DOE/ER-0147/1

Annual Report and Summaries of FY 1983 Activities Supported by the Division of Biological Energy Research

October 1983

U.S. Department of Energy
Office of Energy Research
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Washington, D.C. 20545

INTRODUCTION

The mission of the Biological Energy Research (BER) program is to provide support for fundamental studies in biology oriented towards energy conversion and conservation to underpin future developments in energy related biotechnology. The BER program aims at comprehending biological principles and mechanisms rather than the development of specific technological processes. Implicit in the program is the need for a reciprocal flow of information between BER and those in the technologies about new findings, research needs and trends to avoid isolated paths of endeavor. This is accomplished by emphasizing openness and promptness of reporting research results, participation in interactive meetings and encouragement of investigators to exchange ideas broadly with others and particularly with researchers in industry.

At approximately three year intervals BER sponsors a workshop on research directions to examine the current status of the program, the relationship of recent advances in research to the BER program and what promising new directions are appropriate. In December 1982 the BER program sponsored such a workshop at the Smithsonian Institution's Belmont Conference Center near Baltimore, Maryland. The participants were from academia and industry and represented a number of disciplines. No BER contractors were involved. A number of research areas were pinpointed for attention, including many of those presented in the last year's (Fiscal Year 1982) annual report DOE/ER-0147:

> "Some examples of the research priority areas identified include: 1) definition of stress effects in plants at the biochemical and biophysical levels, and including adaptive mechanisms, 2) the biosynthesis and biodegradation mechanisms of the key natural polymers, cellulose, lignin and other polysaccharides, 3) the development of better understanding of the genetics of those microbes responsible for degradations and fermentations, especially anaerobic microorganisms, 4) the physiological genetics and biochemistry of important processes in both plants and microbial species, and 5) interactive relationships between different species of organisms as with plant host-pathogens, microbial consortia, and plant-soil microbes with emphasis on recognition events, and reciprocal flows of information and substrates. In addition to these areas the BER program maintains a significant stake in photosynthesis (the driving force of biological processes), and other energetics research, plant growth and development mechanisms, genetics of plant systems, microbiological studies on metabolic regulation, thermophily and other areas as will be seen in the following pages of the report."

Also included were a number of research topics to be found in a recent National Academy of Sciences COSEPUP (Committee on Science, Engineering and Public Policy) report. A number of other points were brought out in the BER workshop. There was considerable concern expressed about the need for more collaboration between investigators in the plant and microbiological sciences and those in other disciplines. The rationale for this suggestion is the perceived growing complexity of research problems that necessitate broader thinking and input for more substantial progress. Of particular note was the view that plant scientists, in general, were not availing themselves of state-of-the-art instrumentation and techniques. In some cases they may be unaware of the kinds of instrumentation and capabilities that are available.

Acting on a suggestion of the Belmont meeting participants, a follow-on workshop on Plant Science and Instrumentation was convened at the National Synchrotron Light Source at Brookhaven National Laboratory (BNL) on 14-15 July 1983. The BNL workshop brought a group of plant scientists together with a group of workers deeply involved with instrumentation development and application. The interaction included plant scientists discussing the types of measurements that would be valuable in physiological and biochemical research if suitable techniques and instrumentation were available. The instrumentation oriented researchers discussed the versatility, potentialities and attributes of a number of representative techniques. Out of this meeting emerged several collaborative ideas. The summary report of this workshop, which is expected to be completed in fall 1983, will also contain several suggestions about how the generic issue of plant science and instrumentation might be attacked.

As mentioned above, during the Fiscal Year 1983 additional recognition of the importance of plant science research and the opportunities available was documented in a report under the auspices of the Office of Science and Technology Policy and the National Science Foundation through the National Academy of Sciences COSEPUP. That report confirmed the premises upon which the BER program and certain other research efforts sponsored by NSF and USDA are based. The research agenda identified is broad in scope. The fundamental research issues are relevant not only to agricultural interests but also to research programs in which the long term objectives relate to renewable resources (biomass) destined for use as fuels and chemicals. One inevitable question is how to coordinate work of several agencies that seems to be similar. In this case the question has been resolved. Since 1978 there has been in existence an Interagency Plant Science Committee which was organized not by mandate but by the initiative of individuals from several agencies who recognized the neglected state of plant science research and foresaw the major practical opportunities that would evolve from the support of fundamental plant science research. This interagency group meets monthly

to discuss numerous aspects of plant science research including research areas, manpower, facilities, equipment and a broad array of other topics. There is prompt and free exchange of information about various agency policies and funding so that each agency is apprised of what other agencies are doing. Clearly the mechanism for coordination exists and is operative.

Some representative contributions of BER contractors during the FY 83 period include the following: a) Progress was made in modifying the Agrobacterium tumefaciens Ti plasmid as a vector in the transfer of DNA in higher plants. The plasmid was "disarmed" by genetically altering that information which is responsible for unwanted tumor formation in recipient plants. In addition it was demonstrated that antibiotic resistance as a representative genetic character may be transferred by the Ti plasmid to a recipient plant culture (see project #72). b) By using appropriately constituted genetic lines of maize it has been possible by following male sterility restorer function to measure for the first time the frequency and character of genetic transposition events in a higher organism (see project #35). c) The gene coding for susceptibility to the herbicide atrazine in higher plants has been identified, cloned and sequenced. A mutant gene which is responsible for resistance to the herbicide has likewise been studied. The difference between the two is one nucleotide in the DNA sequence (see projects #99 and #105). d) Amino acid sequences of a number of proteins of the smallest functional photosynthetic particle, known as the reaction center, of the photosynthetic bacterium Rhodopseudomonas capsulata have been determined by cloning and sequencing the corresponding DNA. This information has led to new insights concerning the structural and functional nature of the proteins in photosynthesis (projects #95 and #98).

A number of other activities which are not listed in the summaries were funded during FY 83. These include:

1) Cold Spring Harbor Laboratory, partial support of a workshop entitled "The Genetics and Molecular Biology of Chlamydomonas" which was helc June 26-30, 1983.

2) University of California, Riverside, partial funding for 6th Annual Symposium in Botany and Plant Sciences "Biosynthesis and Function of Plant Lipids", held January 13-15, 1983.

3) University of Minnesota, partial support for the "Fourth International Symposium on Microbial Growth on One-Carbon Compounds" held September 6-10, 1983.

4) Marine Biological Laboratory, partial funding for "A Microbial Ecology Summer Research Program with Special Emphasis on Studies with Anaerobic Microorganisms" held June 19 - August 26, 1983, Woods Hole, Massachusetts.

5) National Academy of Sciences, partial support of "Convocation on Genetic Engineering of Plants: Agricultural Research Opportunities", held at the National Academy of Sciences, May 23-24, 1983.

In the course of FY 83 a total of 71 new research proposals were received and 12 funded. While the BER program continues to grow modestly it is also subject to many of the constraints faced by other programs in active areas.

	No. of Projects	FY 83 Funding (in thousands of $)	% of Total Funds
University Contracts	73	5147	54
Michigan State University Plant Research Laboratory	11	1675	18
National Laboratories Brookhaven National Lab Lawrence Berkeley Lab Oak Ridge National Lab	15	2045	22
Solar Energy Research Inst.	1	105	1
Other Research Institutions (federal, state, industrial, non-profit)	10	422	4
Conferences and miscellaneous	6	106	1
TOTAL	116	9500	

A few words need to be said concerning the manner in which projects are listed as summaries. All projects which were active in Fiscal Year 1983 are listed including several which were funded with FY 82 monies. The funding levels indicate total costs. With respect to projects which are carried on in facilities such as Brookhaven National Laboratory,

Oak Ridge National Laboratory, and the Lawrence Berkeley Laboratory the funding levels will appear deceptively high compared to university projects inasmuch as oftentimes salaries of the principal investigators are included in the total funding figures. If there are any questions about the technical aspects of any of the projects listed these may be pursued by contacting the principal investigator.

There is an obligation, a most pleasant one, to express once again our deep appreciation to the large number of scientists both in the United States and abroad who have provided assistance to the BER program during the past year. Without the help in reviewing the technical quality of proposals, both newly submitted and on-going ones, it would be virtually impossible to achieve the high quality program that exists.

Questions about the overall Biological Energy Research program should be directed to:

Dr. Robert Rabson
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

1. U. S. DEPARTMENT OF AGRICULTURE
Ithaca, New York 14853

REGULATION OF EXPRESSION OF A SOYBEAN STORAGE PROTEIN SUBUNIT GENE. $40,637

John F. Thompson and James T. Madison
Plant, Soil and Nutrition Laboratory

The objective of this research is to understand the regulation of a plant gene. In cotyledon culture, the ß-subunit of the 7S storage protein of soybeans is normally present. Addition of methionine to the basal medium results in the absence of the ß-subunit. Cotyledons will be cultured on basal medium with and without methionine and will be switched from medium without methionine to medium with methionine and vice versa to obtain evidence as to whether the effect is due to transcription, translation, or post translational effects. Messenger RNA from cotyledons grown without and with methionine will be tested for the presence of mRNA for the ß-subunit (by in vitro wheat germ system) and for ß-mRNA precursors (by hybridization to a cDNA clone). The possibility that post-translational processes are inhibited by methionine will be determined by use of ß-subunit specific antibodies.

2. BATTELLE COLUMBUS LABORATORIES
Columbus, Ohio 43201

COMPLEXES OF CHLOROPHYLL DERIVATIVES WITH HEME APOPROTEINS $98,000 (FY 82 funds)

Robert M. Pearlstein
Organic and Polymer Chemistry Section

The chlorophyll (Chl) in either an antenna or a reaction center (RC) protein complex displays a redshift of the longest-wavelength peak in its absorption spectrum (relative to the corresponding monomeric Chl in a solvent) that is difficult to explain in terms of Chl-Chl interactions. Because these anomalous redshifts are probably caused by a mechanism that, in RC complexes, helps to stabilize primary electron transfer, we are investigating the possibility that the redshift results from specific Chl-protein interactions, and exploring any connection between such interactions and the redox properties of primary donor Chl. Initial work with complexes of Chl derivatives and heme apoproteins led to the development of several models for Chl-protein interactions. One of these, which invokes the Coulomb interaction of Chl with charged amino acids in the protein, now appears most promising. We find that the Chl derivative, 3-demethyl-3-(aminomethyl) Chl a, exhibits a reversible 5-nm absorbance shift (to the blue) upon complete protonation of the amine nitrogen. Calculations by others strongly support this model, specifically that point charges selectively placed near the Chl periphery can induce redshifts of the magnitude observed in vivo, and also can have a large effect on the value of the Chl redox potential. We have also synthesized a Chl Schiff base that displays a reversible 23-nm redshift on protonation of the imine nitrogen. The possibility that a Schiff base linkage exists between Chl and an appropriate amino acid (e.g., lysine) in a photosynthetic protein complex is being explored for RC's of Rhodospeudomonas sphaeroides. Studies are planned with additional model compounds that have inducibly chargeable groups, and with techniques for exploring possible Chl-(charged-amino-acid) interactions in Chl-protein complexes from photosynthetic organisms.

3. BOYCE THOMPSON INSTITUTE
Cornell University, Ithaca, New York 14853

CARBON METABOLISM IN LEGUME NODULES $40,034
Thomas A. LaRue

The objective of the project is to determine how the legume nodule uses carbohydrates to provide energy and reductant for nitrogen fixation. Because the plant cells within a nodule are essentially microaerobic, we are investigating the possible role of anaerobic pathways in metabolizing carbohydrates. We have established the utility of changing the gas phase available to bacteroids, detached nodules, or intact plants to manipulate enzyme activity. A brief treatment with pure oxygen decreases nitrogenase activity, and permits study of changes in processes closely related to nitrogenase. The oxidation and reduction of flavins and pyridine nucleotides in intact bacteria can be monitored in situ by changes in their fluorescence.

4. BRANDEIS UNIVERSITY
Waltham, Massachusetts 02254

EFFECT OF LIGHT ON RESPIRATION AND DEVELOPMENT OF PHOTOSYNTHETIC CELLS $48,600
Martin Gibbs
Institute for Photobiology of Cells and Organelles

C.reinhardtii F-60, a mutant unable to fix CO_2, breaks down starch at an anaerobic rate of 12.4 $\pm$ 2.2 µmol glucose in starch consumed·mg^{-1}Chl·$4h^{-1}$. Light decreases the anaerobic rate by a factor of 2 and 4 at 40 and 100 w·m^{-2}. Fermentative products (moles) are, per mole of glucose consumed, in dark vs in light (100 w·m^{-2}): formate 1.8-2.1 vs 0.7-1.2; acetate 0.8-1.1 vs none; ethanol 0.8-1.0 vs 0.05-0.2; glycerol 0.02-0.06 vs 0-0.02; CO_2 0-0.15 vs 2.2-3.4; H_2 0.15-0.4 vs 6-6.8. Carbon recovery is 95-102% (dark) vs 60-75% (light); O/R index is 0.87-1.1 (dark) vs 0.67-1.05 (light); H recovery is 94-104% (dark) vs 65-84% (light). Starch breakdown rate is doubled by 5µM FCCP in dark, and reaches the dark level in light. Molar proportions of the fermentative products are not affected by FCCP in dark, except H_2 which is 0.1; in light, formate is 1.4-2.5, acetate 1.2-1.7, ethanol 0.1, CO_2 1.5-1.9, H_2 4-5.5. In light, 10µM DCMU decreases CO_2 and H_2 to (moles): 0.9-1.3 and 2.5-3.4, and increases formate to 1.5, while having no effect on the other products. Aerobically, ethanol and formate are not consumed in dark or in light. To account for the stoichiometry, the only known pathway is glycolysis followed by pyruvate breakdown into formate and acetyl-CoA. In dark, acetyl-CoA is converted to acetate and ethanol. Light (100 w·m^{-2}) redirects C and H flow since: 1) H_2 and CO_2 evolution is increased, 2) ethanol production is inhibited 3) acetate production is inhibited in absence of FCCP, 4) DCMU does not restore the pattern of fermentation seen in the dark. In addition, it is hypothesized that, in light, acetyl-CoA is converted to fatty acids except when FCCP is present, ethanol formation is prevented by light-induced inhibition of acetaldehyde dehydrogenase, and in the presence of DCMU H_2 formation results from reduced pyridine nucleotides preferentially feeding electrons to photosystem I.

5. UNIVERSITY OF CALIFORNIA AT SAN DIEGO
La Jolla, California 92093

HYDROGENASES, THEIR CLONING, PREPARATION, CHARACTERIZATION AND MODIFICATION FOR USE IN PHOTOBIOREACTORS — $75,000 (FY 82 funds)

Robert G. Bartsch, Martin D. Kamen and Nathan O. Kaplan
Department of Chemistry, A-002

We are seeking to characterize a hydrogenase molecule at the molecular level. The enzyme from Desulfovibrio vulgaris is readily crystallized from ammonium sulfate solution to yield crystals of sufficient size for initial x-ray crystallographic study. Because the enzyme proves to be unstable under the crystallization conditions tested we are studying alternate conditions which utilize low salt concentrations for generating suitable crystals. Based on two partial amino acid sequences with minimal degeneracy in the corresponding DNA sequences, we are synthesizing a mixture of all possible oligonucleotides which could code for the peptide fragments to serve as hybridization probes for identifying restriction enzyme generated gene fragments to be used to clone the hydrogenase gene and ultimately to determine the DNA sequence of the gene and consequently of the corresponding amino acid sequence of the gene product. From the x-ray crystallographic and amino acid sequence studies we propose to determine the three dimensional structure of the hydrogenase molecule. With the aid of this information we hope to be able to understand the enzyme mechanism for catalysis of the seemingly simple chemical reaction typified by the equation: $H_2 = 2H^+ + 2e^-$. Our long range goal is to learn how to incorporate a suitably engineered hydrogenase function into an organism such as a cyanobacterium in the effort to construct an active self-replicating fuel gas generator dependent on water and sunlight for raw materials. In a parallel study we have undertaken to identify possible suitable thermophilic cyanobacteria which might serve as a host for the proposed cell construct.

6. UNIVERSITY OF CALIFORNIA
Los Angeles, California 90024

ENERGY CAPTURE AND USE IN PLANTS AND BACTERIA — $82,000

Paul D. Boyer
Department of Chemistry and Biochemistry

Our studies focus on how plants use sunlight energy and bacteria use biomass energy to synthesize adenosine triphosphate (ATP). This is quantitatively the most prominent chemical reaction in the world. An ATP synthase complex in chloroplast and bacterial membranes forms ATP in a reaction sequence coupled to the use of an electrochemical proton gradient. Our laboratory has developed the energy-linked binding charge hypothesis for ATP synthesis, in which identical catalytic sites on the enzyme participate in coordinated alternation. Experimental evidence favors the view that energy promotes competent binding of inorganic phosphate and adenosine diphosphate (ADP) at one catalytic site and the release of ATP from another. This mechanism appears to be gaining support but needs critical evaluation from many approaches. Studies are underway using ^{18}O-labeling probes of the reversal of bound ATP cleavage with the membrane-bound ATP synthase commplex and the isolated CF_1-ATPase portion, and of the nature and extent of bound intermediates with ^{3}H and ^{32}P-labeled substrates. We will soon initiate fluorescent quench-flow studies of ATP and ATP analog binding. Together with earlier data the results may give a critical evaluation of the proposed existence of a transitorily tightly bound ATP that is rapidly and reversibly hydrolyzed at the catalytic site. Other studies underway arise from our new hypothesis that catalysis must be accompanied by a rotational reorientation of catalytic and noncatalytic subunits. As one probe of this we are studying selected chemical derivitization of subunits in the catalytic and noncatalytic state. Also, studies employing our ^{18}O-exchange techniques have been initiated with mutants forms of the E. coli enzyme with defective noncatalytic subunits to gain further insight on subunit interaction.

7. UNIVERSITY OF CALIFORNIA
Davis, California 95616

FLUORESCENCE PHOTOBLEACHING MEASUREMENTS OF PLANT MEMBRANE VISCOSITY: MECHANISMS OF RESISTANCE TO ENVIRONMENTAL STRESS — $60,000

R. W. Breidenbach, D. W. Rains, and M. J. Saxton
Plant Growth Lab/ Agronomy and Range Science

The purpose of this project is to examine the role of the plasma membrane in determining the responses of different crop species to temperature and salinity: two factors strongly limiting plant productivity. The experiments use fluorescence photobleaching recovery to compare the lateral diffusion rates of proteins and lipids in the plasma membranes of plants differing in their sensitivity to chilling temperatures or high concentrations of salts. Membrane viscosities of sensitive and resistant plants are being compared at various temperatures and salt concentrations. Since many important cellular processes may depend directly upon lateral motion of membrane proteins, this approach provides a direct measure of the relation between the physical properties of the membrane and plant responses to environmental stresses. Understanding of this relationship will provide useful strategies for developing tolerant new plant varieties for production of food, fuel and chemicals.

The techniques we are using will also enable us to examine two other problems in plant biophysics:

1) The effect of the cell wall on lateral diffusion rates
2) The mechanism by which certain polysaccharide fragments trigger the synthesis by the plant of defensive compounds against fungal attack.

8. UNIVERSITY OF CALIFORNIA
Berkeley, California 94720

THE REGULATION OF ENZYME SECRETION IN PLANT CELLS BY CALCIUM — $82,000

Russell L. Jones
Department of Botany

The objective of this project is to define the role of calcium in enzyme secretion from aleurone layers of barley (Hordeum vulgare L.) seeds. The Ca^{2+} concentration of the incubation medium regulates the release of two isozymes of α-amylase, as well as acid phosphatase, protease and ribonuclease from aleurone layers. Using pulse-labelling techniques in conjunction with electrophoretic and serological procedures, I propose to establish the relationship between enzyme synthesis and secretion in the presence and absence of Ca^{2+}. Cytoplasmic calcium levels will be determined with the fluorescent dye quin-2 in suspensions of barley aleurone protoplasts. Organelles involved in the sequestration of Ca^{2+} will be identified by light and electron microscopy. Chlorotetracycline fluorescence will be used to localize the sites of Ca^{2+} deposition in isolated protoplasts by light microscopy. For electron microscopy antimonate will be used to precipitate calcium as electron-dense calcium antimonate in aleurone layers incubated in the presence and absence of Ca^{2+}. Inhibitors of Ca^{2+} transport, Ca^{2+} antagonists and uncouplers will be used to interfere with Ca^{2+} sequestration and thus aid in the identification of those organelles involved in Ca^{2+} homeostasis. The presence of calmodulin in aleurone cells will be determined by extraction of this protein and identification of its activity via the activation of NAD kinase. The existence of other Ca^{2+}-binding proteins will be explored by measuring the Ca^{2+}-binding capacity of proteins purified by electrophoresis.

9. UNIVERSITY OF CALIFORNIA
Irvine, California 92717
Berkeley, California 94720

BIOENERGETICS OF SALT TOLERANCE — $135,000

Janos K. Lanyi
Dept. of Physiology and Biophysics, UC Irvine
Lester Packer
Dept. of Physiology and Anatomy, UC Berkeley

The bioenergetic aspects of cellular salt tolerance include ion transport across membranes, redirection of metabolic pathways for the increased synthesis of osmoregulatory compounds, replacement of some salt sensitive components with salt resistant ones, modification of the photosynthetic apparatus for increased efficiency, and the signals which turn these processes on and off during salt stress. We have developed and adapted methods to study these phenomena in three systems, each uniquely suited to answer specific questions. In membranes of Halobacterium halobium and the cyanobacterium, Synechococcus 6301, we are studying the active and passive transport of sodium, potassium, and chloride ions, and the regulation of the transporters. In whole cells of Synechococcus and in sugar beet (Beta vulgaris) leaf segments, we follow the pathway of carbon dioxide assimilation and the enzymes responsible before, during, and after growth in the presence of salt. In Synechococcus we explore the membraneous and cytoplasmic events which occur during short-term adaptation to salt. In thylakoids of Synechococcus and intact chloroplasts of sugar beet leaves, we are attempting to describe features of the photosynthetic apparatus which are changed by salt stress. Results with these systems so far have yielded interesting and promising results. Using this broad approach, we intend to develop the conceptual basis for a refined description of the physiology of cellular adaptation to salinity.

10. UNIVERSITY OF CALIFORNIA, LOS ANGELES
Los Angeles, California 90024

METHANOGENESIS FROM ACETATE, A KEY INTERMEDIATE IN NATURE — $85,974 (14 months)

Robert A. Mah
Division of Environmental & Nutritional Sciences

The objective of this project is to isolate and characterize new strains and species of methane-producing bacteria, particularly those responsible for converting acetate to methane and carbon dioxide. The approach utilizes samples from anaerobic habitats inoculated into an acetate enrichment system. Sediment from the La Brea Tar Pits, Los Angeles, Ca., a solar saltern, a swine waste lagoon, and a peat bog were examined. Methane evolution was followed by gas chromatographic measurement of the head space. When methane was formed at an exponential rate, a roll-tube dilution of the enrichment is made into habitat-simulating media containing acetate, methanol, or trimethylamine but not H_2. Colonies exhibiting fluorescence under UV epi-illumination are picked and purified. We are characterizing three isolates from these samples. Two of these are hydrogen-oxidizing methanogens which exhibit differences in cationic requirements and/or immunological cross reactivity sufficient to warrant further scrutiny as new species. The third isolate is a halophilic methylotroph which utilizes methanol and trimethylamine but not acetate nor H_2/CO_2 as methanogenic substrates. This latter isolate appears to be widely distributed in hypersaline environments of marine origin. Further characterization of physiological, morphological, and macromolecular properties of these isolates is ongoing.

11. UNIVERSITY OF CALIFORNIA AT SAN DIEGO
La Jolla, California 92093

SELECTION OF MUTANTS INCREASING THE RATE OF FERMENTATION IN YEAST — $50,000

Christopher J. Wills
Department of Biology, C-016

We are using a variety of selective approaches to investigate the role in yeast metabolism of energy transfer mechanisms across the mitochondrial membrane, most particularly in the regulation of glycolysis and gluconeogenesis. An understanding of this regulation will in turn enable us to select yeast strains with greatly increased metabolic rates.

Our primary approach is to select for mutants that can grow well under conditions in which the transfer of energy-rich compounds across the mitochondrial membrane is limiting. One of these conditions is growth on a simple medium with glycerol as a sole carbon source. Mutants able to grow on this medium have apparently increased the efficiency of the malate-aspartate shuttle, one of the shuttles involved in regulating the flow of energy across the membrane. Other shuttles and transport systems are also susceptible to mutational alteration, through growth on limiting media in the presence of various inhibitors. In addition to examining these mutants for altered metabolism, we will screen for altered mitochondrial membrane proteins. Isolation of these latter mutants will enable the genes for these proteins to be cloned, and increase our knowledge of the molecular basis of mitochondrial membrane transport.

12. UNIVERSITY OF COLORADO
Boulder, Colorado 80309

STUDIES OF PLANT CELL WALLS AND OF PLANT-MICROBE INTERACTIONS — $300,000

Peter Albersheim, Alan G. Darvill, Michael McNeil and
Barbara Valent
Department of Chemistry, Campus Box 215

The DOE grants support four major projects in this laboratory. These are: identifying, isolating, and characterizing naturally occurring carbohydrates with biological regulatory functions, developing new methods for the purification and structural characterization of complex carbohydrates, delineating the structures of the polysaccharides present in the walls of growing plant cells, and developing a genetic system for Pyricularia oryzae, the fungus that causes rice blast disease. The latter project has, as its primary goal, the identification of the genes and gene products responsible for race and cultivar specificity in this host-pathogen system. All of these projects, but especially the first three, are closely interrelated. Using newly developed techniques, many of which are being developed in our laboratory, for the purification and structural characterization of complex carbohydrates, we have discovered that the carbohydrates that comprise the plant cell wall are far more structurally complex than had been envisioned. A question that we are attempting to answer is why are these cell wall polysaccharides so complicated. A possible answer comes from our observation that plant cell wall oligosaccharides, when released from the confines of the cell wall, appear to regulate a variety of physiological functions in plants, such as morphogenetic differentiation, growth rate, and defense against pathogens.

13. CORNELL UNIVERSITY
Ithaca, New York 14853

STUDIES OF PHOTOSYNTHETIC ENERGY CONVERSION — $65,000
Roderick K. Clayton
Division of Biological Sciences

We continue to study the photochemical and associated "dark" reactions of photosynthetic bacteria; principally Rhodopseudomonas sphaeroides and Rp. viridis. Our methods include optical absorption and emission spectrometry, measurements with polarized lights, and some specialized spectrometric techniques (EPR, Raman and atomic absorption). We continue to explore the reversible binding of the 800 nm component of the B850 antenna pigment protein in Rp. sphaeroides. Our aim is to learn the nature of the bacterial chlorophyll-protein binding, especially through the competitive binding of bacterial chlorophyll and related molecules. To aid in these studies we have developed a laboratory computer system based on the HP9826 computer.

14. CORNELL UNIVERSITY
Ithaca, New York 14853

EFFECTS OF FREEZING AND COLD ACCLIMATION ON THE PLASMA MEMBRANE OF ISOLATED CEREAL PROTOPLASTS — $42,017
Peter L. Steponkus
Department of Agronomy

The primary objective of the project is to characterize the stress-strain relationship (SSR) of non-acclimated (NA) and acclimated (ACC) protoplasts (Secale cereale L. cv. Puma) to provide a mechanistic basis for the increased tolerance of acclimated protoplasts to large area deformations and, as a result, the decreased incidence of expansion-induced lysis during a freeze-thaw cycle. The large difference in the tolerable surface area increment between NA and ACC protoplasts is the result of differences in the subduction/incorporation of membrane material during contraction/expansion and is not attributable to differences in the elastic properties of the plasma membrane. Whereas in NA protoplasts osmotically induced contraction results in deletion of membrane area by endocytotic vesiculation, contraction of acclimated protoplasts results in the exocytotic extrusion of membrane material that remains contiguous with the plasma membrane. Material subducted into endocytotic vesicles in NA protoplasts is not as readily reintroduced into the plasma membrane as is the material which is exocytotically extruded in ACC protoplasts. As a result, the proportional rate of change in area as a function of tension, $Z(\gamma)$, is substantially higher in ACC protoplasts during expansion following hypertonic-induced contraction. In contrast, $Z(\gamma)$ of NA protoplasts is similar whether the protoplasts are expanded from hypertonic or isotonic solutions. Partial conservation of membrane material in osmotically contracted ACC protoplasts results in a lower, possibly even zero, resting tension (γ_r) in contrast to the reestablishment of γ_r in NA protoplasts. Freeze fracture analysis demonstrates that the protein complement of the PF_p fracture face of the plasma membrane is conserved due to the formation of crystalline arrays and the preferential deposition of lipid material in the interior of the exocytotic extrusions.

15. CORNELL UNIVERSITY
Ithaca, New York 14853

THE IMPORTANCE OF PHYTOALEXIN TOLERANCE AND DETOXIFICATION FOR PATHOGENICITY — $58,377

Hans D. VanEtten and David E. Matthews
Department of Plant Pathology

The production of antimicrobial compounds (phytoalexins) by plants is believed to function as an active mechanism of disease resistance which is effective against at least some microorganisms. However, phytoalexin-producing plants are nonetheless susceptible to some diseases. Our research has indicated that one way in which successful pathogens can overcome this resistance mechanism is by detoxifying their hosts' phytoalexins. The primary host-parasite interaction we have investigated is a root and stem rot disease of pea (Pisum sativum) caused by the fungus Nectria haematococca (Fusarium solani). Virulent isolates of this fungus detoxify pisatin, the major phytoalexin produced by pea. Genetic studies involving crosses between appropriate isolates strongly suggest that the ability to detoxify pisatin is an essential trait for virulence on this host. The detoxification reaction is catalyzed by a substrate-inducible cytochrome P-450 monooxygenase. N. haematococca detoxifies at least two other phytoalexins produced by other legume species of which it is a pathogen. These reactions are also mediated by oxygenases. The objectives of this research proposal are to characterize further the enzymes responsible for phytoalexin detoxification, especially with regard to their substrate specificity, sensitivity to inhibitors, regulation, and relationship to virulence. Both biochemical and genetic analyses will be used to accomplish these objectives. The results will have significance for potential practical applications involving phytoalexins as a means of disease control, as well as for basic theories of plant-parasite interactions.

16. CORNELL UNIVERSITY
Ithaca, New York 14869

MICROBIAL ECOLOGY OF THERMOPHILIC ANAEROBIC DIGESTION — $68,083

Stephen H. Zinder
Department of Microbiology

The anaerobic digester is a microbial ecosystem in which several groups of microorganisms must interact effectively if efficient bioconversion of organic matter to methane is to occur. Our understanding of the organisms involved in anaerobic digestion and their interactions is still rudimentary. The objective of this project is to provide an integrated understanding of the ecology of the microbial populations present in a thermophilic (58°C) laboratory-scale digester being fed a lignocellulosic waste. To do this we have used cultural, microscopic, and radiotracer techniques. We have been especially interested in the ecology of methanogens in the digester. Results obtained thus far include: (1) the discovery and isolation of a two-membered microbial coculture which converts acetate to methane via interspecies hydrogen transfer rather than the aceticlastic reaction; (2) the discovery of a thermophilic Methanothrix. This organism has not been described previously and grows at temperatures over 65°C, contains gas vacuoles, and grows more rapidly than mesophilic strains; (3) Methanothrix eventually displaced Methanosarcina as the dominant aceticlastic methanogen in the digester, possibly because of its low K_s value for acetate (0.3 mM); (4) When Methanosarcina, which does not grow at 65°C, was dominant in the digester an upwards temperature shift to 65°C caused acetic acid buildup and digester failure. When Methanothrix was dominant, the temperature shift did not inhibit methanogenesis from acetate; (5) Methanogenesis from acetate in the digester was completely inhibited by 1 mM 2-bromoethanesulfonate (BES) while 50 mM BES was required for inhibition of methanogenesis from CO_2. Thus, toxicants can affect aceticlastic and CO_2 reducing methanogenic populations differently.

17. FLORIDA STATE UNIVERSITY
Tallahassee, Florida 32306

GUARD CELL BIOCHEMISTRY: RESPONSES TO ENVIRONMENTAL STIMULI CAUSING CHANGES IN GAS EXCHANGE — $74,655

William Outlaw
Department of Biological Science

The objective of this project is to investigate guard cell biochemistry, particularly as it is affected by the environment. (Guard cell pairs, or "stomata", are sprinkled throughout the surface of leaves. Each pair resembles a donut, except the pore size is adjustable, depending on the turgidity of the surrounding guard cells. Virtually all CO_2 for photosynthetic reduction enters the leaf through these pores; likewise, water loss from the leaf also passes through these pores. Thus, the function of these cells is to control gas exchange. Several environmental variables are integrated to optimize aperture size.) Our approaches to this problem are diverse; a particular difficulty is to obtain sufficient quantities of these cells in pure form. One approach is the isolation of guard cell protoplasts. Although we do not have the techniques perfected, we have published one paper on purification of enzymically released cells. Our second approach is to dissect out individual cell samples, which may be subsequently assayed using either quantitative histochemical methodology (one paper on carbon metabolism) or other sensitive techniques (quantification of the phytohormone, ABA). Our third approach is to optically isolate cells. Using this approach, we have unequivocally localized PSII in guard cells (published). In addition, a review article on the role of potassium in guard cell has been submitted; this review complements my recent review on carbon metabolism in these cells.

I hope our work will have practical implications. Production of much of the food, fiber and shelter in this country relies on the functioning of these cells. As important, a better understanding of the regulation of water loss from plants may provide opportunities to conserve this limiting resource.

18. UNIVERSITY OF FLORIDA
Gainesville, Florida 32610

INVESTIGATION OF THE TRANSPOSITION OF MITOCHONDRIAL DNA AND ITS RELATIONSHIP TO FERTILITY IN ZEA MAYS — $68,961

Rusty J. Mans
Department of Biochemistry and Molecular Biology,
J-245, JHMHC

We are looking for altered expression of mitochondrial genes that affect male fertility in maize. These genes are rearranged and their copy numbered altered upon the spontaneous reversion of an S-type cytoplasmically male sterile parental plant to fertility. Sterility in these lines is also suppressed or repressed by the dominant allele of a nuclear gene, i.e. restorer to fertility (Rf3). We have cloned segments of the transposable mitochondrial genes into pB R322 for use as templates in an in vitro transcription system. We have purified an alpha-amanitin sensitive RNA polymerase from maize seedlings that preferentially transcribes cloned DNAs. We will characterize the RNA products accumulated in the system as to size and nucleotide sequence. By comparison of transcripts of appropriate cloned segments of the mitochondrial genome from sterile and fertile revertant plants we will detect altered transcription as a consequence of gene rearrangement in plant mitochondria. These results will facilitate the use of portions of the mitochondrial genome as putative vehicles for genetic engineering of this agronomically important grain.

19. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

ROLE OF CA^{2+} AND CALMODULIN IN PHOSPHORYLATION IN PLANTS — $50,000
Milton J. Cormier
Department of Biochemistry

The long term objectives are to better understand the role of Ca^{2+} and calmodulin in cellular regulation in plant cells. The short term objective is to understand the role of Ca^{2+}-dependent phosphorylation in plant cells. Our demonstration of the presence of high affinity Ca^{2+} binding proteins (such as calmodulin) in plants strongly suggest that free Ca^{2+} is acting as a second messenger in plant cells. Our recent observation that Ca^{2+} and calmodulin are absolute requirements for the activation of plant NAD kinase provide further support for this theory. These findings suggest that free Ca^{2+} may be important in mediating some of the plant responses to stimuli such as light, auxins, gibberellins, and cytokinins. We are examining the Ca^{2+}-dependent regulation of phosphorylation, i.e. phosphorylation of small molecules (such as NAD) and proteins. We are examining these regulatory events in several soybean tissue culture cell lines. Studies on these broad based regulatory functions of calmodulin in plants may lead to the formulation of new growth regulators, new herbicides, and the manipulation of flowering time.

20. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

BIOSYNTHESIS OF A MICROBIAL BIOSURFACTANT: A NOVEL AND UNIQUE PRODUCT — $75,000
W. R. Finnerty
Department of Microbiology

The objective of this project is to determine the mechanism of biosynthesis of a microbial-produced extracellular glycolipid biosurfactant which is effective in the viscosity reduction of heavy crude oil. The approach is to study the enzymology of glycolipid biosynthesis and the genetic relationship between alkane utilization and glycolipid synthesis. Results to date show the glycolipid can be labeled with specific radioactive precursors: glycerol, acetate, propionate, glucose and palmitate. Acyl transacylase activity has been localized in the soluble fraction which acylates the water-soluble backbone of the glycolipid. Glycolipid is synthesized only by alkane-grown cells. The microorganism contains three plasmids of 55, 14.5 and 11.5 Mdal. The genetic and metabolic relationship between alkane utilization and glycolipid formation will be determined. The glycolipid forms stable oil-in-water microemulsions of heavy crude oils with significantly improved rheological properties in the oil. Such bioproducts have application to the microbial desulfurization of high-sulfur crude oil, transportation of heavy oils and enhanced oil recovery.

21. UNIVERSITY OF GEORGIA
Tifton, Georgia 31793

DEVELOPMENT OF INNOVATIVE TECHNIQUES THAT MAY BE USED AS MODELS TO IMPROVE PLANT PERFORMANCE — $36,000

Wayne W. Hanna and Glenn W. Burton
Department of Agronomy

The objectives of this project are to develop techniques for transferring genes from wild species to cultivated plant species, to demonstrate the wealth of germplasm in the secondary and tertiary gene pools that can be transferred to cultivated species and to develop an obligate apomictic pearl millet. Species within the genus Pennisetum are being used as test organisms. The approach utilizes plants of wild species with different genetic backgrounds and ploidy levels (chromosome numbers) crossed and backcrossed with different genotypes of pearl millet, P. americanum, with different ploidy levels to produce fertile interspecific hybrids and derivatives. Germplasm is being transferred to pearl millet through genetic recombination in the interspecific hybrids followed by genome segregation, only genome segregation, and/or by using gamma radiation to produce small translocations (chromosomes with desirable genes) between chromosomes of the wild species and pearl millet. These studies are providing valuable information on the most efficient ways to transfer alien germplasm and could result in the transfer of valuable genes such as pest resistance, drought tolerance, perennial growth habit and apomixis to pearl millet. The overall impact would be on increased, more efficient and more reliable production of food, fiber, and forage.

22. UNIVERSITY OF GEORGIA
Athens, GA 30602

ENVIRONMENTAL STRESS MEDIATED CHANGES IN TRANSCRIPTIONAL AND TRANSLATIONAL REGULATION OF PROTEIN SYNTHESIS IN CROP PLANTS — $84,000

Joe L. Key
Department of Botany

When seedlings of soybean, and a wide range of other crop plants, are subjected to a temperature shift from 28°-30° to about 40° (heat shock or hs), there is a rapid and dramatic shift in the pattern of protein synthesis. Plants synthesize 30 to 40 hs proteins and greatly reduce normal protein synthesis during hs. These hs proteins seem to provide thermal protection to the plant; that is, plants subjected to a tolerant hs temperature (e.g. 40° for soybean) for some 2-3 hr are now tolerant of an otherwise lethal temperature (e.g. 45° for 2 hr). A number of treatments lead to the development of thermal tolerance in soybean: 1) 40° for 2 hr, 2) 45° for 4 to 8 min followed by 2 hr at 28°, 3) gradual increase in temperature (e.g. 3° per hr) from 30° up to 45°-47.5° and 4) arsenite (50 μm) for 2 to 4 hr, a treatment resulting in a fairly common pattern of hs protein synthesis. Thermal protection appears to relate to selective cellular localization of some of the hs proteins during hs.

Cloned cDNAs have been used in an analysis of hs mRNA synthesis. Following hs at 40° to 42°, hs mRNAs are detected within 3 to 5 min; they accumulate for about 2 hr up to levels of 20,000 copies per cell. A shift back to 28° results in a loss of mRNAs with a 1/2 time of about 1 hr. There is a gradual decline of mRNA levels after about 4 hr of continuous 40° hs. A 4 to 10 min hs at 40° results in transient and low level accumulation of hs mRNAs while a 4 to 10 min hs at 45° results in accumulation of hs mRNA over the next 4 to 6 hr at 28° to levels similar to a 2 to 4 hr hs at 40°.

Of physical stresses studied to date (e.g. water stress, anaerobiosis, high levels of hormones, heavy metals), arsenite most nearly mimics hs, though these stresses result in accumulation of detectable levels of hs mRNAs. One set of hs mRNAs (for 4-5 27 kD proteins) which, in contrast to other hs mRNAs, is present in unstressed tissue, is induced to hs levels by such stresses.

23. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

SOYBEAN RIBULOSE BISPHOSPHATE CARBOXYLASE SMALL SUBUNIT GENE FAMILY: GENE STRUCTURE AND REGULATION OF GENE EXPRESSION — $70,000

Richard B. Meagher
Department of Molecular and Population Genetics

Genetic engineering systems in bacteria and in animal cells have made use of well characterized homologous promoter/regulator sequences to control foreign gene expression. It is likely that similar plant control sequences can be used to control foreign gene expression in transformed plant cells. The ribulose bisphosphate carboxylase gene family in soybean is ideally suited to examine strong transcriptional control signals in plants. The gene family is highly diverged and only functionally related regions of DNA would be expected to share homologous DNA sequences. We have completely sequenced one soybean small subunit gene, partially characterized the light induced level of its transcription and identified potential transcriptional control sequences. Furthermore, we have partially characterized the soybean small subunit gene family and shown that it contains several members. We propose to characterize the small subunit gene promoter/regulator sequences in order that they might be used to control foreign gene expression in plant cells. As part of this project we plan to isolate, characterize, and sequence most of the RuBP carboxylase small subunit gene family members in soybean, determine if their mRNA levels are light regulated and if this is due primarily to transcriptional regulation, identify the 5' and 3' ends of their mRNA products, and compare potential homologous promoter/regulator sequences. Quantitative evolutionary measurements will be made in order to determine the degree of divergence of small subunit genes within soybean. Various regulatory elements from the small subunit gene could then be used for transcriptional control of foreign genes manipulated into plants.

24. UNIVERSITY OF GEORGIA
Athens, GA 30602

THE MICROBIOLOGY AND PHYSIOLOGY OF ANAEROBIC FERMENTATIONS OF CELLULOSE — $240,000

H. D. Peck, Jr., and L. G. Ljungdahl
Department of Biochemistry

Investigations into the biochemistry and physiology of the four major groups of microorganisms (primary, ancillary, secondary and methane bacteria) involved in the anaerobic conversion of cellulose to methane and carbon dioxide will be continued. Studies on the primary or cellolytic microorganisms will be focused on the isolation of new thermophilic strains from Iceland, interactions with ancillary bacteria which ferment cellobiose and xylose, growth and modification of fermentation patterns by inorganic pyrophosphate and purification of cellulase. The projected investigations of the ancillary bacteria will emphasize isolation of new strains and increasing ethanol production with T. ethanolicus. The latter research will involve genetic modifications, enzymological studies on the regulation of appropriate enzymes and a study of the effect of inorganic pyrophosphate on growth and fermentation patterns. The enzymology of acetate formation from carbon dioxide by acetogenic bacteria will be studied with initial emphasis on the metabolism of the one-carbon compounds. Further studies with these organisms will include bioenergetics, especially hydrogen metabolism and energy coupling by H_2 cycling, and the structure and function of electron transfer components. Research on secondary bacteria will emphasize the sulfate reducing bacteria from the aspects of H_2 cycling, specificities of electron transfer proteins and enzymes, the mechanism of bisulfite reductase and APS reductase and the enzymology and physiology of new genera of sulfate reducing bacteria. The biochemistry and physiology of both H_2-utilizing and acetate utilizing methanogens will continue to be investigated. The studies with H_2-utilizing methanogens will stress hydrogenase and the effect of inorganic pyrophosphate on growth. The research on the acetate-utilizing methanogens will involve the bioenergetics of sulfite reduction and the mechanism of acetate formation induced by pyrophosphate.

25. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

PHYTOCHROME PROPERTIES AND FUNCTION IN PHOTOSYNTHETICALLY COMPETENT PLANTS — $47,588

Lee H. Pratt
Department of Botany

Phytochrome is a chromoprotein that senses the wavelength distribution of incident light energy and responds to it by modulating the growth and development of green plants. The objective of this program is to characterize phytochrome as it exists in photosynthetically competent plants and to help understand how it influences photosynthetic productivity. This work depends heavily upon application of immunochemical methods for three reasons. (1) The chlorophyll in green plants makes unsuitable the widely used spectrophotometric assays for phytochrome. (2) So little phytochrome is present in green tissues that spectrophotometric assays are generally too insensitive. (3) Antibodies increase greatly the ability to probe the structure and function of a complex molecule such as phytochrome. To ensure maximum specificity and utility of immunochemical approaches, monoclonal antibodies to both pea (Pisum sativum L.) and oat (Avena sativa L.) phytochrome are being produced. These antibodies are in use to quantitate phytochrome in crude extracts from green plants at the femtomol level, to probe the structure of phytochrome as it is isolated from green plants, and to determine the distribution and subcellular localization of phytochrome by immunocytochemistry. Phytochrome in and from both peas and oats is being investigated to determine whether information obtained is of general applicability or is perhaps specific to only one plant or one group of plants. Knowledge deriving from this work will not only help clarify the general question of how phytochrome regulates light-mediated plant growth and development but also the more specific question of how it influences the efficiency with which plants convert solar to chemical energy.

26. HARVARD UNIVERSITY
Cambridge, Massachusetts 02138

UNRAVELING PHOTOSYSTEM II — $76,815

Lawrence Bogorad
Department of Cellular and Developmental Biology

The objective of this project is to identify and characterize genes involved in photosynthesis, particularly photosystem II, by a study of cyanobacteria. The two principal approaches being employed to identify genes for components of photosystem II in cyanobacteria. One is by using antibodies generated against a photosystem II preparation to identify those E. coli strains transformed with a library of cyanobacterial DNA that carry genes for photosystem II components. Another approach is through the use of maize chloroplast genes that hybridize to cyanobacterial DNA as heterologous probes.

27. HARVARD UNIVERSITY
Petersham, Massachusetts 01366

STRUCTURE AND FUNCTION OF FRANKIA VESICLES BY DINITROGEN FIXATION OF ACTINORHIZAL PLANTS — $42,770

John G. Torrey and John D. Tjepkema
Cabot Foundation, Harvard Forest

In root nodules of symbiotic dinitrogen-fixing trees, the actinomycetous endophyte Frankia forms terminal swellings or vesicles which are believed to be the site of the enzyme nitrogenase. When isolated and grown in synthetic culture media lacking combined nitrogen, Frankia also differentiates vesicles and fixes N_2. In our studies we have defined the chemical and physical conditions maximizing vesicle formation and acetylene-reducing (=nitrogenase) activity. Cells are grown in 3-l air-sparged magnetically stirred cultures with doubling time <24 hr. Growth is estimated by protein assay. Maximum acetylene reduction rates are obtained with 5 mM propionate as sole C-source, at pH 6.7 and 28°C. Frankia fixes N_2 under aerobic conditions and our studies have focussed on the mechanisms for O_2 protection of the nitrogenase enzyme. Evidence from kinetic analysis of in vivo enzyme rates suggests a passive gas diffusion barrier. Comparison of O_2 consumption rates in undifferentiated and vesicle-containing cells suggests also a form of "respiratory protection". Further studies of vesicle structure at the light and EM levels are being pursued in the effort to correlate structure with function.

28. THE HELICON FOUNDATION
San Diego, California 92121

CONSTRUCTION AND ANALYSIS OF BACTERIAL STRAINS USEFUL IN CONVERSION OF CELLULOSE TO ETHANOL — $59,722 (FY 82 funds)

Richard W. Armentrout

We will use recombinant DNA technology to construct a plasmid DNA suitable as a cloning vector for Zymomonas mobilis. This vector will be used to transfer genes from E. coli by mating. The expression of various genes which are active in E. coli will be examined in Zymomonas, including the gene for cellobiose utilization. Libraries of cloned DNA fragments from the gram negative cellulose utilizing organism Cellvitrio vulgaris will be screened to identify genes involved in cellulose degradation. These steps are preliminary to the construction of a set of genes which will function in Z. mobilis and allow us to examine a minimal gene set for bacterial cellulose degradation.

29. UNIVERSITY OF IDAHO
Moscow, Idaho 83843

GENETICS AND CHEMISTRY OF LIGNIN DEGRADATION BY STREPTOMYCES — $87,500

Don L. Crawford
Department of Bacteriology and Biochemistry

The objectives of this project are to develop and utilize protoplast fusion and protoplast transformation as recombinant DNA techniques for genetic improvement of lignin degrading Streptomyces, and to characterize the chemical changes in the structure of lignin resulting from the expression of specific lignin catabolism genes. The approach utilizes three lignin degrading Streptomyces strains which differ from one another in their ligninolytic activities and in aromatic compound catabolizing abilities. Protoplast fusion will be utilized to obtain recombinants expressing desirable traits derived from two or more parental strains. Stable lignin degradation enhanced mutants will be isolated and characterized. Genes coding for catabolism of selected aromatic compounds, or for degradation of lignin, will be transformed into protoplasts of other lignin-degrading or nonlignin-degrading strains, and transformants will be characterized to determine how expression of the added genes affects lignin degradation. The technique may be useful in determining the minimum number of genes required for lignin degradation in Streptomyces. Concomitantly, the chemical changes in lignin and the formation of lignin degradation intermediates resulting from degradation will be monitored, and recombinant organisms will be compared with the wildtype parental strains to determine how specific genetic changes affect the catabolic pathway.

30. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

FATTY AND AROMATIC ACID CATABOLIZING BACTERIA IN METHANOGENIC ECOSYSTEMS — $57,354

Marvin P. Bryant
Department of Dairy Science

In complete anaerobic degradation of organic matter to CH_4 and CO_2, three metabolic groups of bacteria are mainly involved; (1) hydrolyzing and fermentative bacteria, (2) obligate proton-reducing acetogens and (3) acetate, methanol, formate and H_2-CO_2-using methanogens. Group 2, includes syntrophs which require methanogens or other H_2-using bacteria to maintain a low concentration H_2 in the ecosystems in order to degrade certain organic compounds. Syntrophomonas β-oxidizes short-chain fatty acids to acetate (or acetate plus propionate) and H_2. Syntrophobacter degrades propionate to acetate, CO_2 and H_2 or formate. In the present work, Syntrophus, which catabolizes benzoate and, probably, hydrocinnamate, to acetate, CO_2 and H_2 and/or formate in association with an H_2-user has been documented. Other syntrophs, one catabolizing phenylacetate, and phenol and, probably, benzoate and hydrocinnamate; and another morphotype catabolizing phenol (other phenolics not yet studied) have been isolated in co-culture with H_2-using Desulfovibrio and with Wolinella (H_2 + fumarate to succinate). A long-chain fatty acid-degrading syntroph from stearate enrichment has been isolated in co-culture with Desulfovibrio using tridecanoate as energy source. The culture contains small amounts of a sugar-fermenting contaminant which does not catabolize fatty acids. The fatty acid-degrading bacterium is similar to Syntrophomonas in morphology and catabolizes butyrate, tridecanoate, stearate and, probably, other saturated fatty acids other than acetate, propionate and isovalerate. It is being purified of the contaminant before further studies are done. These studies add to basic knowledge of methanogenic ecosystems.

31. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

PHOTOSYNTHESIS IN INTACT PLANTS — $116,000
A. R. Crofts
Department of Physiology and Biophysics

In the intact plant, the photochemical reactions, and the reactions of electron transfer and proton transport which they drive, are part of an integrated mechanism which responds to the physiological state of the plant as this is determined by environmental factors. Rates of electron transfer, and the control of cyclic and non-cyclic pathways, can be assayed directly by following the changes on illumination of redox components of the chain, using spectrophotometry, or indirectly using fluorescence techniques. The generation and utilization of the proton gradient can be assayed by following the 515 nm electrochromic change. Our research is aimed at developing instrumentation to facilitate such measurements. A major initial emphasis has been on laboratory-based research designed to develop and test prototype instruments, and to understand in a controlled environment the factors determining the kinetic events we can monitor instrumentally. Our major effort has been on the mechanism of the two-electron gate by which PS II delivers electrons to the plastoquinone pool, but we have also studied reactions on the donor side of PS II with a view to defining conditions for isolating the reactions of the secondary donor Z so that we can characterize its absorption spectrum.

The instruments developed or in prototype include a rapid kinetic fluorimeter; dual-flash kinetic fluorimeter; computer-linked fluorescence induction fluorimeter; kinetic spectrophotometer with flash measuring beam; and kinetic spectrophotometer for measuring pseudo-steady-state kinetics. Three of these instruments are being developed in portable form, and are designed for field use.

32. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

MECHANISM OF PROTON PUMPING IN BACTERIORHODOPSIN — $51,971
Thomas G. Ebrey
Department of Physiology and Biophysics

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 in extreme saline environments. This proposal is to investigate the relationship between the transport of protons across the membrane and structure and conformation of bacteriorhodopsin. We have made an especially intriguing discovery, that the number of protons released by purple membrane sheets by light can be drastically altered by the proteolytic removal of a few amino acids from the C-terminal "tail" of bacteriorhodopsin. Thus we believe that we have a way of specifically controlling the release of some of the protons by altering a small part of the polypeptide chain. In addition the number of protons released has been shown to be a function of the salt concentration, which may in turn affect the protein conformation. We suspect this effect may be localized to the interaction of the C-terminal end of bacteriorhodopsin with the rest of polypeptide. This proposal centers around a set of experiments, based on these observations, to correlate the structure of the purple membrane with its energy transducing function.

33. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

DEVELOPMENT OF A GENETIC SYSTEM FOR BACTEROIDES SPECIES — $63,035
Jeffrey F. Gardner, Abigail A. Salyers
Department of Microbiology

The objective of this project is to develop a method for introducing DNA into Bacteroides. We have constructed a chimeric plasmid (pEG1) which contains a cryptic Bacteroides plasmid (from B. eggerthii B8-51) and pBR328 from E. coli. We have used this chimeric plasmid to test a number of transformation procedures including spontaneous uptake, calcium shock and spheroplasting. Recipients included not only cured B8-51 but also strains of other colonic Bacteroides species. No transformants were detected. We also attempted to transfer pEG1 from E. coli to Bacteroides. P plasmids of E. coli mobilized pEG1 from E. coli to E. coli at high frequency, both aerobically and anaerobically. However, when E. coli was mated with Bacteroides, under a variety of conditions, no transconjugants were obtained. Since it is possible that the selectable markers on pBR328 are not expressed in Bacteroides, we have cloned DNA from B. fragilis which complements trpE in E. coli. This DNA has been inserted in pEG1 to form a new plamsid (pEG100). We will isolate an appropriate auxotroph of B. fragilis to serve as a recipient for pEG100. We will use this strain and pEG100 to test various transformation procedures and conjugation conditions. To aid us in checking mutants and classifying natural isolates, we have developed a method for using cloned DNA fragments to identify colonic Bacteroides. This method could be very useful to a number of workers because procedures normally used to identify colonic Bacteroides species, on the basis of phenotypic traits, are cumbersome and time consuming.

34. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

BIOCHEMICAL AND BIOPHYSICAL STUDIES ON THE E. COLI AEROBIC RESPIRATORY CHAIN — $90,000
Robert B. Gennis
Departments of Chemistry and Biochemistry

Bacteria use a mechanism similar to mitochondria and chloroplasts to conserve energy and convert it to a usable chemical form. In particular, a transmembrane "proton motive force" is generated concomitant with electron flow through an electron transport chain. We are studying the components in the E.coli membrane responsible for generating this electrochemical gradient. E. coli has been selected because of the relative ease with which genetics can be applied to the problem, and also because of the apparent simplicity of the electron transport chain. We are emphasizing the coordinate application of biochemical, immunological and genetics techniques to identify and characterize each component of the aerobic respiratory chain. Our attention has focussed on the cytochrome d terminal oxidase complex which we have recently purified and reconstituted in an artifical, energy-conserving liposome system. Our attentions will now focus on the cytochrome o complex, about which much less is known. We have immunologically characterized this and demonstrated that it contains two b cytochromes, 4 polypeptide subunits, and functions as a "coupling site" or point of proton translocation in the membrane.

35. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

THE ROLES PLAYED BY MITOCHONDRIAL DNA PLASMIDS AND NUCLEAR GENES IN REVERSIONS TO FERTILITY IN S-TYPE MALE-STERILE MAIZE — $72,000

John R. Laughnan
Department of Genetics and Development

These investigations are concerned with the genetic and molecular basis of cytoplasmic male sterility and involve field and laboratory studies of special strains of maize. In particular, genetic reversion from the male-sterile condition to male fertility, the normal condition, is being investigated. Such reversions may occur at the cytoplasmic or nuclear level. Cytoplasmic reversion is associated with disappearance of the S-1 and S-2 mitochondrial DNA plasmids and has been shown to involve transpositional events within the mitochondrial genome. With collaborators we have experiments underway to determine whether the nuclear revertants have a similar basis. We have shown recently that maize nuclear DNA shares some DNA sequences with those of the S-1 plasmid in the mitochondrion. When reversion to fertility occurs at the nuclear level it is associated with transpositional insertion of fertility elements at one or another site within the chromosomal complex, and such elements may undergo secondary transposition to other chromosomal positions. Aspects of this secondary transposition phenomenon are being investigated. Developmental studies of the cytoplasmic reversion event are also being carried out. Since loss of the S-1 and S-2 mitochondrial DNA plasmids is associated with cytoplasmic reversion, but is not instantaneous, analysis of mitochondrial DNA in tissues of revertant plants, and their offspring, offers the opportunity to trace a developmental happening at the molecular level. Certain revertants from male-sterility conditions to male fertility are associated with failure of male-gametophyte or pollen function, but are unimpaired in female transmission. Since it appears that these reversion events involve a research sequence event, and that different chromosomal target sites are involved, they probably represent selected events in which insertions have occurred within genes essential for male-gametophyte function. We have intensified our studies of these revertants since they provide the first opportunity to identify and map genes affecting male gametophyte (pollen) function in maize.

36. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

ACETOPHILIC METHANOGENIC CONSORTIA — $85,000 (FY 82 funds)

Ralph S. Wolfe
Department of Microbiology

The purpose of this research program is to study microbes that participate in the anaerobic degradation of organic matter to acetic acid and of the conversion of acetic acid to methane and carbon dioxide. The experimental approach is to construct, from pure cultures, defined mixtures of bacteria so that the food chain, containing the necessary catalysts for the conversion of carbohydrate to methane, may serve as a model to define microbial interactions at the biochemical level. Knowledge of these interactions is pivotal to the design and efficient operation of anaerobic digesters for the production of methane from biomass.

37. THE INSTITUTE OF PAPER CHEMISTRY
Appleton, Wisconsin 54912

RAMAN MICROPROBE INVESTIGATION OF MOLECULAR STRUCTURE AND ORGANIZATION IN THE NATIVE STATE OF WOODY TISSUE — $30,700
Rajai H. Atalla
Chemical Sciences Division

The objectives of this project are to establish, by Raman microprobe spectroscopy, the variability of molecular structure and organization in the cell walls of native woody tissue. More specifically, the objectives are to determine the structures and molecular orientations of celluloses and lignins at different points within individual cell walls. A Raman microprobe system has been assembled and procedures for recording spectra from 1 μm domains, within cell wall sections, have been established. Polarized Raman spectra have been recorded with polarization parallel and perpendicular to planes of the cell walls. They show evidence of molecular orientation relative to the plane of the cell wall for both cellulose and lignin. Spectra have been recorded for both longitudinal and transverse sections in tissues from black spruce (Picea mariana) and from loblolly pine (Pinus taeda L). Variations in composition have been detected within the secondary wall of an individual cell, as well as between adjacent cells in the same annual ring. Future work will be directed at more complete characterization of molecular organization and variability in native tissues, and at interpretation of the spectral features associated with other components of the cell walls. The information developed is important both for fundamental understanding of the structure of the cell walls, and for analysis and design of industrial processes which use biomass as a primary resource.

38. IOWA STATE UNIVERSITY
Ames, Iowa 50011

PROLINE METABOLISM IN PLANTS UNDER ENVIRONMENTAL STRESSES — $56,980 (15 months)
Cecil R. Stewart
Department of Botany

The objective of this project is to understand the subcellular mechanism which causes proline to accumulate under various environmental stresses. One of the known effects which contributes to proline accumulation is the inhibition of proline oxidation, a mitochondrial process. This process has been characterized with respect to energy metabolism and we have shown that proline uptake into mitochondria is protonmotive. The rate of uptake of proline exceeds the rate of oxidation. An inhibitor of proline oxidation has been identified which mimics the effects of stress. This inhibitor will be useful in determining the effects of stress which inhibit proline oxidation. It is likely that the control on proline oxidation is at the level of uptake into the mitochondria. Control of the rate of proline oxidation is a normal metabolic function, not an abnormal effect of stress.

The effects of salt stress on proline metabolism have been determined and compared to those due to drought. It appears that there is a common metabolic mechanism which causes proline to accumulate under both stresses. Additional experiments have been conducted which support the hypothesis that proline accumulates in response to an osmotic imbalance in the cell. Under stress, inorganic ions are accumulated in the vacuole. Proline accumulates in the cytoplasm to serve as a compatible osmoticum, balancing the osmotic potential between the two cellular compartments.

These results contribute to our knowledge of how plants adapt to adverse environmental conditions. This information is useful in improving plant productivity in marginal environments.

39. UNIVERSITY OF KENTUCKY
Lexington, Kentucky 40546

PHYSIOLOGY/BIOCHEMISTRY OF PHOTOACTIVATION OF OXYGEN EVOLUTION; PROBES FOR THE S-STATE PROTEIN — $28,328 (two years)

George M. Cheniae
Agronomy Department

The objectives of this work are: 1) to determine the minimal oxidation/reduction reactions and sequences involved in the photoactivation of the oxygen-evolving "enzyme" of photosynthesis; 2) to identify the protein(s) synthesized on chloroplast 70S ribosomes that are required in the photoactivation of Photosystem II donor as well as O_2 evolution following photoinhibition of Photosystem II; 3) to identify and characterize the thylakoid polypeptides required for photosynthetic O_2 evolution. Mutational analyses, conventional extraction/reconstitution analyses and affinity/photoaffinity probes are employed in efforts to identify the Mn-containing oxygen evolving enzyme of photosynthesis.

40. CHARLES F. KETTERING RESEARCH LABORATORY
Yellow Springs, Ohio 45387

THE BASIS FOR THE COMPETITIVENESS OF RHIZOBIUM JAPONICUM IN THE NODULATION OF SOYBEAN — $77,740

William R. Evans, Wolfgang D. Bauer
Enhancement of Plant Productivity

The long range goal of our studies is to determine which characteristics would enable an inoculated Rhizobium strain to compete effectively against indigenous soil rhizobia in nodulating soybean. At present, Rhizobium inocula contribute little to nodulation of host plants in the field because most field soils contain indigenous populations of rhizobia that out-compete the inoculated rhizobia. Genetic engineering of Rhizobium inoculum strains to improve their nitrogen-fixing potential will thus be of little value unless competitive inoculum strains are developed. Studies in our laboratory established that the susceptibility of soybean root cells to nodulation is transient and developmentally restricted to small regions located just above the growing tips of the roots. We are attempting to determine the physiological and genetic characteristics that would enable an inoculum strain to colonize the infectible regions of the root system more aggressively than indigenous strains and to infect these regions more rapidly and efficiently. Attachment of rhizobia to the root surface is an important aspect of colonization. Strong attachment of rhizobia to roots, i.e. attachment not broken by gentle washing, appears to be mediated by polar pili in several strains examined. In earlier studies various strains of R. japonicum, the soybean symbiont, were found to differ significantly with respect to their efficiency of infection initiation. We are currently assessing the competitive importance of these differences in efficiency by inoculating seedlings in soil or artificial soil with mixtures of an efficient and an inefficient strain. We are developing new computerized methods for determining and analyzing nodule occupancy by particular strains, and also determining the relative advantage enjoyed by indigenous strains over inoculum strains as a consequence of their uniform distribution throughout the soil.

41. LEHIGH UNIVERSITY
Bethlehem, Pennsylvania 18015

A GENETIC APPROACH TO SECRETION AND HYPERPRODUCTION OF CELLULASE BY TRICHODERMA — $68,500

Bland S. Montenecourt and Jeffrey A. Sands
Department of Biology and The Biotechnology Research Center

The objectives of this project are to delineate the genetics of cellulase secretion in T. reesei through the construction of a series of hypercellulolytic mutants and temperature sensitive mutants and to analyze the genetic lesions through the parasexual cycle and protoplast fusion. It is anticipated that an understanding of the series of events between synthesis of a protein and the eventual secretion of that protein will provide useful information upon which to base new selection systems for the isolation of superior hypercellulolytic mutants. We are also looking at the effect of various secretion inhibitors (cytochalasin A, benomyl, 2-deoxyglucose) on cellulase production by Trichoderma and have isolated a number of mutants resistant to these drugs. One new TS mutant has been studied in considerable detail: at 37°C it grows rapidly but does not secrete cellulase; at the permissive temperature, it hyperproduces endoglucanase (350 IU/ml), showing a 2-fold increase in specific activity of this enzyme.

42. MARTIN MARIETTA LABORATORIES
Baltimore, Maryland 21227

STUDIES OF PHOTOSYSTEM II USING ARTIFICIAL DONORS — $85,630

Richard Radmer
Biosciences Department

The objective of this project is to gain an understanding of photosynthetic O_2-evolution, a poorly understood and profoundly important process. Most of these studies make use of competitive inhibitors of H_2O oxidation, such as hydroxylamine, hydrazine, and their derivatives. (These compounds which are all analogs of two molecules of H_2O, in varying degrees share the ability to compete with and override H_2O oxidation without destroying the O_2 system.) By the use of mass spectrometric and fluorescence techniques we monitor the interactions of these compounds under various conditions with the O_2-evolving (H_2O oxidizing) system of isolated spinach chloroplasts. Our results enable us to infer some chemical and structural characteristics of the O_2-evolving site.

43. UNIVERSITY OF MARYLAND
College Park, Maryland 20742

$62,836

ENERGY-DEPENDENT CALCIUM TRANSPORT MECHANISMS IN PLANT MEMBRANES
Heven Sze
Department of Botany

The objectives of this project are (i) to identify and characterize active calcium (Ca) transport systems in plants and (ii) to understand the mechanisms that regulate Ca fluxes. Plant tissues include tobacco tissue callus (Nicotiana tabacum), oat roots (Avena sativa) and corn roots (Zea mays). We are using an in vitro approach developed previously in our laboratory to study Ca transport. Organelles and specific membrane vesicles are isolated and purified by differential and density centrifugation; and 45-Ca uptake is measured by a filtration procedure. At least two types of ATP-dependent Ca transport systems have been identified in microsomal vesicles using specific inhibitors and stimulators: one requires a pH gradient and one does not. These two types of Ca transport systems will be separated and characterized and the membrane identity will be determined. To understand how Ca fluxes are regulated, the effect of phyto-hormones and fungal toxins on the various Ca transport systems will be studied. One promising system is the Helminthosporium maydis T toxin which affects Ca transport into isolated mitochondria in vitro. The effect of toxin on active proton and Ca transport into other subcellular membranes will be determined. The mode of toxin action in decreasing Ca uptake into mitochondria will be examined. Ca levels are important in regulating various physiological and biochemical processes. These studies of active Ca fluxes are central to our understanding of not only the mechanism and regulation of solute transport but also the regulation of plant growth and development.

44. UNIVERSITY OF MASSACHUSETTS
Amherst, Massachusetts 01003

$77,000
(16 months)

CONVERSION OF CELLULOSE TO ETHANOL BY MESOPHILIC BACTERIA
Ercole Canale-Parola
Department of Microbiology

The objectives of this project are i) to obtain from natural environments, or by means of mutagenic techniques, strains of mesophilic bacteria capable of fermenting cellulose with maximum production of ethanol, and ii) to study the metabolic processes responsible for the anaerobic conversion of cellulose to ethanol by these bacteria. The procedures used for the isolation of ethanol producers are designed to select for diverse cellulolytic bacteria and involve culture media of varied composition, inocula from different natural environments, different types of cellulosic materials as growth substrates, and a variety of cultural conditions. The fermentation products and the phenotypic characteristics of the isolated strains are determined. Ethanol production from cellulose is studied under different cultural conditions, in pure cultures and in mixed cultures with Zymomonas mobilis. Studies on the inducibility of the cellulase systems of the isolates by pentoses, hexoses, and disaccharides are carried out. Another experimental approach used is aimed at cloning cellulase system genes from mesophilic cellulolytic bacteria into a suitable vector plasmid and then introducing that plasmid into cells of Z. mobilis. The objective of this part of the research is to obtain Z. mobilis strains capable of fermenting cellulose. The bacterial strains isolated in the course of this research and the information obtained on their metabolism will be useful in the development of efficient industrial processes for the conversion of cellulosic materials to ethanol.

45. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824-1312

CONTROLLED MEDIA FOR PLANT TISSUE CULTURE — $55,000
Norman E. Good
Department of Botany and Plant Pathology

The objective of this research is to produce plant tissue culture media which are reasonably stable during the rather long culture periods required. The intent is to develop complexes of the plant hormones which can serve as generous reservoirs which break down at various predictable rates, thus providing a range of steady-state levels. At the same time metabolically inert hydrogen ion buffers with suitable low pKa's are being developed so that the hydrogen ion concentrations of media can be more closely regulated. A large number of indoleacetyl amino acids have been prepared already to serve as auxin sources and these show considerable promise as determinants of diverse callus growth and organogenesis. For instance, indoleacetyl-L-alanine releases free indoleacetic acid relatively quickly and supports massive callus growth, indoleacetyl-L-phenylalanine releases free indoleacetic acid slowly and supports primarily shoot production. Complexes of cytokinins with sugars are also being prepared but have not yet been tested. Intermediates for the synthesis of promising buffers are being assembled.

46. UNIVERSITY OF MINNESOTA
Minneapolis, Minnesota 55104

PHYSIOLOGY AND GENETICS OF METHANOTROPHIC BACTERIA — $70,066
Richard S. Hanson
Gray Freshwater Biological Institute

The objectives of this project are to develop genetic techniques for studying methylotrophic bacteria, to map several genes to biosynthetic and catabolic pathways and to characterize specific and non-specific methane monooxygenases. Three bacteria are employed; Methylobacterium organophilum strain XX, a type II facultative methylotroph; Organism SB1, a type II obligate methylotroph and Methylomonas 761, a type I methylotroph that obligatorily requires methane or methanol as an energy source but its growth rate is increased 10 fold by the addition of glucose or amino acids as carbon sources. Methylomonas 761 is a unique type I methylotroph with a complete glycolytic pathway and Krebs cycle. A conjugative plasmid from Organism SB1 that mobilizes chromosomal markers to Pseudomonas species has proven very useful for the in vivo cloning and mapping of several amino acid markers from this methylotroph. A restriction map of this plasmid is nearly complete. The particulate and soluble methane monooxygenases from this species have very different substrate specificities and are being characterized by comparing their subunit composition and antigenic similarities. A newly constructed plasmid vector containing a cosmid gene, genes for kanamycin and tetracycline resistance, seven unique restriction sites and the replicon and mobilization functions of pRK 290 have proven useful for cloning genes of the methanol operon of M. organophilum strain XX.
We now have tools for studying the genetics of amino acid biosynthesis and one carbon metabolism in three different groups of methylotrophs. We intend to complete a map of genes that code for enzymes and regulatory functions involved in one carbon metabolism, to further investigate the unusual properties of Organism SB1 in order to understand its requirement for one carbon energy sources.

47. UNIVERSITY OF MINNESOTA
St. Paul, Minnesota 55108

CORN STORAGE PROTEIN - A MOLECULAR GENETIC MODEL — $70,000
Joachim Messing
Department of Biochemistry

The objective of this proposal is to study the Zein multigene family in Zea mays. The approach uses a new cDNA cloning procedure to isolate full length zein cDNA clones. Clones of different zein subfamilies are found and the microheterogeneity within and between subfamilies is studied by DNA sequencing. The comparison of cDNA and genomic sequences of the A30 subfamily shows some very interesting results. The Z4 genomic clone is an active gene because it matches with one cDNA clone very closely. There are two size classes of zein proteins within one subfamily which differ by a 32 amino acid residue repeat in the middle of the protein. Two poly-A addition signals appear in the 3' end of the zein messages and both are used. A second subfamily, A20, will be analysed the same way, and then compared with the A30 subfamily. It will be seen, how the 5' leader sequences and the 3' non-translated region are conserved between the two subfamilies. Differences in the signal sequence, the repeated structure, and the carboxyterminus will be of interest in the understanding of the zein protein structure. Zein proteins determine the protein yield and the nutritional quality of corn, a major crop in our agricultural industry.

48. UNIVERSITY OF MINNESOTA
Minneapolis, Minnesota 55455

THE MECHANISM OF SWITCHING FROM AN ACIDOGENIC TO A BUTANOL-ACETONE FERMENTATION BY CLOSTRIDIUM ACETOBUTYLICUM — $73,000
Palmer Rogers
Department of Microbiology, Medical School

The overall goal of the experimental work proposed is to elucidate in molecular terms the regulation of the four-branched fermentation pathway in Clostridium acetobutylicum. The roles and interactions of the regulating devices in the overall physiologic program of this obligate anaerobe will be investigated.

The experiments are designed to provide knowledge of how the switching of the fermentation between the acidogenic and solventogenic phases is related to H_2 and NAD(P)H on the one hand or to the sporulation program on the other. The specific modes of regulation and allosteric modulators for the enzymes in the four branches leading to butanol, acetone, butyrate and acetate will be determined. A selection system for isolating acid-less mutants in anaerobic plate culture will be developed and mutants that are asporogenous will be isolated. The mutants will be used to develop a genetic system in Clostridium acetobutylicum based on protoplast fusion.

It is hoped that the fundamental knowledge gained will provide a clearer picture of the normal regulation circuitry of this important anaerobe so that a scientific basis is established for future genetic programming of the solventogenic fermentation.

49. UNIVERSITY OF MINNESOTA
St. Paul, Minnesota 55108

FEASIBILITY OF MOLECULAR HYBRIDIZATION TECHNIQUES IN STUDYING GENOMIC ORGANIZATION — $60,055

Irwin Rubenstein, Department of Genetics & Cell Biology
Ronald L. Phillips, Department of Agronomy and Plant Genetics

We propose to develop physical methods to determine the chromosomal location of isolated genomic segments. The first procedure will use a modified dot hybridization technique combined with the use of DNAs from a series of trisomic, disomic and monosomic maize plants. We will attempt to demonstrate the capability to distinguish an expected 2:1:3 hybridization ratio versus a 1:1:1 ratio depending on whether or not a particular sequence is in that chromosome. The second procedure to be developed is that of in situ hybridization using immunofluorescent antibodies or 125I-Labeled probes to detect sequences present at low reiteration frequencies. Our objective will be to develop a reasonably rapid technique to determine the chromosomal location of unique sequences. We hope that these approaches will allow us to map any gene sequence cloned by recombinant DNA techniques.

50. UNIVERSITY OF MISSOURI
Columbia, Missouri 65211

PHOTOSYNTHESIS AND CLONING IN CYANOBACTERIA--A SYSTEM FOR THE BIOCONVERSION OF SOLAR ENERGY — $72,129

Louis A. Sherman
Division of Biological Sciences

The objective of this study is to identify and clone genes coding for photosynthetic membrane proteins in the cyanobacterium, Anacystis nidulans R2. This strain can be transformed with exogenous DNA and it possesses indigenous plasmids. We have constructed hybrid cloning vectors that can replicate in both A. nidulans and Escherichia coli and that can confer ampicillin and chloramphenicol resistance to both organisms. We have cloned the rRNA operon from A. nidulans onto one vector (pSG111) and shown that the recombinant plasmid can be stably maintained extrachromosomally. We have also isolated temperature-sensitive photosynthesis mutants and herbicide-resistant mutants of A. nidulans. We are now using two approaches for the identification and cloning of specific photosynthesis genes. We have produced a library of A. nidulans chromosomal DNA in the vector pSG111. By transforming this library into specific mutants, we can identify genes by complementation. This has successfully been accomplished in at least two cases. We are also using heterologous probes from chloroplast and E. coli DNA to identify photosynthesis genes. This procedure has enabled us to clone the A. nidulans genes coding for the large and small subunits of RuBP carboxylase, three subunits of the ATPase complex, a membrane-bound hydrogenase, and a Ca^{+2}-binding protein homologous to calmodulin. Additionally, we have found that sequences homologous to these probes are located on plasmid as well as chromosomal DNA. Most of these plasmid-encoded genes are on the 33 Mdal plasmid; however, we have detected the presence of a very large plasmid (>120 Mdal) that also encodes photosynthesis genes. The gene coding for the chloroplast 32kDa herbicide-binding protein hybridizes to three chromosomal and two plasmid sequences. The three chromosomal genes are also present in two DCMU-resistant mutants; despite the presence of multiple genes, wild-type cells can be readily transformed with the DCMU resistance trait.

51. MOUNT SINAI SCHOOL OF MEDICINE OF THE CITY
UNIVERSITY OF NEW YORK
New York, New York 10029

THE RESPIRATORY CHAIN OF ALKALOPHILIC BACTERIA — $78,020
Terry Ann Krulwich
Department of Biochemistry

Obligately alkalophilic bacteria contain extraordinarily high concentrations of membrane-bound respiratory chain components and an apparently complex respiratory chain, with many carriers that are distinguishable on the basis of their midpoint potentials. It has been proposed that the alkalophile respiratory chain possesses structural/functional properties, of which the complexity is a part, which facilitate particularly effective energy transduction at high pH. Initial studies of H^+ translocation during respiration by whole cells of alkalophilic Bacillus firmus RAB support this hypothesis. Further studies of H^+ pumping will be conducted using whole cells and reconstituted systems. The latter will first involve a study of cytochrome oxidase purified from B. firmus RAB. This respiratory chain complex has been purified to homogeneity. It will now be characterized and studied in proteoliposomes.

52. NATIONAL INSTITUTE OF GENERAL MEDICAL SCIENCES
National Institutes of Health
Bethesda, Maryland 20205

GENBANK (TM), THE GENETIC SEQUENCE DATA BANK — $40,000
Christine Carrico

GenBank (TM), the Genetic Sequence Data Bank, is an internationally available repository of all reported nucleotide sequences greater than fifty nucleotides in length, annotated for sites of biological interest and checked for accuracy. As of June 1, 1983, GenBank contained 1.70 million bases, comprising 2082 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, co-sponsored by the National Institute of General Medical Sciences, National Cancer Institute, National Institute of Allergy and Infectious Diseases, and Division of Research Resources, all of NIH, and the Department of Energy and the National Science Foundation, is of particular interest to geneticists and molecular biologists. A copy of the database is available for a modest fee on computer-readable magnetic tape to anyone requesting it. Dial-up on-line access is also available to anyone, but only a limited number of users can be accommodated at any one time. The first yearly hard copy edition of the database will be available in early 1984.

53. UNIVERSITY OF NEBRASKA-LINCOLN
Lincoln, Nebraska 68583-0718

BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERIZATION OF HIGHER PLANTS WITH REDUCED PHOTORESPIRATION — $53,949

Raymond Chollet
Department of Agricultural Biochemistry

This project is designed to further our understanding of mechanisms which may reduce photorespiration in higher plants. Leaf anatomical/ultrastructural features and CO_2 exchange characteristics of the crucifer Moricandia arvensis are intermediate between those of C_3 and C_4 plants and show similarities to the C_3-C_4 intermediate species, Panicum milioides. In comparison to C_3 plants, both species exhibit reduced rates of photorespiration [Holaday et al. (1982) Plant Sci. Lett. 27, 181-189]. Low in vitro activities of certain key C_4-related enzymes suggest that a limited C_4-like CO_2 concentrating mechanism is not responsible for reducing photorespiration in either M. arvensis or P. milioides [Holaday et al. (1981) Biochim. Biophys. Acta 637, 334-341 and unpublished data]. Pulse-chase $^{14}CO_2$ incorporation experiments with these intermediate species indicate that the pathway for carbon assimilation is similar to that in the C_3 plants, M. foetida and Glycine max [Holaday & Chollet (1983) Plant Physiol., in press]. There is NO evidence of C_4 photosynthesis. After a 6-s exposure to air containing 310 µl $^{14}CO_2$/L, only about 6% of the total ^{14}C fixed is present in malate and aspartate in both M. arvensis and P. milioides. During subsequent chase periods in $^{12}CO_2$-air there is little apparent turnover of these C_4 acids. However, there are notable differences among the species examined in the labelling of glycine: with M. arvensis after 36 s, 20% of the ^{14}C is found in this key photorespiratory metabolite compared to 15% with M. foetida, 14% with P. milioides and 9% with G. max. Additionally, following a similar chase period at subatmospheric levels of CO_2 (100 µl/L), the percentage of ^{14}C in glycine increases 2- to 3-fold in P. milioides and the C_3 plants, but only 0.2-fold in M. arvensis. These apparent differences in glycine metabolism may relate to the mechanism by which photorespiration is reduced in M. arvensis. Future investigations will focus on photorespiratory glycine and glycolate metabolism in this intermediate species.

54. UNIVERSITY OF NEBRASKA
Lincoln, Nebraska 68583-0722

VIRUSES OF EUKARYOTIC GREEN ALGAE — $62,000

James L. Van Etten
Department of Plant Pathology

We have recently isolated and partially characterized four distinct dsDNA viruses which replicate in Chlorella-like green algae symbiotic with Hydra and Paramecium. One of these viruses, PBCV-1, also synchronously infects and replicates in two culturable Chlorella. This has allowed, for the first time, the production of mg quantities of a eukaryotic algal virus as well as the development of a plaque assay for PBCV-1. The PBCV-1 Chlorella system is the first example of a virus infecting any eukaryotic plant which can utilize procedures directly adapted from those used to study bacteriophage.

The objective of this proposal is to determine the structural organization and properties of the large viral genome (ca. 280 kbp) and the nature and function of the viral gene products. The research has several potentially important long range implications. Studies on PBCV-1 will: (i) provide an opportunity to study a new type of virus-host relationship, (ii) determine the role it plays in symbiosis, (iii) demonstrate the usefulness of PBCV-1 or PBCV-1 DNA as a vector for transferring genes into other algae or higher plants, and (iv) determine if PBCV-1 or PBCV-1 lysates contain a new source of plant cell wall degrading enzymes. Finally studies on the regulation and expression of the dsDNA viral genome in the host will provide new information on gene regulation in eukaryotic plants.

55. NEW YORK STATE DEPARTMENT OF HEALTH
Center for Laboratories and Research
Albany, New York 12201

METHANE PRODUCING BACTERIA: IMMUNOLOGICAL CHARACTERIZATION — $47,000
Conway de Macario, E.,* Macario, A.J.L.,* and Wolin, M.J.°
*Laboratory of Immunology
°Laboratory of Environmental Biology and Field Services

The objective of this project is to use immunologic methods for identification and classification of methane-producing bacteria (methanogens). A comprehensive panel of calibrated antibody probes is derived from rabbit antisera. These probes are used to elucidate the antigenic fingerprint of reference methanogens. A standard chart of antigenic fingerprints is generated and is utilized as a reference to classify new strains on the basis of their own fingerprints. Isolates from a variety of ecologic niches in this country and abroad are fingerprinted. The data are examined together with other immunologic results (e.g. cross-adsorption and serial dilution) and studied considering information obtained in other laboratories, such as nucleic acid homologies, physiologic characteristics, and cell-wall chemistry to establish the phylogenetic position of the new isolate. Immunochemical dissection of taxonomically important surface molecules (antigenic determinants) is carried out with antibody probes by means of inhibition-blocking experiments and direct binding assays. These studies are being applied to identification and enumeration of methanogenic bacteria in practically important methane producing systems, i.e. systems for production of natural gas from biomass, waste decomposition systems and natural ecosystems where methane production is an important component of the carbon cycle. The antigenic fingerprints have demonstrated that several methanogens that have been isolated recently belong to new taxonomic groups and are distinctly different from all other previously characterized methanogens. Immunochemical dissection has also helped to identify several antigenic cell surface components of methanogens.

56. STATE UNIVERSITY OF NEW YORK AT BINGHAMTON
Binghamton, New York 13901

GENE-ENZYME RELATIONSHIPS IN SOMATIC-CELLS AND THEIR ORGANISMAL DERIVATIVES IN HIGHER PLANTS — $90,000
Roy A. Jensen
Center for Somatic-cell Genetics & Biochemistry

Comprehensive and systematic approaches will be taken to isolate regulatory mutants which excrete tyrosine, phenylalanine or tryptophan in cultured cells of tobacco (Nicotiana silvestris). Mutants will be isolated by selecting for resistance to various antimetabolite analogs of aromatic amino acids in initially haploid cell populations. Resistant mutants will be sought whose phenotype of analog resistance is temperature-sensitive, genetically stable, and which will regenerate (at least at low temperature). Mutants will be screened for pathway deregulation by scoring the ability of candidate callus fragments to cross-feed appropriate Bacillus subtilis auxotrophs. Clonal purity of mutants will be rigorously established through several rounds of protoplast plating. Biochemical and enzymological characterization of key aromatic pathway enzymes of wild type will be completed to allow the acquisition of a collection of genetic markers that are rigorously defined biochemically. Isolation strategies are proposed based upon the current enzymological data available, the in vitro and in vivo effects known for some analogs, and upon the availability of an in vivo inhibitor of phenylalanine-ammonia lyase activity. The expression of particular mutations at the cell-culture level will be compared with the impact of the same mutations at the organismal level whenever possible. Dominance relationships can be assessed following formation of somatic-cell hybrids through protoplast fusion. The gene dose relationships of regulatory mutations with ploidy state will be examined by comparing specific activities of appropriate enzymes and through measurement of excretion rates of aromatic amino acids. The detailed study of pathway arrangement and regulation in wild type in comparison with deregulated mutants will contribute heavily to an overall appreciation of a metabolic pathway which is the focal point of a very large fraction of higher plant metabolism.

57. UNIVERSITY OF NORTH CAROLINA
Chapel Hill, NC 27514

THE ISOLATION AND CHARACTERIZATION OF β-GLUCOSIDASE GENE AND β-GLUCOSIDASE OF TRICHODERMA VIRIDE — $85,000

Darrel W. Stafford
Department of Biology

mRNAs have been isolated from Trichoderma reesei QM9414 cultures grown in the presence of cellulose for varying times. Electrophoretic analysis of in vitro translation products which had been precipitated with antibody to cellobiohydrolase I (CBH) I) indicated high levels of CBH I mRNA at 20hr, 42hr, and 72hr post-induction. No translational product was precipitated by CBH I antibody from translation incubations using mRNA isolated from cultures induced for 7 days. A β-glucosidase immunoprecipitable translation product was detectable only at 20hr post-induction.

The amino terminal portion of CBH I has been published by Pettersson. A mixture of oligonucleotides designed to be specific for amino acids 16-19 of CBH I was synthesized, 5'-labeled with ^{32}P and used to prime the synthesis of cDNA by reverse transcription of mRNA from 20hr induced cultures. The resulting reverse transcript of single strand DNA was fractionated by acrylamide electrophoresis and the DNA was sequenced by the Maxam and Gilbert technique. The sequence (when translated into protein sequence) agreed perfectly with the amino acid terminal sequence published by Pettersson. Genomic and cDNA clones for CBH I were identified using this authentic CBH I cDNA as an hybridization probe. The genomic and cDNA clones of CBH I are being sequenced.

We have also synthesized oligonucleotides for probes for CBH II and endo-glucanase II and have selected, in a first screen, phage which hybridize to the probe designed to be specific for CBH II.

58. OHIO STATE UNIVERSITY
Columbus, Ohio 43210

DEVELOPMENT OF GENETIC SYSTEMS FOR ANALYSIS OF THE OBLIGATE ANAEROBE METHANOBACTERIUM RUMINATIUM PS — $63,196

John N. Reeve and James I. Frea
Department of Microbiology

The goal of this project is to develop techniques which will facilitate genetic analyses of methane producing micro-organisms. We are isolating antibiotic resistant mutants to provide selective genetic traits. DNA prepared from antibiotic resistant strains is being used to determine if methanogens can be transformed by direct exposure to DNA or following conversion of methanogens to protoplasts. The size and structure of the DNA of several Methanococcus species is being characterized by use of restriction enzymes and two dimensional agarose gel electrophoresis. Several genes have been cloned by in vitro recombinant DNA procedures from Methanobrevibacter smithii, Methanosarcina barkeri, and Methanococcus vanniellii which are functionally expressed in Escherichia coli as demonstrated by complementation of auxotrophic mutations of Escherichia coli. The fine structure of these genes is being determined for comparison with the known structure of the analogous genes already characterized from bacteria. Availability of specific cloned methanogen genes has led to the instigation of a search for mutations in these genes in the methanogenic species from which the genes were originally isolated. Ultraviolet light and mitomycinC are being used as mutagens. If such mutants can be isolated then they will be exposed to preparations of DNA containing the cloned genes, as donor DNA, in procedures designed to develop genetic transformation systems. Analyses of cloned methanogen DNAs show that there are several repetitive sequences in the genomes of methanogens. Current studies are aimed at determining if these sequences show the characteristics of transposable elements and if they can be modified by in vitro enzymatic procedures to act as vectors for introduction and integration of DNA into the genomes of methanogens.

59. UNIVERSITY OF OKLAHOMA
Norman, Oklahoma 73019

METABOLISM OF FATTY ACIDS BY SYNTROPHOMONAS WOLFEI — $36,231
Michael McInerney
Department of Botany and Microbiology

The objective of this project is to study the metabolism of fatty acids by the anaerobic hydrogen-producing fatty-degrading bacterium, Syntrophomonas wolfei. The degradation of fatty acids is often the rate-limiting steps in the conversion of organic matter to methane and carbon dioxide. However, little is known about the metabolism of the bacteria responsible for these reactions because they have very slow growth rates and can only be grown in co-culture with hydrogen-using bacteria. Methods to grow S. wolfei in pure culture by gas recycling after hydrogen removal will be developed as well as techniques for mass-culturing S. wolfei in pure culture or in co-culture. Methods will also be developed to obtain cell-free extracts of S. wolfei free from contamination by cells of the hydrogen user by selective lysis of S. wolfei cells with lysozyme. These methods will be used to determine the pathways for fatty and degradation, hydrogen production and poly-β-hydroybutyrate formation in S. wolfei. The implications of this research are of primary importance to the optimization of any commercial process involving anaerobic microorganisms for fuel gas production or waste treatment.

60. UNIVERSITY OF PENNSYLVANIA
Philadelphia, PA 19104

FACTORS GOVERNING LIGHT DRIVEN ELECTRON AND PROTON TRANSLOCATION IN PROTEINS ACROSS MEMBRANES — $80,000
P. Leslie Dutton
Department of Biochemistry & Biophysics

The goal of this project is to reveal the properties of the individual steps that lead to electrogenic charge separation and recombination across membrane proteins. The photochemical reaction center from Rhodopseudomonas sphaeroides, cytochrome c and quinones represent the principal system used. The means to the goal of this project is to achieve a closer experimental access to the electrical properties of the electrogenic reactions. This will be done by organizing the proteins in the form of planar bilayers or in the form of protein mono- and multilayers deposited on planar substrates and electrode material. Use of flash activation and kinetic analysis (in several cases down to helium temperatures), use of systematic biochemical alteration of the ΔG of key reactions, and use of the effects of applied potentials should provide information on the biochemical nature of the electrogenic events and provide much needed experimental contributions that will be useful to understanding the factors that govern electron and proton translocation.

61. PURDUE UNIVERSITY, Dept. of Horticulture
West Lafayette, Indiana[1]
NATIVE PLANTS INC.; Salt Lake City, Utah[2]

REGULATION OF PROTEIN AND mRNA METABOLISM IN SALT TOLERANT AND INTOLERANT CULTURED HIGHER PLANT CELLS — $80,000

R.A. Bressan[1], A.K. Handa[1], P.M. Hasegawa[1], T.H. Ulrich[2], G.J. King[2], T. Helentjaris[2]

The proposed work has the following objectives: 1) to identify specific gene products (protein and mRNA molecules) which are associated with salt shock and eventual tolerance in cultured plant cells 2) determine if such proteins and mRNAs are the same as those elicited by other stresses, e.g. heat shock 3) determine if such proteins and mRNAs are the same as those associated with PEG-induced water stress tolerance in tobacco and other plant species 4) determine whether abscisic acid can affect the salt tolerance of cultured cells and whether it can induce changes in protein and mRNA synthesis which are associated with tolerance and measure the changes in ABA levels in cells as they undergo adaptation and become tolerant 5) determine to what extent salt shock and/or tolerance specific proteins are associated with cell membrane fractions and organelles 6) determine how the synthesis of salt shock and tolerance specific mRNAs and proteins is regulated e.g. all at transcription, in a coordinated fashion, etc. 7) determine the genomic organization of salt shock and tolerance genes, e.g. their relative chromosomal locations, level of amplification, if any, and regulatory and sequence similarities.

62. PURDUE UNIVERSITY
West Lafayette, Indiana 47907

STUDIES ON THE STRUCTURE AND EXPRESSION OF GENES IN HIGHER PLANTS — $120,000

Brian A. Larkins and Donald E. Foard
Department of Botany and Plant Pathology

The objective of this project is to isolate and characterize the genes encoding the low molecular weight protease inhibitors from soybean (Glycine max L. Merr). This group of proteins includes the Bowman-Birk protease inhibitor and its related family of isoinhibitors, PI I-IV. Characterization of these polypeptides reveals that they are rich in sulfur-containing amino acids and that their amino acid sequences are highly conserved. A major aspect of the research is to prepare a cDNA probe for the BBI by synthesizing complementary DNA from messenger RNA. This cloned probe is used to isolate the corresponding gene from a soybean genomic library. Information which has been obtained reveals that the Bowman-Birk inhibitor is encoded by a single gene in the soybean genome which is under developmental regulation. Nucleotide sequence analysis of this gene reveals that it contains no intervening sequences. The results obtained from this research will be useful in gene transfer experiments designed to improve the nutritional value of seeds and increase the natural plant protection by engineering multiple copies of the BBI gene in soybean.

63. THE ROCKEFELLER UNIVERSITY
New York, N.Y. 10021

EXPRESSION OF NUCLEAR GENES ENCODING THE SMALL SUBUNIT OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE. $85,000

Anthony R. Cashmore
Laboratory of Cell Biology

The aim of this proposal is to characterize the nuclear genes from pea encoding the small subunit of ribulose-1,5-bisphosphate carboxylase. Two of these genes have been isolated and sequenced. In collaboration with Luis Herrera-Estrella and Marc Van Montagu in Gent, Belgium, we have examined the expression of these genes in tobacco cells after transfer with Agrobacterium. A 900 nucleotide 5' non-coding fragment from the small subunit gene was linked to the coding region of the bacterial gene chloramphenicol acetyltransferase. Expression of this hybrid gene was observed in tobacco cells, and furthermore the expression was dependent on, and inducible by light. One aspect of future work will involve in vitro mutagenesis studies aimed at defining the DNA sequence requirements necessary for this light induction.

64. RUTGERS - THE STATE UNIVERSITY OF NEW JERSEY
New Brunswick, New Jersey 08903

CELLULASE - A KEY ENZYME IN FERMENTATION $52,347

Douglas E. Eveleigh
Department of Biochemistry & Microbiology
Cook College

The objective of this project is to enhance the production of cellulase by the fungus Trichoderma reesei through removal of factors regulating the secretion of the enzyme. The study involves clarification of the discrete stages in the secretory mechanism, and encompasses a study of the T. reesei physiology (Rutgers Medical School), of secretory mutants (Lehigh University) and of cellulase methodology (Cook College). Hypersecreting mutants have been isolated and one class of them shown to possess enhanced amounts of endo-plasmic reticulum. Our immediate results at Cook have shown improved methods of purification of the several components of cellulase, and of identification of the products via high precision liquid chromatography (HPLC).

65. UMDNJ-RUTGERS MEDICAL SCHOOL, Piscataway, NJ 08854 and LEHIGH UNIVERSITY, Bethlehem, PA 18015 and COOK COLLEGE, New Brunswick, NJ 08903

BIOMASS UTILIZATION: BASIC SECRETORY PHENOMENA AND THE CONSTRUCTION OF HYPERCELLULOLYTIC MUTANT OF TRICHODERMA REESEI — $119,894 (FY 82 funds)

B. K. Ghosh & A. Ghosh, Dept. Physiology & Biophysics UMDNJ, Rutgers Medical School; and B.S. Montenecourt & G.I. Sheir-Neiss, Biotechnology Research Center, Lehigh Universtiy.
Douglas E. Eveleigh, Cook College

Cellulases are important industrial enzymes having potential application in the recycle and use of renewable biomass. Little is known in fungi about their site of synthesis, co- or post-translational modification (e.g., glycosylation, selective proteolysis, etc) and the transport from the site of synthesis to the cell exterior. The objective of this project is to study the phenomena of secretion of the cellulase enzyme complex in Trichoderma reesei wild type (QM6a) and a hypercellulolytic mutant (Rut-C30). Rut-C30 compared to QM6a exhibits: (i) 3-5 fold increase in the yield of cellulase; (ii) enhanced secretion of the cellulase; (iii) proliferation of the endoplasmic reticulum during the logarithmic phase of growth and (iv) resistance to repression of cellulase biosynthesis by glycerol. The specific time sequence of secretion has been pinpointed. Synthesis and secretion of endoglucanase and cellobiase terminate at the end of the logarithmic phase of growth. In contrast, synthesis and secretion of the filter paper activity continue throughout stationary phase. Concomitant releases of other intracellular marker enzymes do not occur suggesting lack of cell lysis. Thus, cellulase secretion is not the result of autolysis. Preliminary cytochemical observations suggest that the endoglucanases may be located in vesicles near the plasma membrane prior to secretion. This will be precisely determined by immunocytochemical methods. Gel electrophoresis (SDS) patterns indicate that the enzymes of the mutant are slightly larger than those of QM6a. Isoelectric focusing of the enzymes shows a greater charge heterogeneity for the endoglucanases of Rut-C30 than QM6a. The Rut-C30 enzymes during the time course of fermentation do not show as much microheterogeneity as the batch culture enzymes. Cause of this microheterogeneity (i.e. glycosylation, proteolysis, etc.) is under investigation.

66. SMITHSONIAN INSTITUTION Washington, D.C. 20560

A PRIMARY LIGHT HARVESTING SYSTEM: PHYCOBILISOMES AND ASSOCIATED MEMBRANES — $60,342

Elisabeth Gantt
Radiation Biology Laboratory
Rockville, MD 20852

Absorption of light for photosynthesis is greatly enhanced in red and blue-green algae by the accessory pigments in the phycobilisome antennae. A large part of the energy absorbed is transferred directly to the reaction centers in the photosynthetic membranes. The structural diversity between the green plants and red and blue-green algae is becoming more apparent. In phycobilisome-containing plants the two photosystems appear to be integrated and not sequestered in separate regions of the thylakoids as in green plants. A spatially integrated system allows for potentially better energy utilization which would be especially important under light limiting conditions. We are using the apparently simpler photosynthetic membranes of the red alga Porphyridium cruentum to determine the topological relationship of the photosystems (I and II), and their relationship with the phycobilisomes. A combination of approaches, involving non-ionic detergent fractionation, antibody production against purified polypeptides of phycobilisome anchor protein(s), and chlorophyll-binding proteins of photosystems I and II, is being applied. Directly related with this are our investigations on photosynthetically active photosystem II-phycobilisome particle preparations which appear to be virtually free of photosystem I. By comparing the composition of the PSII-particles we expect to identify the functional components of the polypeptides catalizing the photosystem II reactions. These investigations are expected to contribute to our understanding of the photo-oxidation of water in oxygen evolving plants.

67. SOUTHERN ILLINOIS UNIVERSITY
Carbondale, Illinois 62901

REGULATION OF ALCOHOL FERMENTATION BY ESCHERICHIA COLI. $60,000
David P. Clark
Department of Microbiology

Our objective is to identify the genes involved in the production of ethanol by the facultative anaerobe Escherichia coli and to elucidate their regulation. Strains in which the two enzyme activities acetaldehyde:CoA dehydrogenase and alcohol dehydrogenase are expressed constitutively in high levels have been used as the starting point for the isolation of mutants lacking these enzymes or with thermolabile enzymes. Mutants lacking only one enzyme are much less frequent than those lacking both activities, suggesting some functional interaction between these two enzymes. This possibility is being investigated by two different approaches. First, the adh operon is being cloned, in order to characterize the various adh genes and their products more closely. Secondly, the two enzymes are being purified, both by classical methods and also by dye ligand chromatography using Cibacron Blue and Procion Red which specifically bind enzymes using NAD as cofactor. The regulation of gene expression is being approached, not only by the use of gene cloning, but also by the isolation of operon fusions in which the regulatory sequences for the adh operon are fused to the β-galactosidase structural gene. Such fusions confirm the anaerobic control of the adh operon and will also allow the isolation of novel regulatory mutants. Understanding the operation and genetic regulation of alcohol fermentation at the molecular level should allow the optimization of alcohol production by fermentation.

68. STANFORD UNIVERSITY
Stanford, California 94305

CLONING AND MAPPING OF EARLY SYMBIOTIC GENES OF RHIZOBIUM MELILOTI $76,630
Sharon R. Long
Department of Biological Sciences

Our research concerns the genes used by Rhizobium meliloti to form nitrogen fixing root nodules on its symbiotic host, alfalfa. We have identified a region which is required for early stages of nodule formation; we have obtained clones representing the DNA of this region and have generated five mutants with lesions in the DNA fragments where the nodulation genes appear to be located. In the coming year, we will generate a fine structure map of that area, by placing transposon insertions at frequent intervals in the locus and by DNA sequencing. We will mutagenize adjacent fragments of DNA also, in order to determine whether any nodulation genes are present there. In addition, we will attempt to clone and map other genes involved in rhizobial infection; we will use the direct-complementation strategy which we have developed for obtaining cloned nodulation genes. This procedure will be carried out on five other nodulation mutants, which appear to be defective in genes other than those already identified and studied by us. The relationship of infection genes to host range genes is another topic which we are interested in. We are beginning a series of experiments designed to clone directly the bacterial genes responsible for host-plant choice. The identification of such genes will be an essential prerequisite for studying their effect on the symbiosis and for manipulating the symbiosis for greater agronomic usefulness.

69. STANFORD UNIVERSITY
Stanford, California 94305

CARBON DIOXIDE AND THE STOMATAL CONTROL OF WATER BALANCE AND PHOTOSYNTHESIS IN HIGHER PLANTS — $66,000
Eduardo Zeiger
Department of Biological Sciences

Stomata provide higher plants with a primary means to adjust to their continuously changing environment. Stomatal control of gas exchange also has a major impact on photosynthetic rates and the water balance of the vegetation.
CO_2 is an important factor affecting mesophyll photosynthesis and stomatal conductance yet its mode of action on stomata remains unknown. Stomata open in low CO_2 concentrations and close in high CO_2, both in light and darkness. The light response is of special interest because of its possible role as a coupling factor between the photosynthetic and stomatal responses of the leaf.
The modulation of light-dependent stomatal responses by CO_2 has been classically assumed to be mediated by guard cell chloroplasts but their recently reported lack of RuBP carboxylase is inconsistent with that hypothesis. However, using fluorescence spectroscopy techniques on guard cell chloroplasts, we found that fluorescence transients associated with photophosphorylation are specifically inhibited by CO_2. These data indicate that, through a yet to be characterized metabolic pathway, CO_2 could modulate the supply of energy for stomatal movements by controlling photophosphorylation rates in guard cell chloroplasts. A role of these organelles in stomatal responses to CO_2 is further indicated by gas exchange studies of the orchid Paphiopedilum, having achlorophyllous stomata, in comparison with its normal relative, Phragmipedium. The CO_2 sensitivity of Paphiopedilum stomata was markedly reduced in the light. These findings are providing us with new research tools to investigate the role of CO_2 in stomatal function. Further progress on these questions should increase our ability to evaluate the impact of CO_2 on plant productivity and the possible consequences of atmospheric CO_2 enrichment on the biosphere.

70. UTAH STATE UNIVERSITY
Logan, Utah 84322

BIOENERGETICS OF THE METHANOGENIC BACTERIA — $81,310
J. R. Lancaster, Jr.
Department of Chemistry and Biochemistry

The objectives of this project are to (1) determine the biochemical components involved in methanogenesis and delineate their functional relationships to each other, and (2) determine the mechanism(s) whereby methane formation results in energy production (ATP synthesis) by the cell. Our approaches involve the application of modern biophysical and biochemical techniques for the measurement of electron transport- and transmembrane ion gradient-linked bioenergetic phenomena. Our initial studies focus on membrane-associated enzymatic activities, including hydrogenase and ATPase. Since the sole function of methane formation by these unique organisms is the production of cellular energy such a perspective should provide a fruitful experimental approach toward the delineation of methanogenic processes in general.

71. WASHINGTON UNIVERSITY
St. Louis, Missouri 63130

GENETIC ENGINEERING WITH A GENE ENCODING A SOYBEAN STORAGE PROTEIN — $63,000

Roger N. Beachy
Department of Biology, Box 1137

The objectives of our studies are to understand the mechanisms that control the expressions of genes encoding the β-conglycinin seed storage proteins of soybeans. Part of that study involves transferring one or more of the storage protein genes to a new plant species and subsequently determining if the gene is expressed, and following the fate of the gene product. During the past year a gene encoding the α'-subunit of β-conglycinin was characterized by DNA sequence analysis. Since the expression of other eucaryotic genes transcribed by RNA polymerase II is controlled by DNA sequences in front of (5') of the gene, we generated a number of DNA fragments that contained the structural gene and varying amounts of DNA 5' of the gene. The amount of DNA varied from (+) 9,000 nucleotides to (-) 14 nucleotides. Each of these DNA fragments (eight in total) are being transferred via a six-step conjugation and transformation sequence, into the Ti-plasmid of Agrobacterium tumefaciens. These bacteria will be used to transform tobacco cells. The Ti-plasmid chosen for this work has been mutated to permit the regeneration of intact, fertile plants from transformed cells. Subsequent experiments will determine if the engineered gene is expressed and whether it is devlopmentally regulated. We will also determine the fate of the RNA and protein products of the expressed gene. Concurrent with these experiments the gene, under control of a constitutive promoter, will be transferred petunia cells and its expression will be monitored in a similar manner.

72. WASHINGTON UNIVERSITY
St. Louis, Missouri 63130

DEVELOPMENT OF A TUMOR-INDUCING (TI) PLASMID VECTOR FOR PLANT GENETIC ENGINEERING — $70,000

Mary-Dell Chilton
Department of Biology

The Agrobacterium Ti plasmid is a natural gene vector that inserts T-DNA, a specific sector of the plasmid, into host plant nuclear DNA causing tumorous growth. Tumor cells can be genetically engineered by introducing foreign DNA into T-DNA, which then carries it into the host plant. An important problem with this gene vector system is that tumor cells do not regenerate into plants as can normal plant cells. We have constructed a MICRO-Ti plasmid that solves this problem. It is a relatively small plasmid carrying the left and right borders of T-DNA but lacking all of the interior (tumor-inducing) genes of T-DNA save one, the innocuous nopaline synthase gene. MICRO-Ti plasmid has a unique cloning site within T-DNA where foreign genes of choice can be inserted. It is clear that MICRO-Ti can transfer its T-DNA to plant cells when helped by a large plasmid carrying VIR (virulence) genes: although we see no tumorous outgrowth, the plant cells at the site of inoculation synthesize nopaline, a sign that they possess the nopaline synthase gene. It appears that MICRO-Ti can thus be complemented in trans by a VIR helper plasmid. This small vector plasmid, with several refinements, will provide the basis for a convenient and useful genetic engineering vector for crop plants.

73. WASHINGTON STATE UNIVERSITY
Pullman, Washington 99164

REGULATION OF TERPENE METABOLISM — $66,918
Rodney Croteau
Institute of Biological Chemistry, 6340

The volatile plant oils (essential oils) and plant derived resins are finding increasing use as replacements for petroleum based industrial feedstocks, and the potential for the large scale production and expanded use of these renewable resources is considerable. To realize this potential, fundamental knowledge of the biochemistry of essential oil and resin terpenes is needed, particularly as it applies to the regulation of terpene metabolism. This research project is intended to provide an understanding of such regulatory mechanisms at the enzyme level. This objective is being accomplished through the intensive investigation of two model systems (camphor metabolism in Salvia officinalis and menthone metabolism in Mentha piperita) which allows us to probe the control of both biosynthetic and catabolic processes involved in monoterpene accumulation. The pathways of biosynthesis of both monoterpene ketones are being established, as are the sequences of reactions involved in the conversion of both compounds to their water-soluble derivatives which are transported out of the oil gland sites of synthesis to the roots. The enzymes of these pathways are under study. A major focus of the research is the possible hormonal mediation of developmental enzyme level changes (biosynthetic activity decreases as the leaf reaches full expansion) and the possible inductive processes mediated by the terpenes themselves (catabolic activity may be initiated by collapse of the mature oil glands with the monoterpenes "flooding" the surrounding epidermis and adjacent mesophyll tissue). These lines of investigation are being pursued, as is the ultimate fate of the terpene conjugates on transport to the roots. The new information gained from this research will have important consequences for the yield and composition of terpenoid oils and resins that can be made available for exploitation as renewable sources of chemical feedstocks.

74. WASHINGTON STATE UNIVERSITY
Pullman, Washington 99164

DNA TRANSFORMATION OF PLANT CELLS DEFECTIVE IN NITRATE REDUCTASE ENZYME ACTIVITY — $45,144
Andris Kleinhofs and Paul F. Lurquin
Department of Agronomy and Soils and Program in Genetics and Cell Biology

The objective of this research is to develop procedures for plant cell transformation equally useful for dicotyledonous and monocotyledonous plants. The approach uses nitrate reductase-deficient mutants which cannot grow on nitrate as the sole nitrogen source, but can be propagated on reduced nitrogen sources such as amino acids, urea, or ammonia. These mutants provide a strong selection permitting the isolation of rare events. Genes coding for the molybdenum cofactor functions essential for nitrate reductase activity were isolated from Escherichia coli. Previous experiments showed that these genes code for functions which produce a product that can interact in vitro with the plant nitrate reductase apoprotein to regenerate a functional enzyme. These genes are being physically mapped with restriction enzymes and sequenced. The sequence information is important to combine the E. coli genes with a promoter functional in plant cells. The constructed chimeric plasmid will be introduced in the plant cells via liposomes and microinjection. Integration of the foreign gene into the plant cell genome will be facilitated by homologous recombination and transposable elements. The procedures developed will lead to genetic engineering of plants. This will in turn lead to the development of plants more suitable for biomass or specific product (oils, carbohydrates, proteins, secondary metabolites) production.

75. UNIVERSITY OF WASHINGTON
Seattle, Washington 98195

STUDIES ON THE CONTROL OF PLANT CELL ENLARGEMENT BY CELLULAR PARAMETERS — $62,753

Robert E. Cleland
Department of Botany, KB-15

The goal of this research is to determine how plant cell enlargement is controlled and regulated at the cellular level by the hormone auxin. Previous studies have indicated that auxin causes cells to excrete protons into the cell wall, where the resulting acidic pH activates "wall loosening" processes. One part of our research concerns the nature of this wall loosening process. Isolated cell walls of the oat coleoptile, when under tension, extend in response to acid. We are characterizing this process in terms of its dependence on pH, tension, temperature, and prior history of the tissue. A second aspect of this research is to study the relationship between the energy metabolism of cells and the auxin-induced proton excretion. The short-term effects of auxin on nucleotide di- and triphosphates are being measured for the first time, and inhibitors are being used to probe the relationship between various types of respiration and proton excretion. Finally, the relationship between the turgor pressure of cells and the rate of cell enlargement is being studied by measuring turgor pressure directly with a micro-pressure probe under conditions where the growth rate is being varied. These results should provide us with a clearer understanding of the mechanisms which control cell enlargement in plants, and thus their primary productivity.

76. UNIVERSITY OF WASHINGTON
Seattle, Washington 98195

THE FERMENTATION TO ETHANOL OF XYLOSE PRESENT IN BIOMASS BYPRODUCT SOLUTIONS — $92,000

Benjamin D. Hall, Mary Lidstrom, Clement E. Furlong
Departments of Genetics, SK-50 and Microbiology & Immunology, SC-42

The goal of this project is to develop a yeast strain that will fermentatively convert xylose to ethanol. Preliminary studies on the pentose fermentative pathway in the yeasts S. cerevisiae and S. pombe suggests an inability to isomerize xylose to xylulose. These yeasts can fermentatively utilize xylulose. In order to overcome the xylose isomerase deficiency, we are presently attempting to express the xylose isomerase gene of E. coli in these yeasts. Using a gene bank from an E. coli xylose positive strain, we have isolated a clone that complements the xylose isomerase defect of a xylose isomerase negative E. coli mutant. From this clone, we have isolated and determined the sequence of a 1.4 kb fragment of DNA that complements the xylose isomerase defect. In addition, we have purified to homogeneity the E. coli xylose isomerase, and determined the partial n-terminal amino acid sequence of this protein. Comparison of the protein and DNA sequence data conclusively proved that the clone contains the xylose isomerase structural gene. We have fused this gene to the promoter fragment of Adh 1 and Adh gene from S. cerevisiae and S. pombe respectively. The transcriptional and translational expression of the hybrid gene in these yeasts is being examined. Clones of S. cerevisiae and S. pombe that produce functional xylose isomerase enzyme will be analyzed for ethanol production. These yeast transformants will then be genetically manipulated in order to select mutants which will efficiently ferment xylose in spent sulfite liquor into ethanol.

77. UNIVERSITY OF WASHINGTON
Seattle, Washington 98195

A STUDY OF THE GENETICS AND REGULATION OF METHANE OXIDATION — $49,712
Mary Lidstrom
Department of Microbiology and Immunology SC-42

The objective of this project is to study genetics and regulation of C-1 specific functions in methanotrophic bacteria. We have isolated and characterized the plasmids found in nine different obligate methanotrophs. They range in size from 26 to 120 Mdal, and are at present entirely cryptic. We have shown that they do not contain the structural genes for nitrogen fixation, and that they do not contain antibiotic resistance markers. DNA-DNA hybridization analysis has revealed homology only in the case of the plasmids from two different Methylosinus species, and only a small region of each plasmid is homologous. We have used a series of broad host range cloning vectors derived from pRK290 and a mobilizing plasmid (pRK2013) to move genes between methane and methanol utilizing bacteria. Our approach for the remainder of this project involves using cloned C-1 genes from facultative methanol utilizers to identify and isolate genes with similar functions in obligate methanotrophs. This work is of primary importance for the manipulation of methylotrophic bacteria for commercial chemical production.

78. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

DEVELOPMENT OF NITROGEN-FIXING MONOCOT-BACTERIA ASSOCIATIONS — $68,000 (FY 82 funds)
Winston J. Brill
Department of Bacteriology and Center for Studies of Nitrogen Fixation

The objective is to understand the ecology and physiology of nitrogen-fixing bacteria that associate with corn roots. With plants grown in the phytotron, an increase of nitrate concentration increases the rate of acetylene reduction by the associated bacteria. Most probable number estimations of Azospirillum-like bacteria showed that there was no significant differences between the numbers of nitrogen-fixing bacteria observed on roots of active versus inactive corn lines. Many unidentified bacteria are observed in the extracellular mucilage, and typically have an electron-lucent zone surrounding them. Bacteria are seldom seen in epidermal cells, and rarely in cortical cells. The location of the bacteria suggest that the primary site of colonization is the epidermal slime layer, not the interior of the root, as has been postulated by most investigators. It is hoped that manipulation of these microbes and the host plant will allow plants, such as corn, to obtain a significant percent of their required nitrogen from atmospheric nitrogen through associative nitrogen fixation.

79. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE — $41,306
Jerry L. Kermicle
Laboratory of Genetics

We seek to identify and map components of the R gene in corn which govern the presence, distribution and timing of anthocyanin pigmentation. A simplex R allele (not duplicated) is composed of a tissue specific segment of short genetic length and a longer segment that is common to alleles of different tissue specific activities. This inference derives from three mutations induced by the transposable element Dissociation, (Ds), analyzed in recombination tests with alleles of contrasting tissue specific effects. This analysis is being extended to include spontaneous and chemically induced mutants as well as to a larger group of Ds variants. Components underlying a heritable interaction between alleles (paramutation) are being mapped similarly. We want to relate the genetic components responsible for tissue specific control to those responsible for paramutations.

80. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

STARCH SYNTHESIS IN THE MAIZE ENDOSPERM AS AFFECTED BY STARCH-SYNTHESIZING MUTANTS — $55,430
Oliver E. Nelson
Department of Genetics

Our objective in this project is to utilize the numerous mutants of maize to gain increased knowledge of the important process of starch biosynthesis in cereal seeds. During the past year, we've been able to establish that the sugary mutant of maize (the usual sweet corn of commerce) whose principal carbohydrate storage product is not starch but the highly branched water-soluble polysaccharide, phytoglycogen, lacks a debranching isoenzyme [an α-(1-6)-glucosidase] present in nonsugary endosperms. This suggests that the amylopectin component of starch is the result of an equilibrium between enzymes introducing α-(1-6) linkages into starch and enzymes cleaving those linkages. We have also detected the existence in developing maize endosperms of two oligosaccharide synthases-enzymes capable of synthesizing short chain length maltodextrins from glucose-1-Po_4. These oligosaccharide synthases may be responsible for the primers which all starch synthases require to function and hence would carry out the initial step of starch synthesis.

81. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

CARBON ISOTOPE FRACTIONATION IN PLANTS — $71,593
Marion H. O'Leary
Departments of Chemistry and Biochemistry

Plants fractionate carbon isotopes during photosynthesis in ways which reflect photosynthetic pathway and environment. 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 (diffusion, carboxylation, etc.) vary with species, environment, and other variables. To this end, we have developed quantitative models for carbon isotope fractionation which describe this process in terms of rates of diffusion, carboxylation, and other components. We have developed experimental approaches which focus on the initial events in carbon dioxide fixation and enable us to determine the relative rates of the various individual processes involved. During the current project period (June, 1983), we have been analyzing malic acid samples obtained from plants showing Crassulacean acid metabolism following nocturnal exposure of these plants to $^{13}CO_2$ or $^{13}C^{18}O_2$. From these samples we will determine the activity of carbonic anhydrase in these plants and we will determine the extent to which fumarase randomizes malate during nocturnal CO_2 fixation.

82. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

PHYTOCHROME FROM GREEN PLANTS: ASSAY, PURIFICATION AND CHARACTERIZATION — $50,000
Peter H. Quail
Botany Department

The objective of this project is to purify and characterize the phytochrome molecule(s) from light-grown chlorophyllous tissue of higher plants and algae. The approach involves the use of (a) purification procedures that greatly reduce chlorophyll levels (principally polyethyleneimine precipitation) to permit spectral analysis, and (b) immunological procedures for further characterization. The difference spectrum of the phytochrome from green Avena is distinct from that of phytochrome from etiolated shoots in that the Pr maximum is shifted to shorter wavelengths. Immunoprecipitation of 'green' phytochrome with antibodies directed against 'etiolated' oat phytochrome distinguishes two populations of spectrally active phytochrome. 25% of the 'green' phytochrome is recognized by this IgG, but requires 2.5-fold more IgG than for 'etiolated' phytochrome to maximize immunoprecipitation; the remaining 75% is not recognized. Mixing experiments indicate that these apparent immunochemical differences between 'green' and 'etiolated' phytochrome are neither the result of posthomogenization modifications nor of interfering substances in the green tissue preparations. The significance of these data is that they suggest the presence in cells of light-grown tissue of one or more phytochrome species distinct from the well-characterized molecule that predominates in etiolated tissue. Together with the absence of detectable levels of immunoprecipitable phytochrome apoprotein among the in vitro translation products of poly(A) RNA from green tissue (Proc. Natl. Acad. Sci. US 80: 2248, 1983), these data suggest the possibility of more than one phytochrome gene.

83. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

MOLECULAR MECHANISM OF ENERGY TRANSDUCTION BY PLANT MEMBRANE PROTEINS — $61,440

Michael Sussman
Department of Horticulture

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 which, 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 that is essential for the uptake of minerals and nutrients. This protein has unique molecular properties. Since it contains a single polypeptide of M_r=104,000 that is phosphorylated during the reaction cycle, its chemical structure is very similar to that of cation-translocating ATPase's found in the plasma membrane of animal cells (e.g., the NaK-ATPase of kidney, the HK-ATPase of stomach and the Ca-ATPase of muscle). However, since it only translocates protons, it is functionally more similar to the membrane ATPase found in bacteria, mitochondria and chloroplasts.

In this project, protein modification and sequencing techniques are used 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: (1) an ATP-binding site and (2) an ion-binding site. The primary structure of these sites will be determined by application of automated solid phase sequencing techniques. Essential amino acids will be further identified by comparing the amino acid sequence obtained with enzyme isolated from many plant species. Results derived from the study of this unique protein will be used to define a molecular mechanism for protein-mediated energy transduction.

84. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

ONE CARBON METABOLISM IN ANAEROBIC BACTERIA: ORGANIC ACID AND METHANE PRODUCTION — $106,000

J. Gregory Zeikus
Department of Bacteriology

This research aims to identify the pathways and regulation of carbon and electron flow during one carbon metabolism by acetogenic and methanogenic bacteria. The approach involves comparison of growth parameters, enzyme activities and end products formed in relation to the fermentation of H_2-CO_2, HCOOH, CO, CH_3OH and acetate. Emphasis is placed on determination of the biochemical mechanism of acetate synthesis from one carbon compounds or the synthesis of methane from acetate by specific carbon tracer analysis (i.e., ^{14}C, ^{13}C-NMR, and ^{18}O), enzymatic analysis (i.e., one carbon oxidoreductases, such as CO dehydrogenase) and mutant analysis (i.e., metabolic lesions in one carbon transformation reaction). The results are of importance to biotechnology because acetic acid is a major chemical commodity and methane is a major fuel source.

85. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

BIOCONVERSION BY PHOTOSYNTHETIC SYSTEMS — $85,000
John L. Bennett

The objective of the research is to determine the effects of environmental factors on the structure, function and formation of chloroplasts of plants and algae. Adaptive control mechanisms are being sought in these systems. Three areas of control are being explored: (1) regulation of chloroplast formation at the levels of gene transcription and protein turnover, (2) homeostatic control of photosynthetis by reversible phosphorylation of chloroplast proteins, and (3) adaptation of the photosynthetic apparatus to extreme environmental conditions through mechanisms involving the regulation of gene expression and/or post-translational controls. Although special emphasis will be given to light as an environmental variable (including changes in light intensity, spectral quality and daily duration), the modifying effects of high and low temperature, water stress and disease will also be studied. Current studies of the regulation of gene transcription involve the use of cloned DNA probes prepared against the gene for major chloroplast proteins such as ribulose bisphosphate carboxylase/oxygenase and the light-harvesting chlorophyll a/b protein (LHCP). In the future it is hoped to include genes for other chloroplast proteins in the study. Current aspects of research on chloroplast protein phosphorylation include the regulation of excitation energy distribution within thylakoids by the reversible phosphorylation of LHCP, the control of PSII activity by the phosphorylation of PSII polypeptides, the regulation of cyclic and non-cyclic electron transport, and the regulation of CO_2 fixation. Emphasis is being given to the purification of the protein kinases, both thylakoid-bound and soluble, which play central roles in these regulatory phenomena.

86. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

PLANT MOLECULAR GENETICS — $200,000
Benjamin Burr and Frances A. Burr

The primary research project of the laboratory has been an investigation of the molecular structure and genetic characteristics of maize transposable elements. The major portions of one large element, Dissociation (Ds), have been cloned. This particular receptor element is over 20,000 bp in length. The segments cloned include the important junction regions between the insertion and the wild-type sequences. Using the clones as hybridization probes, we have deduced that at least part of the Ds insertion is not repeated in the genome. Based on genetic evidence, Ds had been anticipated to be present in multiple copies. By careful analysis of several Ds mutations at the Shrunken locus, we have found that the structure of Ds can differ significantly. We believe that these differences are the result of structural rearrangements that the element undergoes in the process of transposition or while at a fixed position, the latter being responsible for a "change-of-state" of the phenotype. We have also been working with another transposable element family in maize called Mutator. These elements are genetically distinct from the controlling elements described by McClintock. Two Mutator (Mu) elements have been cloned and their structures analyzed. One of these is detected only in strains possessing Mutator activity and, in contrast to Ds, it appears to be a stable structure. These characteristics suggest that it would be an ideal element to use for isolating genes by transposon tagging. Mutants at a number of loci have been obtained using Mutator. The Mutator cloned from the Shrunken locus is currently being used as a probe to select random clones from strains with Mutator at the Bronze locus.

87. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

MECHANISMS OF ENERGY CONVERSION IN PHOTOSYNTHESIS — $270,000
Geoffrey Hind
Biology Department

The goal of this project is to discover how energy is transformed from electron potential to chemiosmotic potential in photosynthetic systems. Processes involving cyclic electron transport are of prime concern and are studied in (1) intact chloroplasts of the C-3 plant, Spinacia oleracea, (2) bundle sheath cells of the C-4 plant, Zea mays and (3) isolated heterocysts from the filamentous cyanobacterium Anabaena 7120. In these systems, electron flow from reduced plastoquinone to the photochemically generated oxidant, $P700^+$, is mediated by a cytochrome b/f complex. The polypeptide compositions of complexes from these organisms are being compared and their activities in situ or following detergent extraction are being studied using flash, steady state and low temperature spectrophotometry. Special interest attaches to the means by which, in cyclic flow, electrons return to the cytochrome complex from the strong reductant generated by photosystem 1. Passage of electrons through the complex is coupled to vectorial H^+ transport, possibly by a form of "Q-cycle" as postulated by Mitchell; the stoichiometry of this coupling and its dependence on ambient redox poise are being studied to elucidate the coupling mechanism. The source of reductant needed to poise the cycle is also of concern; in C-3 photosynthesis, activity of photosystem 2 is normally adequate to poise the cycle and the problem is rather to ascertain what mechanisms are involved in preventing total inhibition of cyclic flow by competition with non-cyclic reductions of $NADP^+$ and O_2. These investigations will provide knowledge of the factors limiting photosynthetic reduction of CO_2 and N_2. In showing how the cytochrome complex serves as an efficient energy transformer, they are also of presumed relevance to future design of biomimetic energy conversation devices.

88. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

THE REGENERATION OF PLANTS FROM CROWN GALL TUMORS — $140,000
Daniela Sciaky
Biology Department

The genetic engineering of plants using the T-DNA of Agrobacterium tumefaciens requires that the engineered plant cell be regenerated into a fertile plant. Two separate occurrences are being studied with respect to understanding how crown gall callus can organize into shoots. One event is the spontaneous formation of shoots from Nicotiana otophora cells transformed by pTiB6806. The T-DNA in these shoots is no longer colinear with the T-DNA of undifferentiated transformed cells. Fragments of T-DNA from the shooting tumors will be isolated to determine how the T-DNA is organized in these tumors and to understand how the T-DNA was rearranged. The second event is the localization of a 2.7 kb insert in the T-DNA of pTiA66 leading to the requirement for auxin to induce tumors in some plants. When these tumors are placed in tissue culture they sometimes form shoots and lose their auxin requirement. Sequences homologous to the 2.7 kb insert are present in other regions of the Ti plasmid of A66 and in the A66 chromosome. The primary structure of the junction between the insert and T-DNA indicates that the insert has the structure of a transposable element. Studies are being initiated to determine whether this structure can act as a transposable element in plants and, how this structure regulates the appearance of shoots in tumors.

89. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

THE PHYSIOLOGY AND BIOCHEMISTRY OF CYANOBACTERIA — $185,000
Harold W. Siegelman
Biology Department

The organism Microcystis aeruginosa is probably the most successful cyanobacteria in freshwaters, and it occurs worldwide. It is usually responsible for algal blooms and may cause serious water management problems. The success of the organism is probably associated with an efficient photosynthetic mechanism and a gas vacuole system which permits vertical movement in the water column. The molecular structure and composition of the photosynthetic energy collection systems including phycobilisomes, chlorophyll, carotenoids, and reaction centers are being characterized in several strains. The cells grow successfully over a very wide range of light intensities. The effects of light intensity are being examined with turbidostat and low cell density cultures. Phycobilisomes from cells grown at several light intensities are characterized by agarose cell filtration, hydrophobic-interaction chromatography, electron microscopy, phycobiliprotein stoichiometry, and fluorescence and absorbance spectroscopy. The cell walls of two widely-separated geographic strains have different compositions and additional strains are being examined. The number and composition of the pentapeptide toxins of M. aeruginosa are strain specific, and their biosynthesis is inversely related to cell division rate. The toxin structures are being determined by amino acid analysis and mass spectroscopy. Pathological and hematological studies indicate that the toxins require an in vivo activation process and then cause thrombocytopenia. The toxicity is apparently due to pulmonary thrombosis. The lipid and carbohydrate composition of several strains of M. aeruginosa are being examined by chromatographic and mass spectral methods. The compositional analysis of several strains of M. aeruginosa for lipids, carbohydrates, carotenoids, and toxic pentapeptides may provide a useful basis for strain identification or classification of morphologically-similar isolates of a single species. These components may provide useful intermediates for chemical feedstocks.

90. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

PLANT CELL GENETICS — $20,000
Harold H. Smith
Biology Department

The objective of this project is to gain an understanding of genetic controls in plant development through cell and tissue culture and the analysis of genetic tumors. The genetic basis of spontaneous tumor formation in certain Nicotiana plants is being studied by isolating particular chromosomes that, when transferred to distantly related species, cause tumors to develop. These chromosomally-defined, tumor prone plants are being grown in tissue culture to determine their differences from normal plants in growth factor requirements, and with regard to differences in superoxide dismutase, an enzyme characteristically deficient in mammalian tumors. Transmission genetics of the alien tumor-controlling chromosome, from the fourth to the ninth segregating generation, reveals that: (1) about half the plants produced by self pollinating such 2n+1 plants have the extra chromosome (a high rate of transmission), and (2) only about half the plants with this extra chromosome actually form tumors (a low rate of penetrance). The total enzyme activity of superoxide dismutase is found to be as high in these tumorous genotypes as in the nontumorous ones, a result that is contrary to findings with many animal tumors. The Nicotiana tumor system provides a unique material for further studies on genetic controls of differentiation.

91. LAWRENCE BERKELEY LABORATORY
Berkeley, CA 94720

RESONANCE STUDIES IN PHOTOSYNTHESIS — $60,000
Alan Bearden
Donner Laboratory

The first objective of this work has been the further study of the role of iron-sulfur proteins in the acceptor structure of chloroplast Photosystem I. Studies by low-temperature electron paramagnetic resonance (EPR) spectroscopy with partially oriented membranes from the green halophilic alga, Dunaliella parva have shown the existence of an iron-sulfur cluster with an orientation different from the previously known acceptors, Cluster A and Cluster B. Concomitantly time-resolved EPR spectra of whole chloroplasts and photosynthetic particles isolated with enhanced Photosystem I activity, clearly indicate a more complicated set of early reaction in the primary/secondary dynamics of this Photosystem. Preliminary publication of these results has been in Biochimica Biophysica Acta 720-722, 16-19 (1983) and 723, 23-25 (1983). Secondly, we have completed during this fiscal year the first experimental demonstration of "Hopfield" charge transfer band arising from tunneling processes in the electron-transfer in the photosynthetic bacterium, Chromatium. These results have been accepted for publication in the Proceedings of the National Academy of Sciences. The generality of these latter studies to problems in biophysics and solid state physics has been recognized and a first paper has been published in the Physical Review B 27, 7431-7439 (1983); papers for Physical Review Letters and the Journal of Molecular Biology are in preparation.

92. LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
Berkeley, California 94720

PHOTOCHEMICAL CONVERSION OF SOLAR ENERGY — $110,000
Lester Packer, Rolf J. Mehlhorn, Alexander T. Quintanilha
Membrane Bioenergetics Group, Applied Science Division

This program seeks to characterize the molecular events associated with photochemical conversion mechanisms. Bacteriorhodopsin is a light- and temperature-stable protein, which operates via a photocycle to develop a proton current, contains retinal as a chromophore, and is the only protein found in the purple membrane isolated from Halobacterium halobium. Chemical modification and electron spin resonance techniques will be used to study the topography of carboxyl residues of bacteriorhodopsin. We seek to characterize buried carboxyl groups located in hydrophobic protein domains near the retinal chromophore, and at least 16 Å from the membrane surface, and on the carboxyl-terminal tail, and to determine whether modification of these groups perturbs the light-induced proton pumping activity. The data will be compared with several models of bacteriorhodopsin's tertiary structure to determine if, like other membrane proton pumps, a buried carboxyl group is required for activity.

93. LAWRENCE BERKELEY LABORATORY
Berkeley, California 94720

CELL CYCLE DEPENDENT EXPRESSION OF GENES CODING FOR PHOTOSYNTHETIC PROTEINS IN EUGLENA GRACILIS — $28,000

James C. Bartholomew
Chemical Biodynamics Division

In this project we will map the genes coding for photosynthetic proteins in Euglena gracilis into the nuclear or chloroplast domain. Plasmids containing DNA homologous to mRNA'es for reaction center proteins will be used to study the regulation of expression of the photosynthetic genes during the cell cycle and during plastid development. We will use in vitro translation systems to study the factors that effect the synthesis of these proteins. We hope to develop from these studies an understanding of how the genetic information coding for the photosynthetic apparatus is organized and controlled during plastid development.

94. LAWRENCE BERKELEY LABORATORY
Berkeley, California 94720

PLANT BIOCHEMISTRY: REGULATION OF PHOTOSYNTHETIC METABOLISM AND SECONDARY BIOSYNTHESIS — $280,000

James A. Bassham
Chemical Biodynamics Division,

The objectives of this project are to determine photosynthetic and biosynthetic paths in green plants and the mechanisms of regulation in these paths. Both metabolic regulation (feedback inhibition, energy charge, etc.) and regulation of gene expression are studied in plant tissue, including whole plants and organs, cells and organelles isolated from leaves, and plant tissue culture. Subtask I (Regulation of Metabolism and Gene Expression) focuses on key enzymes and processes of metabolism in photosynthetic cells, and is directed to obtaining a better understanding of control of primary carbon and nitrogen metabolism in these cells, including specialized cells found in certain plants (C_4 plants). Subtask II (Secondary Plant Metabolism: Plant Pigments) includes similar goals focussed on secondary products and also investigation of structures and mechanism of action of non-chlorophyllous plant pigments.

95. LAWRENCE BERKELEY LABORATORY and the
UNIVERSITY OF CALIFORNIA, BERKELEY
Berkeley, California 94720

MAPPING PHOTOSYNTHETIC GENES IN RHODOPSEUDOMONAS CAPSULATA — $82,000

John E. Hearst
Department of Chemistry and the Laboratory of Chemical Biodynamics

The objectives of this project include the mapping and sequencing of light harvesting and reaction center genes in Rhodopseudomonas capsulata. The regulation of these genes and a study of their phylogenetic relationship to similar genes in other photosynthetic bacteria, algae and higher plants are anticipated. In this fiscal year, five structural genes coding for the reaction center (RC) L, M and H subunits and the two light harvesting (LH) I polypeptides, LHP1 and LHP2, have been mapped on two restriction fragments from the R-prime plasmid, pRPS404. The genetic map of the R-prime plasmid has been aligned with the restriction map. It had been shown that enhanced near-infrared fluorescence mutants of R. capsulata typically lack RC or LH I polypeptides and that these lesions are marker rescued by two restriction fragments from the R-prime plasmid: the 7.5 kb Eco F fragment and the 4.75 kb Bam C-Eco B fragment. We have now determined the nucleotide sequence of these two restriction fragments and have found that the Bam C-Eco B fragment carries the structural genes for the RC L and M subunits and both LH I polypeptides. Thirty kilobasepairs (kb) away from this locus, the Bam F fragment (within the Eco F fragment) carries the RC H subunit.

96. LAWRENCE BERKELEY LABORATORY
Berkeley, CAlifornia 94720

WATER SPLITTING AND OXYGEN EVOLUTION IN PHOTOSYNTHESIS — $105,000

Melvin P. Klein
Chemical Biodynamics Division

The details by which manganese participates in the photosynthetic oxygen evolution reactions are obscure. Our efforts in this area are directed at the use of X-ray spectroscopy, low temperature EPR and Electron Spin Echo spectroscopy to extract structural information about the manganese centers of photosynthetic membranes. These structural parameters and the changes that occur during function of the membrane apparatus are to be used as a framework for the elucidation of mechanistic details of the water splitting process. Previous studies using X-ray absorption techniques in our laboratory have provided information about the ligand structure and average oxidation state of manganese in active dark adapted (resting) photosynthetic membranes. Significant advances have recently been made in that stable, active sub-chloroplast membrane preparations from spinach containing only the photosystem II light reactions are now available. Secondly, a photo-induced EPR signal rich in manganese hyperfine structure has recently been observed at low temperature in chloroplast preparations and is interpreted as arising from reactions close to the oxygen evolving complex. X-ray spectroscopic data of these same preparations indicates an oxidation and ligand state of manganese distinctly different from that of dark adapted membranes.

97. LAWRENCE BERKELEY LABORATORY
Berkeley, California 94720

STUDY OF THE STRUCTURE AND MECHANISM OF ACTION OF PHYTOCHROME — $82,000
Henry Rapoport
Department of Chemistry, University of California

The objectives of this project are to study the structure and mechanism of action of the non-chlorophyllous plant protein-pigment phytochrome. Phytochrome is the morphogenically active plant pigment which controls all developmental aspects of plant growth. It does this by undergoing a photochemical interconversion upon light excitation to a new, active form. The specific structural features of this isomerization, as well as some other structural aspects of phytochrome, remain unknown. It is essential that these structural questions be unambiguously established in order to have a full understanding of phytochrome's function. Understanding the underlying mechanism by which phytochrome functions as a reversible biological switch may allow us to control growth, flowering, and fruiting. The action of far red light causes phytochrome to change from the inactive P_R form to the active P_{FR} form. The two most reasonable hypotheses for its mode of action in the P_{FR} form are through gene activation or by affecting membrane permeability. However, structural information on exactly what changes accompany the transformation to the P_{FR} form is totally lacking. We plan to provide this information by applying detailed NMR analysis to this transformation.

98. LAWRENCE BERKELEY LABORATORY
Berkeley, California 94720

CHARGE SEPARATION IN PHOTOSYNTHETIC LIGHT REACTIONS — $168,000
Kenneth Sauer
Chemical Biodynamics Division

We are using electron paramagnetic resonance spectroscopy (EPR) and fluorescence lifetime measurements to monitor the dynamics and mechanism of charge separation associated with the primary light reactions of photosynthesis. Transient EPR measurements exhibit electron spin polarization that reflects the chemical nature of the molecular species involved as well as their distance of separation and their orientation in the photosynthetic membranes. Fluorescence decay kinetic studies elucidate the pattern of excitation transfer among the light-harvesting chlorophyll molecules and charge recombination in the reaction centers. Analysis of the several exponential decay components shows that they can be distinguished with respect to their response to excitation intensity, added redox agents, divalent cations and conditions that result in membrane phosphorylation. In addition to providing knowledge about the electron carriers involved, these studies are informative about the membrane organization and how it is subject to control by secondary constituents.

We are also investigating the structure of reaction center complexes of photosynthetic bacteria. We have isolated the reaction center proteins from Rps. capsulata and are sequencing oligopeptides derived from them by selective cleavage. We are preparing to crystallize these complexes for detailed structure determination.

99. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

ANALYSIS OF PHOTOSYNTHETIC MEMBRANES — $219,000
C.J. Arntzen
MSU-DOE Plant Research Laboratory

Chloroplast membranes are the site at which the energy trapped from light during photosynthesis is converted into the stable chemical intermediates $NADPH_2$ and ATP. The photosynthetic light reactions require the concerted interaction of pigments that sensitize the process as well as electron carriers and enzymatic proteins. A central theme of this task is to understand the structural organization of the chloroplast membranes, which allows us to understand the structural organization of the chloroplast membranes, which allows us to understand compartmentalization of individual functions and ultimately the regulation of efficiency and activity of the overall process. The approaches used in this research include ultrastructural analysis of developing and mature membranes, detergent fractionation of isolated membranes to isolate individual chloroplast membrane components, and characterization of both light-harvesting and electron transport partial reactions in the intact membranes or membrane subfragments. Current research emphasis is being directed upon the covalent modification of light-harvesting pigment-proteins via protein phosphorylation. The phosphorylation is involved in regulating the efficiency of energy trapping via the antennae pigment bed. We are currently testing the concept that this regulatory process involves the lateral mobility of a specific pigment-protein complex within various distinct regions of the chloroplast membrane system. We are also characterizing the functional activity of the photosystem II complex, with special attention being directed at the reaction center polypeptides and those polypeptides which appear to participate in the removal of electrons from water. The long term objective of this research program is to identify mechanisms which control the quantum efficiency of photosynthesis with the idea that understanding of these controls will allow us to successfully manipulate the photosynthetic process to optimize biomass productivity.

100. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

DIFFERENTIAL GENE EXPRESSION IN RHIZOBIUM — $157,000
Barry Chelm
MSU-DOE Plant Research Laboratory

The interaction of bacteria of the genus Rhizobium 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 objective of this project is to identify the underlying molecular mechanisms by which the expression of the bacterial genome is regulated during this process. We have focused primarily on the R. japonicum/soybean system. Specific genes whose expression is regulated during nodule development or by other physiological changes have been isolated by hybridization screening of an R. japonicum DNA fragment library carried in an E. coli phage lambda vector. The genes isolated thus far include those encoding the nitrogenase subunits, glutamine synthetase I and the large subunit of ribulose bisphosphate carboxylase. 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. Other developmental stage specific R. japonicum genes are being isolated through the use of stage specific hybridization probes. These stage specific probes are being prepared from total genomic DNA by first removing sequences which are present in other cell types via RNA excess hybridization followed by RNA saturation with RNA from the developmental stage of interest and will be used to characterize the stage specific RNA population in detail by kinetic analyses and to isolated stage specific genes from genomic libraries. The isolated genes will be genetically manipulated and reintroduced into rhizobia in order to establish the importance of these genes for the process of nodulation. These further understandings of the processes regulating legume nodulation and nitrogen fixation should enlighten the viewpoint from which the symbiotic relationship might be further optimized.

101. MICHIGAN STATE UNIVERSITY,
East Lansing, Michigan 48824

RESISTANCE OF CROP PLANTS TO ENVIRONMENTAL STRESS — $94,000
Andrew D. Hanson
MSU-DOE Plant Research Laboratory

This project seeks to identify metabolic pathways which are activated by environmental stress and which have adaptive significance, and to understand the genetic and environmental control over these pathways. The aim is to use natural or induced genetic variation in the expression of such adaptive pathways in breeding stress-resistant crops. (1) Anaerobic stress and glycolytic pathways in imbibed barley aleurone and embryo tissues. These seed tissues -- unlike other tissues of the barley plant, -- can withstand complete anoxia for several days, during which both lactate dehydrogenase (LDH) and alcohol dehydrogenase (ADH) activities are induced and lactate and ethanol accumulate as glycolytic end-products. Both LDH and ADH systems are being subjected to isozyme and immunological analysis, as first steps in characterizing their genetic and environmental regulation. (2) Betaine accumulation in barley. Natural variability for the activity of the stress-induced betaine synthesis pathway has been surveyed in wild and cultivated barley. The available diversity found (a two- to three-fold range) has proven adequate for inheritance studies which have shown the betaine trait to be highly heritable. Accordingly, a program has been started to develop isopopulations, low and high in betaine, for a direct test of the value of high betaine levels in stressed field environments.

102. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

ACTION AND SYNTHESIS OF PLANT HORMONES — $219,000
Hans Kende
MSU-DOE Plant Research Laboratory

The principal objective of this task is to gain knowledge on the mode of action of the plant hormones ethylene and cytokinin. These hormones regulate processes related to stress and senescence in plants. Ethylene promotes aging and the appearance of stress symptoms, cytokinins retard them.

a) Regulation of stress-ethylene synthesis. Plants under stress produce ethylene. Ethylene synthesis is very rapidly induced through the enhancement of the activity of 1-aminocyclopropane-1-carboxylate (ACC) synthase. This enzyme is de novo synthesized during induction of ethylene synthesis. Research will mainly concentrate on the mechanism by which ACC synthase is regulated. Antibodies against ACC synthase will be produced for the isolation and further characterization of the enzyme. Using these antibodies, we shall also study the level of control of this enzyme and the mode of its turnover. We are also studying the role of stress ethylene in rice plants. When rice is flooded, it perceives this as a stress and synthesizes ethylene in response to it. Ethylene induces growth in rice and permits it to maintain its foliage above the water. We are investigating the mechanism by which ethylene, perhaps in conjunction with other growth regulators, controls growth in rice plants.

b) Localization of the site of action of cytokinins. We are synthesizing a cytokinin photoaffinity probe, 8-azido-N^6-benzyladenine, in radioactive form to localize the site of action of this hormone. This will be attempted using two plant systems: moss protonemata and cultured tobacco cells. Cytokinins induce bud formation in mosses and are accumulated by target cells of this organism. In tobacco cells, cytokinins regulate cell division. We shall attempt to isolate the tagged receptor from both types of cells in order to characterize it and localize it in the cell.

103. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

CELL WALL PROTEINS $124,000
D.T.A. Lamport
MSU-DOE Plant Research Laboratory

Our major objective is to determine the function of extensin, a hydroxyproline-rich cell wall glycoprotein of higher plants. Recently we proposed a new model of the cell wall consisting of two concatenated polymeric systems: a cellulose "warp" interpenetrating a crosslinked extensin "weft". Such mechanical coupling between two polymers provides a new basis for understanding cell extension. Current approaches involve (i) isolation of two precursors (P1 and P2) of the firmly bound structural protein extensin from tomato cell suspension cultures. (ii) Structural elucidation of the possible role of the crosslinked amino acid isodityrosine (IDT) in extensin. (i) we propose to characterize the precursors kinetically, compositionally and electron microscopically. Kinetic experiments will involve pulse-chase experiments followed by isolation of the labelled precursors to determine turnover rates at different stages of growth. This will help distinguish between possible rate-limiting steps in wall assembly; secretion or attachment. We propose to peptide map and sequence P1 and P2 as an approach to a determination of their relationship, and function. Sequence data will help to predict IDT crosslink domains and hence determine network size and porosity predicted by the new cell wall model. Electron microscopic approaches will help define those network parameters which depend on IDT crosslinkages. (ii) We shall attack the questions of IDT crosslinkages by isolation of small multimers of extensin. We predict these are formed (a) during the attachment process in muro, and (b) by an in vitro peroxidatic crosslinkage system to be developed.

104. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

REGULATION OF FLOWERING $94,000
Anton Lang
MSU-DOE Plant Research Laboratory

This project deals with the regulation of flower formation by hormone-like (i.e. translocatable) substance, the main focus currently being on inhibitors of flower formation ("antiflorigen"). The existence of antiflorigen in some long-day plants has been conclusively demonstrated, in previous research on this project, by grafting experiments with species of Nicotiana and Hyoscyamus. Antiflorigen, like the promotive hormone-like factor(s) of flower formation ("florigen, floral stimulus"), is not specific in either the taxonomic or the physiological sense, being interchangeable between different species and genera, and between plants in which flowering is dependent on different environmental (photoperiod) requirements. This physiological evidence argues strongly that both antiflorigen and florigen are ubiquitous in flowering plants. The next logical step is isolation and chemical identification of these agents. We are currently working on this problem, with emphasis on antiflorigen since - among various reasons - solution of this problem may be helpful in the search for florigen which so far has been unsuccessful. For isolating antiflorigen, we are using both solvent extraction and phloem diffusion, for assay mainly explants from tobacco inflorescence stems which initiate flower buds in vitro. Continued grafting experiments have indicated that our principal shortday plant (Maryland Mammoth tobacco) does not seem to produce antiflorigen, at least not in the amounts produced by the long-day plants mentioned above.

105. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

THE MOLECULAR-GENETIC BASIS FOR THE DEVELOPMENT OF PHOTOSYNTHETIC COMPETENCE — $157,000
Lee McIntosh
MSU-DOE Plant Research Laboratory

The aim of this research project is to delineate the molecular-genetic basis of photosynthetic competence in higher organisms and to genetically modify specific polypeptides involved in photosynthesis. To accomplish these goals we are (1) isolating chloroplast and nuclear genes whose products are integral photosynthetic components, (2) investigating the regulatory regions of the genes and functional domains of their products, and (3) employing gene mutations to artificially alter photosynthetic capacity in the transformable photosynthetic system. The first two chloroplast gene mutations we are characterizing are those which transmit resistance to streptomycin and the herbicide atrazine. Our recent results indicate that resistance to atrazine appears to be due to a single amino acid substitution in the primary sequence of a 32 Kilodalton (Kd) thylakoid membrane polypeptide. The 32 Kd herbicide binding protein has also been shown to display lower efficiency of electron transport in several species of weeds with atrazine resistant biotype. We intend to employ the mutant "resistance" gene to transform the naturally transformable cyanobacterium Synechococcus R2. The purpose of these experiments is to introduce a chloroplast gene into cyanobacteria and to test for functional integration of the foreign gene product into the photosynthetic apparatus. We are also investigating the possibility of altering (reducing) photorespiration - the net inhibition of photosynthesis due to oxygen competition with CO_2 at ribulososebisphosphate carboxylase (rubisco) - by the direct alteration of the gene encoding the catalytic (large) subunit of rubisco. Our first approach is to employ the bacterial large subunit gene from Rhodospirillum rubrum which is cloned, expressed and functional in E. coli. We have completed the DNA sequence of the gene and are now changing specific bases within the gene to give site-specific amino acid substitution.

106. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

SENSORY TRANSDUCTION IN PLANTS — $141,000
Kenneth L. Poff
MSU-DOE Plant Research Laboratory

The objective of this project is to understand the mechanims whereby plants sense the state of their environment through the perception of light and temperature. Light is sensed by two major systems in plants: the blue light photoreceptor pigment, and phytochrome. We are studying phytochrome through a continued selection for and physiological characterization of mutants with altered pathways for the production of phytochrome and for its utilization. The blue light photoreceptor pigment regulates phototropism through which light direction is measured. For such a measurement, a gradient in light intensity must exist through the organism. Mutants and inhibitors are being used in corn, sunflowers, and soybeans to distinguish between a gradient produced via focusing and one produced via screening.

Thermotaxis in Dictyostelium discoideum is being used as a model system in an attempt to understand the mechanisms whereby an organism measures temperature or fluctuations in temperature. We can now demonstrate thermotaxis by the individual amoebae and, based on the physiological characterization of this response and on the response of several mutants, can conclude that slug thermotaxis is a manifestation of the thermotactic response of the component amoebae. Thus, we can safely ignore the organizational complexities of the multicellular slug and search for the "biothermometer" at or below the cellular level.

An understanding of photosensory transduction in plants is of importance since this environmental sensing preceeds and ultimately regulates photosynthetic capacity. Knowledge of thermosensing is of critical importance because of the major thermal limitations on plant productivity and the fact that heat is the ultimate product of any energy production and utilization.

107. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

IDENTIFICATION OF POSSIBLE TARGETS FOR GENETIC ENGINEERING IN PLANTS — $157,000
C.R. Somerville
MSU-DOE Plant Research Laboratory

The overall objective of this task is to develop genetic systems for the analysis and modification of specific physiological processes in plants. We are using this approach to investigate three separate areas of plant biology in which single gene variation may be a strong determinant of plant productivity.

A) Based on the premise that photorespiration is deleterious to plant productivity we are attempting to create useful or informative variation in the responsible enzyme by modifying the corresponding gene in vitro. We are also attempting to resolve several problems in our understanding of photorespiratory metabolism by the isolation of specific classes of mutants which have eluded discovery, and by characterization of a CO_2-concentrating mechanism by which some algae avoid photorespiration.

B) Disease is a major factor in yield of many crop species. Although genetic variation in disease resistance is available in most crop species, it is not known what "disease resistance" genes encode. We are exploring the possibility that novel immunological techniques (monoclonal antibodies) can be used to characterize the biochemical basis of the genetic differences between disease resistant and susceptible cultivars of soybeans. Identification of responsible factors would permit rational assessment of disease management strategies and novel approaches to genetic manipulation of the resistance genes.

C) Many species of economic importance are extremely chilling sensitive whereas related species are often quite tolerant of chilling temperatures. We are exploring the mechanisms involved in chilling resistance by the isolation and biochemical characterization of single gene mutations which dramatically alter the chilling sensitivity of a model plant (Arabidopsis thaliana).

108. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

DEVELOPMENTAL BIOLOGY OF NITROGEN-FIXING ALGAE — $157,000
C.P. Wolk
MSU-DOE Plant Research Laboratory

Certain cyanobacteria (blue-green algae) trap solar energy via photosynthesis and utilize the chemical reducing power to fix atmospheric nitrogen gas (N_2) to produce ammonia which then is utilized as the nitrogen source for cellular growth. The objective of this project is to understand the cellular differentiation processes which are required to allow N_2 fixation to proceed. The assimilation of N_2 and the incorporation of the reduced nitrogen into organic molecules occurs to specialize cells called heterocysts. These cells have been isolated and biochemically characterized. The following projects are currently being pursued in an effort to understand further the development of the specialized cells. Using recombinant-DNA technology we have constructed DNA clones designed to be capable of transfer to, and replication and selection in cyanobacterial cells, and to circumvent problems of genetic restriction. Such clones have been successfully transferred from Escherichia coli to, and replicated and selected in, unicellular as well as nitrogen-fixing filamentous cyanobacteria. With partial support from N.S.F., we are also constructing a physical-genetic map of the Anabaena genome using the cosmid cloning technique. Thus, we have made substantial progress toward developing tools for the genetic manipulation of photoautotrophic, nitrogen-fixing cyanobacteria. The significance of this work is that it opens the way to increased understanding of cellular differentiation and to construction of modified strains which would be particularly suitable for commercial application of biological solar energy conversion.

109. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

ENVIRONMENTAL CONTROL OF PLANT DEVELOPMENT AND ITS RELATION TO PLANT HORMONES — $141,000

Jan A.D. Zeevaart, MSU-DOE Plant Research Laboratory

Environmental factors such as daylength, temperature and soil moisture content have pronounced effects on plant growth and development. Plant growth substances are often involved as intermediaries between the perception of an environmental factor and the ultimate morphological expression. The objectives of this program are to characterize the hormones involved, how their synthesis and catabolism are regulated by the environment, as well as to understand their mode of action. The following projects are currently under investigation.

a) Regulation of stem and leaf growth in the long-day rosette plant spinach by gibberellins (GAs). The aim is to determine how long days control the synthesis of the biologically active GA_{20} and how this in turn causes stem elongation.

b) Regulation of stem growth and flowering in dwarfed mutants of Arabidopsis thaliana and tomato which are deficient in gibberellins. The aim is to determine the steps at which the GA biosynthetic pathway is blocked in the various mutants.

c) Regulation of abscisic acid (ABA) synthesis, catabolism and compartmentation in response to water stress. Although ABA controls the loss of water by closing stomata, the bulk ABA in a leaf often does not correlate with stomatal aperture. The distribution of ABA at the organ, cellular, and subcellular level is therefore being studied in Xanthium strumarium and Vicia faba (supported in part by the National Science Foundation).

110. OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37830

ENERGY AND NUTRIENT UTILIZATION EFFICIENCY IN INTENSIVE FOREST BIOMASS PRODUCTION: A STUDY OF SITE-SEEDLING INTERACTIONS — $200,000

S. B. McLaughlin, D. W. Johnson, N. T. Edwards, R. J. Luxmoore, R. F. Walker, Environmental Sciences Division

This study addresses plant physiological and soil-plant nutrient processes which are important considerations in the utilization of marginal land for silvicultural production of energy. The primary objective of this work is to provide physiological criteria for quantifying yield potential of biomass candidate species, to determine the role of soil-plant nutrient dynamics on productive potential of these species, and to provide important insights into the physiological basis of species' tolerance and adaptability to environmental stress. Four tree species, yellow poplar (Liriodendron tulipfera), loblolly pine (Pinus taeda), sweet gum (Liquidambar styriciflua) and black locust (Robinea pseudoacacia) are being studied in a 20 ha managed population. Treatments being examined include mycorrhizal preconditioning, variable rate and timing of nitrogen supply, and mulching. Plant physiological parameters being measured include whole tree allocation of energy between metabolic pools, plant water status, leaf photosynthetic rate, photosynthate allocation by foliage, and root and soil respiration rate. The kinetics of nitrogen mobilization, and movement through soil-plant nutrient cycles are being examined to quantify nutrient conservation mechanisms under varying rates of nutrient supply. These data will provide important insights into the interactions involved in seasonal allocation of carbon, water, and nutrients for representative biomass candidate species and aid in the selecting of criteria for enhancing biomass production potential.

111. SOLAR ENERGY RESEARCH INSTITUTE
Golden, Colorado 80401

BASIC PHOTOBIOLOGY RESEARCH $105,000
Paul F. Weaver, Stephen C.T. Lien, Michael Seibert
Photoconversion Research Branch

This work encompasses three areas of electron-transport studies associated with photobiological H_2 metabolism. Task 1 will determine the degree of integration among H_2 metabolism, N_2 fixation, photosynthesis, and the multiplicity of energy-conversion mechanisms present in photosynthetic bacteria, particularly Rhodopseudomonas and Rhodospirillum species. A broad spectrum of mutants is being generated with defective redox components operative in autotrophic or heterotrophic, anaerobic and aerobic, and light or dark growth modes. These defects are being characterized by pleiotrophic losses of other growth modes, spectral and enzymatic properties, and protein separation and quantitation techniques. The data will be used to amplify H_2 metabolism in organisms utilizing less energy intensive, ammonia-insensitive hydrogenase reaction rather than the nitrogenase pathway. Task 2 has led to the first purification of hydrogenase from a eucaryotic source (Chlamydomonas reinhardtii). Characterization of the physical, chemical and catalytic properties of the algal enzyme will be continued. The results obtained will be compared with those from procaryotic hydrogenases. In addition, special emphasis will be placed on developing a mechanistic model for the formation of a catalytically active complex between hydrogenase and various electron-mediators. Task 3 will investigate the water-splitting apparatus of oxygenic photosynthetic systems using biochemical techniques, electron microscopy and physical methods such as fluorescence induction, flash spectroscopy and direct, quantitative detection of O_2 yields. Chloroplasts and four types of O_2-evolving PS II preparations are being characterized by polypeptide-composition analysis, Mehler-reaction activities, EPR-detectable PS I, low-temperature fluorescence, steady-state and flash yields of O_2, and stability of O_2-production activity over extended time periods.

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and Summary Number

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Department of Energy
Washington, DC 20545

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