolution.

SOUTHERN ILLINOIS UNIVERSITY - Carbondale, IL 62901

113. Regulation of Alcohol Fermentation by *Escherichia coli*

D.P. Clark, Department of Microbiology $63,780

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*. Focus is on the two final steps in alcohol synthesis, which are catalyzed by alcohol dehydrogenase and acetaldehyde CoA dehydrogenase. We have isolated a series of mutations affecting the expression of these enzymes. Some of these mutations are in the structural genes for these enzymes; others affect the regulation of the *adh* operon. We have recently cloned the genes coding for these enzymes and are now studying the effect of multiple copies of the *adh* gene on fermentative growth and its regulation. A recently invented technique, proton suicide has allowed the selection of a variety of novel mutants affecting fermentation which are presently being characterized. We have isolated a comprehensive collection of operon fusions in which the *lacZ* structural gene is fused to promoters that are inactive aerobically but active anaerobically. Although these genes (like *adh*) are only expressed under anaerobic conditions, the level of induction varies from two-fold to nearly 100-fold. The nitrogen source, medium pH, nature of the buffer, presence of alternative electron acceptors (e.g., nitrate), and other factors exert a great effect on the expression of many of these genes. In the near future we will investigate control mechanisms common to the *adh* operon and other anaerobically regulated genes.

STANFORD UNIVERSITY - Stanford, CA 94305

114. Genetic Engineering of Corn and Other Higher Plants

R.W. Davis, Department of Biochemistry $100,000

The project objective is to apply to higher plants the recent developments of molecular biology in mammals and microorganisms by the development of DNA transformation systems for higher plants. We have developed an electroporation technique for the introduction of DNA into carrot cells. All assays are for transient gene expression of the chloramphenicol acetyl transferase gene from bacteria, which is connected to various plant promoters and polyadenylation sites. Since we plan to use the transient expression assay to dissect the critical *cis*-acting elements for a plant promoter, it is essential to understand the physiological state of the plant protoplast. It is likely that the formation of a plant protoplast induces the wound response. We have investigated this by examining the transcriptional state of the hydroxyproline-rich glycoprotein (HRGP). This gene is induced upon the wounding of a

plant. We have found that upon formation of a plant protoplast, a 1.5 kilobase mRNA homologous to this gene is induced. However, the PAL gene, which is inducible by ethylene, was not induced in carrot protoplasts. This makes it possible to investigate the critical elements in the PAL promoter that are responding to ethylene. A major effort has been made using the electroporation technique to investigate the effects of anti-sense RNA. We have found that if sufficient anti-sense RNA is produced during transient gene expression, sense strand synthesis on an accompanying DNA molecule can be suppressed. This could have important application in the agricultural field in reducing the expression of particular endogenous genes or by reducing the expression or invading viral sequences.

115. Host range and other symbiotic genes of *Rhizobium meliloti*

S.R. Long, Department of Biological Sciences **$99,080**

The ability of certain plants to host nitrogen-fixing *Rhizobium* bacteria allows the plants to thrive without costly and petrochemical-intensive nitrogen fertilizer. We are studying *Rhizobium* genes used in selecting the plant host and in creating an effective nitrogen-fixing symbiosis. During the past year, we have carried out an extensive analysis of a DNA segment borne on recombinant plasmid pJT5. This clone includes *Rhizobium meliloti* genes which control the bacteria's selection of alfalfa as its host. Eighty-nine mutations were generated by transposon Tn*5* insertion, and mapped by digestion of DNA with restriction enzymes. These strains were tested for symbiotic phenotype: each mutant was inoculated onto at least 10 sets of 2-3 alfalfa plants, and number of nodules was scored at intervals of several days. This revealed several DNA segments where mutations cause a delay in nodule formation, or decreased numbers of nodules, or both. In one region, mutations caused a severely decreased nodulation response. This DNA region, designated *nodH*, has been completely sequenced, revealing an open reading frame of 247 amino acids. Having previously established that the "common" nod genes *ABC* are induced by alfalfa exudates, we have now analyzed that exudate and have demonstrated an active fraction to be the flavone, luteolin. We have also examined control of nodulation, host range, and other genes. Clone pJT5 appears to encode several regulatory genes which control *nodABC*, in addition to the host range loci. With the analysis of these genes and their control, we come closer to an understanding of how these beneficial bacteria invade specific host plants.

116. Carbon Dioxide and the Stomatal Control of Water Balance and Photosynthesis in Higher Plants

E. Zeiger, Department of Biological Sciences $52,909 (7 months)

This research project studies the stomatal responses to CO_2 and their interaction with other factors controlling stomatal movements. A central rational for our studies has been the postulate of a chemiosmotic mechanism controlling ion content in guard cells. Recent work in our laboratory has provided direct empirical evidence for this notion. These studies have characterized a specific, blue light-induced opening in intact leaves, with its kinetic and photobiological properties correlating with blue light-induced proton extrusion by guard cell protoplasts. Analysis of the response with the electrophysiological technique of patch clamping has shown that blue light activates a proton pump at the guard cell plasmalemma. Current studies are aimed at the characterization of the control of proton pumping by light and its modulation by other stimuli. Measurements of the blue light response in the intact leaf have shown that its magnitude depends on prevailing carbon dioxide concentrations; analysis of this interaction under patch clamp conditions could provide insight on the sensory transduction of the CO_2 response. In addition, we are analyzing the interaction between blue light-induced proton extrusion and a recently characterized, red light-dependent alkalinization, under the rationale that the alkalinization is the expression of photosynthetic CO_2 fixation in guard cells, which is hard to observe under white light conditions because of metabolic regulation.

TEXAS A&M UNIVERSITY - College Station, TX 77843

117. Metabolic Mechanisms of Plant Growth at Low Water Potentials

J.S. Boyer, Department of Soil and Crop Sciences $69,260

In higher plants, growth is more frequently limited by water than by any other environmental resource. The project objective is to identify the underlying molecular mechanisms causing the limitations, by focusing on the processes of photosynthesis and cell enlargement. The inhibition of photosynthesis by low leaf water potential was caused primarily by losses in chloroplast activity in sunflower (*Helianthus*). Many of the effects could be simulated by preincubating chloroplasts at Mg^{2+} concentrations expected to be present at low water potentials. Moreover, exposure to high light intensities during the development of low water potentials had no effect on the inhibition indicating that chloroplast activities were determined by direct effects of leaf water status and not by photoinhibition. We are further investigating chloroplast responses *in vivo* to test whether Mg^{2+} regulation plays a role in the inhibition. Our studies of cell enlargement show that, in localized growing regions, photosynthate accumulates and can maintain turgor completely at

moderately low water potentials. The primary signal causing decreased growth was a loss in the gradient in water potential required for water entry into the growing cells. We are exploring the subsequent role of several metabolic factors using hormone mutants, a pressure probe, and a newly designed thermocouple psychrometer. This work will further understanding of the mechanisms of growth inhibition with limited water and may indicate ways to reduce the limitation.

VIRGINIA POLYTECHNIC INSTITUTE & STATE UNIVERSITY - Blacksburg, VA 24061

118. Enzymology of Acetone-Butanol-Isopropanol Formation

J.-S. Chen, Department of Anaerobic Microbiology **$64,310**

Four alcohols (C_2 to C_4) have been suggested as feedstocks that can support a major fraction of the U.S. chemical industry using currently available technology and facilities. These alcohols are products of microbial fermentation. Two of the alcohols, isopropanol and n-butanol. plus acetone are products of the anaerobe *Clostridium beijerinckii* (formerly, *Clostridium butylicum*), the organism currently under study. DNA homology studies show that *C. beijerinckii* is distinct from the acetone/butanol-producing *Clostridium acetobutylicum*. Despite their industrial uses, these anaerobes have unresolved biological problems, one being the instability of their producing ability (strain degeneration as well as occasional failure to undergo the metabolic transition to initiate alcohol/solvent production). An essential step toward understanding the nature of these problems is to elucidate the enzymology of the normal fermentative processes. We have been studying the enzymes specifically involved in the formation of acetone, butanol and isopropanol. Alcohol dehydrogenase (ADH) has been isolated from a butanol/isopropanol-producing (B/I) and a butanol-producing (B) strain of *C. beijerinckii*. The 150-fold purified ADH from the (B/I) strain still shows both butanol and isopropanol dehydrogenase activities. The butanol/isopropanol dehydrogenase shows a M_r of 98,000 and is specific for NADPH. ADH from the B strain shows only butanol dehydrogenase activity. a M_r of 53,000, and a higher activity with NADPH than NADH. The two ADH's also show a 1000-fold difference in their Km for butyraldehyde. The molecular properties and the control mechanism for the expression or these ADH's are being studied.

WASHINGTON UNIVERSITY - St. Louis, MO 63130

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

R.N. Beachy, Department of Biology $70,130

The β-conglycinins are soybean seed storage proteins and are made up of three subunits, α', α, and β-subunits. We are investigating the regulated expression of genes encoding the α' and β-subunits in transgenic plants. Each gene has been introduced into petunia and tobacco plants on a modified Ti-plasmid in *Agrobacterium tumefaciens*. Both the α' and β-genes are expressed in developing embryos of the transgenic plants in much the same manner as in soybean embryos. Our research deals with identifying the DNA sequences that control the expression of these β-conglycinin genes. The transcriptional promoter that controls the expression of the α'-subunit gene was identified by mutating DNA sequences 5' of the gene, introducing each of the mutant genes into tobacco and petunia plants by transformation, and comparing the expression of mutant and wild type genes in transgenic plants. By such experiments we determined that no more than 210 nucleotides 5' of the gene are required to obtain gene expression in tobacco and petunia plants. Within this region sequences between 150 and 210 nucleotides 5' of the gene were to be critical for the high level expression of the α'-gene. We are currently investigating the possibility that these nucleotides act as an enhancer sequence for the α'-subunit gene by creating a series of gene construction to test the importance of position and orientation of this sequence on the function of several different promoters and several different genes. This information will be critically important for, ultimately, identifying the mechanism(s) that govern the tissue specific expression of these genes.

120. Hydroxyproline-Rich Glycoproteins of the Plant Cell Wall

J.E. Varner, Biology Department $99,086

The cell walls of plants, particularly of dicots, characteristically contain glycoproteins rich in hydroxyproline. It has been presumed that, in analogy with the collagen matrix of animal tissues, the plant glycoprotein(s)-extensin(s)-has an important structural/developmental role. The goal of this project is to check this presumption. Towards this end we have 1) isolated and purified to homogeneity one hydroxyproline-rich glycoprotein from aerated carrot slices, 2) characterized the conformation of this glycoprotein as an extended polyproline II helix--it is a rod 80 nm long and visible by electron microscopy, 3) isolated and purified the mRNA for carrot extensin, 4) made cDNA against the mRNA, and 5) used the cDNA to isolate the gene for a carrot extensin. This gene has been sequenced and the amino acid sequence derived from the base sequence. The transcripts from this gene increase in abundance following wounding (slicing and aeration of the carrot root). Another, non-homolo-

gous, transcript coding for a 33 kilodalton peptide rich in proline and poor in leucine also accumulates following wounding. This second transcript encodes several repeats of - His Lys Pro Pro Val - and - Try Thr Pro Val. Current work includes the study of 1) the control of the expression of the extensin gene, 2) the control of the expression of the 33kd protein, 3) the chemistry of the reactions responsible for insolubilizing the extensins in the cell wall and 4) the characterization of a glycine-rich protein found abundantly in pumpkin seed coat cell walls and in lesser abundance in other tissues.

WASHINGTON STATE UNIVERSITY - Pullman, WA 99164

121. Regulation of Terpene Metabolism

R. Croteau, Institute of Biological Chemistry $78,000

Oils and resins from plants are important renewable resources. Knowledge of the biochemistry of these terpenoid substances is needed to deduce regulatory mechanisms at the enzyme level. The objective of this project is to provide such understanding through the intensive investigation of two model systems: (1) camphor metabolism in *Salvia officinalis* and (2) menthone metabolism in *Mentha piperita*. These systems allow probing the control of both biosynthetic and catabolic processes involved in monoterpene accumulation. The pathways of biosynthesis of both monoterpenes have been established. As the plant matures, both terpenoids undergo catabolism by a pathway involving conversion to a glycoside and transport of this derivative to the root/rhizome. Following hydrolysis, the terpenoid undergoes oxidative degradation to acetate, which is recycled into other lipids of the developing root/rhizome. During the catabolic phase, the epidermal oil glands collapse as terpenes are removed from the extracellular cavity. The ultrastructural basis for this process is under investigation. The current focus is centered on factors that may mediate developmental changes in the levels of biosynthetic and catabolic enzymes present. Several growth regulators and bioregulators increase terpene yield by delaying the onset of catabolism and by altering the levels of the cyclase enzymes responsible for producing the parent monoterpene types. The mixture of terpene stereoisomers produced and the extent of terpene oxidation/reduction can also be influenced by bioregulators. Results will have important consequences for yield and composition of terpenoid oils and resins that can be made available for industrial exploitation.

UNIVERSITY OF WASHINGTON - Seattle, WA 98195

122. Studies on the Control of Plant Cell Enlargement by Cellular Parameters

R.E. Cleland, Department of Botany $73,788

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 identified four major control points for cellular expansion: 1) the rate of auxin-induced H^+-excretion, 2) the capacity of the walls to undergo acid-induced wall loosening, 3) the rate of osmoregulation, and 4) the value of the effective turgor (i.e., the turgor in excess of the wall yield threshold). Changes in any one of these four control points will lead to changes in the growth rate. We continue our studies on each of these control points to determine the role of each in the modulation of the rate of cell enlargement. Studies on H^+-excretion proceed along two lines: 1) purification of plasma membrane ATPase via a two-phase system, and reconstitution into synthetic vesicles, and 2) studies on the importance of proton excretion in cell enlargement in maize coleoptiles, where severe doubts about its role have been raised. Studies on wall loosening are concentrated on the role of calcium in the mechanical properties of the cell wall, and the effects of auxin on calcium removal and release to the wall. Studies on osmoregulation and turgor center on the question of whether solute uptake is a driver or a consequence of enhanced cell enlargement.

123. RNA Polymerase III Transcription in Higher Plants

B.D. Hall, Genetics Department $70,000

Wheat germ and developing wheat will be used to obtain an *in vitro* transcription system for the synthesis of 5S rRNA and pre-tRNA molecules. Whole cell and nuclear extracts from wheat embryos will be fractionated by methods which yielded active RNA polymerase III (polIII) systems from yeast, *Xenopus*, and human cells. Transfer RNA- and 5S rRNA-specific transcription factors will be identified and partially purified using a DNA binding assay based on gel electrophoresis. The assay detects specific binding of the transcription factors to their promoter sequences and has been employed successfully in our studies of the corresponding transcription factors (TFIIIA and TFIIIC) from yeast. Once isolated, the wheat transcription factors will be combined with wheat polIII and tRNA and 5S rRNA gene templates to form the basis of the cell-free transcription system. Additional protein fractions from the wheat extract will then be added as necessary until specific and accurate tRNA and 5S rRNA synthesis is obtained *in vitro*. Preincubation and competition experiments will be carried out with the *in vitro* system to determine which factors are specific for each class of gene and to define intermediates in the polIII transcription pathway.

124. The *Rhizobium meliloti* Exopolysaccharide: Biosynthesis and Role in Nodulation

J.A. Leigh, Department of Microbiology & Immunology $50,000

Of all the nitrogen fixing symbioses known, the *Rhizobium* legume association is the most complex and the most important economically. 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 how polysaccharides, produced by *Rhizobium*, induce and control events in the establishment of this association. We are following up on the recent observation that mutants of *R. meliloti* which fail to produce the acidic extracellular polysaccharide fail to enter nodules. The conclusion that the exopolysaccharide is required for nodule invasion is based on highly consistent results with a large and genetically diverse set of mutants. We are studying the mutants biochemically to determine where they are blocked in the pathway of polysaccharide biosynthesis and whether the mutations affect the synthesis of other poly- or oligosaccharides. We are studying the interactions of the mutants and of the polysaccharide itself with the plant to determine the precise function of the polysaccharide in nodule invasion. These experiments include investigating the effects of isolated polysaccharide on nodule invasion, carrying out nodulation experiments with temperature sensitive mutants in polysaccharide synthesis, inoculating plant roots with different bacterial strains simultaneously, assaying attachment of the mutants or of the polysaccharide to plant roots or extracted components of plant roots, and probing nodules with antibody to the polysaccharide. We have also found that degradation of the polysaccharide by *Rhizobium* takes place, and we are studying this phenomenon genetically and biochemically to determine the possible role of degradation in nodulation.

UNIVERSITY OF WISCONSIN - Madison, WI 53706

125. Role of Transit Peptides in the Proper Localization of Nuclear-encoded Chloroplast Proteins

K. Keegstra, Department of Botany $50,000

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 pre-

cursor proteins imported into isolated intact chloroplasts. The localization of imported proteins will be examined by chloroplast fractionation studies. We currently are studying the precursors for ferredoxin and plastocyanin; these soluble proteins are located in the stromal space and the thylakoid lumen respectively. We also have a precursor gene for LHCP, a thylakoid membrane protein, and are isolating precursor genes for proteins destined for the chloroplast envelope membranes. The role of the transit peptides will be 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 will determine whether the transit peptide influences the ultimate location of the polypeptide.

126. Organization of the *R* Chromosome Region in Maize

J.L. Kermicle, Department of Genetics

$54,093

Alleles of the *R* gene confer specific patterns of anthocyanin pigmentation to tissues of the corn plant and seed. We seek to identify, map and characterize components which govern the distribution, intensity, and timing of pigmentation. Mutational and recombinational analysis of alleles existing in geographically diverse races uncovers variation at two levels. The *R* gene is represented more than once in some strains due to chromosome segment duplication. Each representative (genic element) functions independently, governing pigmentation of particular plant or seed parts. A genic element is made up of a unique region which is responsible for its tissue-specific action and a common region which can be substituted between elements of contrasting tissue-specific effects. Spontaneous mutations as well as variants isolated following chemical mutagenesis and insertion of transposable elements are utilized in the analysis. These studies provide a map of *R* pigmenting components. We are characterizing other phenomenon of *R* gene regulation, such as paramutation, in similar terms. We intend to relate the components for such phenomena to the map of basic pigmenting determiners.

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

O.E. Nelson, Department of Genetics

$62,000

The goal of this project is to investigate the processes of starch synthesis in the developing maize endosperm utilizing mutants affecting starch synthesis as experimental probes. One such mutant for which no biochemical lesion has previously been detected is *su2*, although it has been known that seeds homozygous for *su2* synthesize

only ca. 90% as much starch as nonmutant seeds and that the amylose content of *su2* starch is elevated (ca. 35%) compared to nonmutant starch (ca. 25%). We have found that the phosphofructokinase activity of mutant endosperms is much lower than that activity in nonmutant endosperms. Following DEAE-cellulose chromatography of nonmutant endosperm extracts, two peaks of enzyme activity are detected while mutant extracts yield only a reduced first peak and lack the second peak. Also, following polyacrylamide gel electrophoresis, nonmutant extracts produce two bands of activity, but the *su2* extracts have only one. It is not clear that the loss of a phosphofructokinase isozyme is the primary effect of the mutation, but if so, it would be the first instance of a biochemical lesion not directly in the path of starch synthesis having an effect on starch production. It is not clear, either, how the loss of this isozyme might affect starch production, but since nonmutant extracts contain ca. twice as much ATP and FBP as mutant extracts, a possible reason is that the blocking of the path from the triose phosphates through PEP to oxaloacetate which would sustain the citric acid cycle results in a diminution of the supply of ATP to the system. This reduced supply of ATP is reflected in the lower production of starch.

128. Carbon Isotope Fractionation in Plants

M.H. O'Leary **$72,166**

Plants fractionate carbon isotopes during photosynthesis in ways which reflect photosynthetic pathway and environment. This fractionation is a complex product of fractionations due to individual steps (diffusion, carboxylation, etc.). The object of our work is to develop methods for using this isotope fractionation to give information about how the components of the carbon fixation process vary with species, environment, and other variables. These studies provide important information regarding environmental control of the efficiency of photosynthesis as well as information regarding changes in photosynthesis which can be expected in coming years as atmospheric carbon dioxide levels increase. To this end, we study quantitative models for carbon isotope fractionation which describe this process in terms of rates of diffusion, carboxylation, and other steps. We measure isotope fractionations associated with the initial events in carbon dioxide fixation, from which we can determine the relative rates of the various individual processes involved. Our approaches are unique in that they provide a view of the carbon isotope fractionation process over a period of a few hours, whereas combustion methods used by previous investigators provide only a long-term view of the carbon isotope fractionation process. We are currently using these methods for studies of environmental variables in isotope fractionation in a variety of plant species, including C_3, C_4, and CAM plants. We have also developed other methods using stable isotopes which rely on NMR and mass spectral measurements to study plant metabolism.

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

P.H. Quail, Department of Botany $56,760

This project is directed at characterizing phytochrome from fully-green, light-grown tissue. We showed previously that such tissue contains a predominant molecular species of Mr-118,000 that differs immunochemically and spectrally from etiolated-tissue phytochrome and a lesser-abundant 124,000-Mr molecule that is apparently similar to etiolated-tissue phytochrome. Attempts, to purify green-tissue phytochrome have resulted in development of a protocol involving a combination of polyethylene glycol precipitation, hydroxyapatite, hydrophobic and ion exchange chromatography that yields a preparation that is ~200-fold enriched. Two major obstacles that we have now overcome were (a) degradation of the Mr-118,000 polypeptide by proteases not inhibitable by inhibitors known to be effective against etiolated-tissue proteases, and (b) separation of the phytochrome from ribulosebisphosphate carboxylase, the major protein contaminant in these preparations. Investigation of the biogenesis of the green-tissue phytochrome species indicates that the amount of the Mr-118,000 polypeptide increases during early seedling development in the light.

130. Molecular Mechanism of Energy Transduction by Plant Membrane Proteins

M.R. Sussman, Department of Horticulture $54,834

The focus of this project is a protein that converts chemical energy into electrical energy. This protein is known as a $[H^+]$-ATPase, or proton pump, and is found in the plasma membrane of fungi and higher plants. Its function is to generate a proton electrochemical gradient across this membrane. The gradient is mainly an electrical potential that, in root hair cells of higher plants and hyphal cells of mycelial fungi, can exceed 200 to 250 millivolts, interior negative. In these cells it generates a protonmotive force essential for the uptake of minerals and nutrients. The protein has unique molecular properties. Since it contains a single polypeptide of Mr = 100,000 that is phosphorylated during the reaction cycle, its chemical structure is very similar to that of cation-translocating ATPases found in the plasma membrane of animal cells (e.g., the $[Na^+, K^+]$-ATPase of kidney, the $[H^+, K^+]$-ATPase of stomach, and the $[Ca^{2+}]$-ATpase of muscle). However, since it only translocates protons, it is functionally more similar to the membrane F_oF_1 ATPase found in bacteria, mitochondria, and chloroplasts. We are using protein modification and sequencing techniques to study how the enzyme functions. Radioactive probes that react covalently with essential amino acids are used to characterize the enzyme's two active sites (an ATP-binding site and an ion-binding site). The ATPase is phosphorylated *in vivo* and *in vitro* by a plasma membrane protein kinase. We are studying the possible role of this phosphorylation in regulating the ATPase activity. The enzyme is being isolated from many plant species (oats, tomato, potato, and carrot) and compared using sequencing and immunological techniques. Results will be used to define a molecular mechanism for protein-mediated energy transduction.

UNIVERSITY OF WISCONSIN - Milwaukee, WI 53201

131. Genetics in Methylotrophic Bacteria

M.E. Lidstrom, Center for Great Lakes Studies $67,340

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 concentrated on the methanol oxidation system since it constitutes a coordinately regulated gene set that contains at least some highly conserved functions. We have cloned and characterized ten genes necessary for methanol oxidation in *Methylobacterium* AM1. These genes include the structural genes for methanol dehydrogenase and cytochrome c_L, four genes involved in regulation or stability;, three genes involved in attachment or positioning of the methanol dehydrogenase cofactor, PQQ, and one gene involved in excretion. We have cloned a 1.5 kbp fragment containing the putative methanol dehydrogenase promoter region into a broad-host range promoter probe vehicle, and are in the process of characterizing this regulatory region. An internal fragment of the methanol dehydrogenase structural gene has been used as a probe to clone a putative methanol dehydrogenase gene from a methane-utilizer.

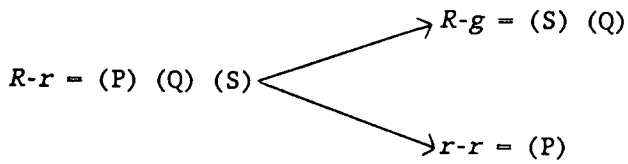
YALE UNIVERSITY - New Haven, CT 06520

132. Molecular Cloning and Structural Characterization of the *R* Locus of Maize

S.L. Dellaporta, Department of Biology $67,340

The *R* locus of maize is one of several epistatic genes involved in the synthesis of pigments in maize. It may act as a tissue-specific regulator of certain genes in the anthocyanin biosynthesis pathway. Last year we were able to obtain a putative *R* clone by association with the maize controlling element *Activator (Ac)*. We have confirmed this clone to be *R*-specific by several criteria including: i) the ability to detect the integration and excision of several transposable elements at *R*; and ii) the cross-hybridization of this clone with other *R* components including the self-color (Sc), plant (P), seed (S), and leaf color (Lc) components. Our most detailed analysis of the molecular genetics of *R* has been a study of the organization of the *R-r:standard* allele in collaboration with Dr. Jerry Kermicle (University of Wisconsin). This allele has been characterized by genetic analysis as a tandem duplication with the (P) component on the proximal member and the (S) component on the distal member of the chromosomal duplication. By unequal crossing-over *R-r* derivatives can be obtained that retain just the (P) or (S) component. We examined

R-r and several (P) and (S) derivatives by genomic blot analysis using the *R* probe. These results suggest that in addition to the (P) and (S) homology, a third sequence that participates in meiotic recombination with the (P) duplication is responsible for (P) and (S) derivative alleles. We propose to name this cryptic component "(Q)". Our present model for intralocus recombination at the *R-r* allele is shown below. We are currently cloning *R-r* and several derivatives to perform a fine structure molecular analysis aimed at elucidating the mechanism of intralocus recombination.



133. Membrane Vesicles as a Simplified System for Studying Auxin Transport

M.H.M. Goldsmith, Department of Biology $70,500

Indoleacetic acid, (IAA), the auxin regulating growth is transported polarly in plants. IAA stimulates a rapid increase in the rate of electrogenic proton secretion by the plasma membrane. This not only increases the magnitude of the pH and electrical gradients providing the driving force for polar auxin transport and uptake of sugars, amino acids and inorganic ions; but by acidifying the cell wall, also leads to growth. We find that auxin uptake by membrane vesicles prepared from actively growing plant tissues exhibits some of the same properties as by cells: the accumulation depends on the pH gradient, is saturable and specific for auxin, and enhanced by herbicides that inhibit polar auxin transport. The saturable uptake of IAA by the vesicles exceeds the uptake predicted from the magnitude of the pH gradient by several fold. We are monitoring the presence of a transmembrane potential using fluorescent cyanine dyes; however, an involvement in accumulation of auxin by the vesicles appears unlikely since a K^+ diffusion potential across the vesicle membrane is not affected by increasing the ionic strength of the medium, whereas uptake of auxin is. On the other hand, reducing intravesicular volume osmotically does not proportionally reduce IAA uptake. The available evidence suggests that a saturable intravesicular auxin binding site is responsible for the excess IAA accumulation by the vesicles.

134. Mechanisms of Potassium Transport in Plants and Fungi

C.L. Slayman, Department of Physiology **$91,390**

Previous research on bacteria (J. Biol. Chem. 255:433, 1980) and on yeast (Biochem. Biophys. Acta 772:51, 1984) postulated cotransport of K^+ ions and protons to be a major mechanism for potassium accumulation in these walled organisms. Experiments of Rodriguez-Navarro, Blatt, & Slayman (J. Gen. Physiol. *In press*, 1986) have demonstrated such a mechanism in the mycelial fungus *Neurospora*, operating with a maximal velocity of >10 mmols/kg cell water min. and K_m values in the range 1-10 um. The mechanism operates measurably only in potassium-deprived cells, requires the presence of ca. 100 um Ca^{++} ions. and functions both the sequester potassium and to balance charges during the acute proton efflux which follows cytoplasmic acid loading.

New experiments are being carried out to determine the system's kinetic response to intracellular K^+ and H^+ ions, and its mode of control by intra- and extracellular calcium ions. These experiments rely mainly on electrophysiological techniques, and the resultant electrical-kinetic data (current-voltage curves) are analyzed by means of simple, generalized reaction models. In addition, standard radioisotope (^{42}K, ^{86}Rb, ^{45}Ca) flux measurements will be made, to obtain complementary chemical kinetic data. Experiments are also planned to isolate the *Neurospora* K^+-H^+ symport protein and characterize it when reconstituted into bilayer lipid membranes.

Identification and characterization of similar systems in higher plant tissues is being attempted via cultured cell preparations from *Arabidopsis thaliana*. Cultured cells have been chosen for initial experiments, because they should provide both cleaner kinetic data and easier access to genetic techniques than will conventional excised tissue preparations. Understanding of the mechanism of potassium accumulation in higher plants is critical in studies of growth, turgor regulation, diurnal movement, and transpiration.

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