DOE/ER-0147/2

Annual Report and Summaries of FY 1984 Activities Supported by the Division of Biological Energy Research

October 1984

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

INTRODUCTION

Fiscal Year 1984 marked the fifth anniversary of the Division of Biological Energy Research (BER). The Division's program fulfills the need in the Department of Energy for a fundamental biological research activity designed to generate the information essential to development of biosystems related to energy matters. The research areas that the BER program supports are primarily in the bioconversion realm with primary emphasis on basic mechanistic studies.

In the five years of its existence, the BER program has been instrumental in enhancing the research activities in a number of specific areas of importance to the program. Most notable of these are: i) basic studies on methanogenesis, including the physiology, genetics and biochemistry of methanogens and of organisms that tie into the anaerobic digestion processes; ii) mechanisms of microbial breakdown of cellulose and other members of the lignocellulosic complex; iii) the plant cell wall, its structure and function; and iv) the biochemical changes associated with the responses of plants and microorganisms to physical stresses, temperature, moisture, pH and salinity.

Other major areas of emphasis in the botanical and microbiological areas, as will be noted on reading this report, include:

1) photosynthesis, the primary conversion of solar to chemical energy as carried on by green plants and bacteria.

2) the regulatory processes in growth and development of plants that determine ultimate productivity.

3) the metabolic regulatory mechanisms in plants that determine how the photosynthetic assimilated resources are utilized.

4) bioconversions by microorganisms involving fermentation mechanisms.

5) anaerobic and/or thermophilic microbial conversion mechanisms leading to materials useful as fuels or industrial chemicals.

6) the genetic and cellular processes that will serve as the foundation for genetic manipulation of plants and microorganisms in the future.

7) the basis of organismal interactions in symbiosis, host-pathogen and other interrelations with emphasis on recognition as well as information and metabolic transfer mechanisms.

The BER program is constantly evolving as the status of research problems changes, as new techniques and approaches to problems are developed, and in response to the changing perceptions of national research needs and opportunities.

Among the facets of the BER program that are considered of significance are the various efforts to improve or modify research techniques and tools for research in the plant and microbial sciences. This includes, where possible and appropriate, the employment of relatively sophisticated instrumentation

to attack problems. In areas where the pool of available researchers is small, opportunities are provided for training. For example, BER has been providing support for introducing both undergraduate and graduate students to the intriguing world of the microbiology of non-medical anaerobic organisms, including microbial ecology, through the partial support of a summer research and training program at the Marine Biological Laboratory at Woods Hole, Massachusetts.

As has been the case since the beginning of the BER program, there has been an effort made to support, wholly or partially, workshops, symposia, and conferences on topics that are appropriate to the program. It is felt that such scientific meetings are critical for exchange of results and ideas, but, in addition, to provide forums to pursue topics in depth and to stimulate research and sometimes provide the setting for the generation of new approaches as well as for encouraging collaborations. The activities supported in FY 1984 included:

A) American Society for Microbiology - partial funding for a conference on "Genetics and Molecular Biology of Industrial Microorganisms" September 30-October 3, 1984, at Indiana University, Bloomington, Indiana.

B) University of California, Davis - partial funding of conference on "Molecular Basis of Plant Disease" August 19-23, 1984, at the University of California, Davis.

C) University of California, Davis - partial funding of conference on "Bicarbonate Utilization by Photosynthetic Organisms" August 18-22, 1984, at Asilomar Conference Center, Pacific Grove, California.

D) University of California, Riverside - partial funding for 7th Annual Symposium in Plant Physiology entitled "Structure, Function and Biosynthesis of Plant Cell Walls", January 12-14, 1984, University of California, Riverside.

E) University of Illinois, Urbana - partial funding of conference "Plasmids in Bacteria", May 14-18, 1984, University of Illinois, Urbana.

F) University of Minnesota - partial funding for "Implications of Molecular Genetics In Plant Breeding Symposium", June 4-6, 1984, University of Minnesota, St. Paul.

G) University of Tennessee - partial funding of "Plant Tissue Culture Symposium", September 9-13, 1984, University of Tennessee, Knoxville.

H) Cold Spring Harbor Laboratory - partial funding of a meeting on "Molecular Biology of the Photosynthetic Apparatus", May 9-13, 1984, Cold Spring Harbor, New York.

One particularly interesting and exciting workshop convened by BER was held 20-21 May 1984 to discuss the importance of complex carbohydrates in biological research and industrial processes. The principal focus was on analytical techniques for determining sequence and conformation of complex carbohydrates which are tremendously more difficult than analyses of either proteins or nucleic acids. The participants expressed the view that complex carbohydrates are being appreciated to have many more significant roles in biological function and in industry, (this recognition is expected to grow), and that structural information is now achievable with some very powerful and sophisticated, but expensive, techniques. The conclusion was that within the next few years, a small network of regional research and service centers for performing these analyses should be established. Such structural information should impinge on an extraordinarily broad array of disciplines and accordingly should stimulate the interest of many investigators in the scientific community.

Some representative contributions from investigators supported by BER funding may be highlighted:

a) Significant new insights about how gas exchange occurs in plants adapted to flooded conditions have been attained using deep water rice as a model. Leaf surface contours were shown to be critical for the diffusive transport of oxygen to roots while other reactions involving ethylene stimulated growth were demonstrated to be integrated into an adaptive pattern for adjusting to flooding stress.

b) The role of phosphorylated proteins in photosynthesis regulation is now much more evident as a result of some investigations on the control of the activity balance of the two photosystems in photosynthesis. The light harvesting chlorophyll a/b-protein complex appears to be phosphorylated by a kinase that is activated and deactivated depending on the oxidation-reduction state of the plastoquinone pool. This mechanism thus affords a more balanced distribution of excitation energy between Photosystems I and II.

c) During the course of the development of the microbial activity within an anaerobic digestor where methane gas is produced, it has been observed that the microbial populations change appreciably before stabilization is achieved. These dynamics have now been documented. The development of highly specific immunologic agents produced against various species of methanogenic bacteria has made identifications easier, faster and more reliable.

d) Working collaboratively with investigators in Belgium, one BER investigator has succeeded in the transfer of DNA coding for the synthesis of one of the major photosynthetic proteins (small subunit of ribulose 1,5 bisphosphate carboxylase) from one plant to another species of plant and

having the message expressed. What is intriguing is that the DNA message transferred also contains coding for a light receptor that governs the development of chloroplast proteins. This finding affords new opportunities for studying developmental reactions that are controlled by light.

During FY 1984 the funds available to the BER program were distributed as follows:

	# of Projects	FY 84 Funding (in thousands of $)	% of Total Funds
University Contracts & Grants	83	5924	56
Michigan State University Plant Research Laboratory	11	1750	17
National Laboratories Brookhaven National Lab Lawrence Berkeley Lab Oak Ridge National Lab	12	2191	21
Solar Energy Research Institute	1	105	1
Other Research Institutions (federal, state, industrial, non-profit)	8	454	4
Conferences and miscellaneous	10	96	1
TOTAL	125	10520	

In FY 1984, a total of 86 new research proposals were received. Of these proposals, 15 were funded over and above the on-going projects. It must be pointed out that numerous fine proposals, judged to be worthy of funding in the review process, could not be accommodated into the program because of lack of funds. On-going projects are peer reviewed, usually on a three-year rotation and in competition with new proposals.

The proposal review process, site visit reviews, and other review activities in FY 1984 for the BER program involved over 550 scientists in this country and abroad. These individuals represented a broad cross section of disciplines as well as institutions. Without these many contributions by the research community, the BER program could not achieve and maintain the quality program that exists. For the many, many hours of effort that went into these reviews, we thank those involved most sincerely.

Any questions pertaining to the technical aspects of any research project should be addressed to the principal investigator.

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 — $43,000

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

The goal of this research is to learn the mechanism by which added methionine turns off the expression of one soybean storage protein subunit gene. When immature soybean cotyledons are grown in organ culture, the addition of methionine to the culture medium prevents the appearance of the ß subunit of the 7S storage protein. Cotyledon culture experiments have led to the following conclusions. First, the effect of methionine persists for at least three weeks. Second, the methionine effect is reversible since the ß-subunit increased normally after removal of methionine. Third, methionine does not promote the degradation of the ß-subunit. When methionine is present in the cotyledon culture medium, no translatable ß-subunit mRNA is detected [Creason, Holowach, Thompson and Madison, Biochem. Biophys. Res. Commun. 117, 658-662 (1983)] indicating that methionine inhibits transcription or the processing of the initial transcript. Effects of methionine on translation or on the turnover of ß-subunit mRNA appear to be minor. Hence the next step is to learn whether methionine inhibits transcription or a post-transcriptional modification of the initial transcript.

2. BATTELLE-C.F. KETTERING RESEARCH LABORATORY
Yellow Springs, Ohio 45387

THE BASIS FOR THE COMPETITIVENESS OF RHIZOBIUM JAPONICUM IN THE NODULATION OF SOYBEAN — $78,000

Wolfgang D. Bauer and William R. Evans

The objective of this research is to identify characteristics of the nitrogen-fixing bacterial symbiont, Rhizobium japonicum, which are crucial to its ability to compete effectively as a seed inoculant against indigenous rhizobia in nodulating the host plant, soybean. The ability of the bacterium to attach to the host root is likely to be an important characteristic with regard to both colonization of the root surface and the initiation of infections. The variables affecting root attachment capabilities are being determined. Attachment appears to be primarily mediated by bacterial pili (fimbriae) rather than binding of host root lectin. Antibodies against purified pili have been obtained and are being used to screen for mutants with altered attachment capabilities. Such mutants will be used to rigorously test the role of pili and attachment in competition. Quantitative nodulation assays have revealed that various strains of R. japonicum have markedly different efficiencies of nodulation, and that such efficiency depends on culture age. Various results indicate that nodulation efficiency, i.e. the number of bacteria required to generate a given number of nodules, may be related to synthesis of exopolysaccharide capable of binding soybean lectin. Transposon induced mutants of the bacteria are being screened for isolates incapable of synthesizing such polysaccharide in order to test this notion and its relevance to competition between strains. All Bradyrhizobium isolates tested thus far are able to remain viable and infective upon prolonged storage in "pure" water. We are seeking to identify the nutrients that support such viability to assess their use in inoculum preparation.

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

CARBON METABOLISM IN LEGUME NODULES $42,000
Thomas A. LaRue

Symbiotic nitrogen fixation in legume nodules consumes more photosynthate than can be accounted for by the known energy requirements for nitrogenase. The object of our research is to determine how the legume nodule metabolizes carbohydrate to provide energy and reductant for nitrogen fixation. Because the plant cells within a nodule contain very little free oxygen, we are investigating the possible role of anaerobic pathways of carbohydrate metabolism.

We have established that plant enzymes characteristic of anaerobic metabolism do occur in nodules, and that the nodule contains low concentrations of alcohol and acetaldehyde. Both these carbon compounds can be used by the bacteroid to provide energy and reductant for nitrogen fixation.

Succinic acid is a major constituent of nodules, and is a substrate for the bacteroids. We are now determining if it synthesized by an aerobic or anaerobic pathway. We are studying the mitochondria isolated from nodules to learn how they function at the low concentration of oxygen. The oxidation and reduction of flavoproteins and pyridine nucleotides in intact mitochondria and bacteroids is monitored in situ by changes in their fluorescence.

4. BRANDEIS UNIVERSITY
Waltham, Massachusetts 02254

EFFECT OF LIGHT ON RESPIRATION AND DEVELOPMENT OF PHOTOSYNTHETIC CELLS $55,215
Martin Gibbs
Institute of Photobiology

One goal of this project is to determine the pathways of fermentation in algae with emphasis on organisms adapted to a hydrogen metabolism. The pattern of starch fermentation (mole product per mole glucose in starch consumed) in light vs dark is: ethanol 0.1, 0.9; acetate 0.0, 1.; formate 0.9, 2.1; CO_2 2.5,0: and H_2 6.9, 0.4. In light, DCMU decreases CO_2 and H_2, increases formate while having little effect on the other products or the rate of starch breakdown. Both the uncoupler, FCCP and DBMIB, an inhibitor of plastoquinone, doubles the rate of starch breakdown. The former increases acetate and H_2 photoevolution but not ethanol production while DBMIB increases ethanol production to 1.2 and reduces H_2 photoevolution. Alcohol and CoA dependent acetaldehyde dehydrogenases are found in cellular extracts. Light redirects C and H flow at the level of two carbon metabolism. In light, acetyl CoA is used anaerobically in biosynthesis: glycolytic NADH, the result of triose-P oxidation is channeled to plastoquinone and H_2 at the expense of ethanol and this route of chloroplast respiration is reversed by DBMIB removing the rate limiting step of pyridine nucleotide turnover. Isolated chloroplasts couple H_2 uptake to CO_2 assimilation (photoreduction) in the presence of DCMU at rates one-third of the parent cell. Photoreduction in contrast to photosynthesis is inhibited by rotenone suggesting that electrons from H_2, donated at the level of plastoquinone are required for photoreduction to occur. Current studies include enzyme profiles in order to determine if the fermentation sequence is chloroplast-localized and the pathways of H_2 in photoreduction and the oxyhydrogen reaction are also chloroplastically localized.

5. UNIVERSITY OF CALIFORNIA
Riverside, California 92521

CATALYTIC MECHANISM OF HYDROGENASE FROM AEROBIC N_2-FIXING MICROORGANISMS — $45,000

Daniel J. Arp
Department of Biochemistry

This project deals with elucidation of the catalytic mechanism of hydrogenase from aerobic N_2-fixing organisms. Several properties of these hydrogenases make them ideal to function in an environment in which all of the available substrate is generated in situ, e.g., a very low rate of the back reaction, hydrogen evolution, and a low K_m for H_2. We are particularly interested in the enzyme from Rhizobium-induced root nodules because of the potential for increased legume productivity in symbioses expressing this enzyme. The specific goals of the proposed research are fourfold: to 1. determine the mechanism of the isotope exchange reaction of Rhizobial hydrogenase; 2. investigate Rhizobial hydrogenase catalyzed para-H_2 to ortho-H_2 conversion; 3. purify and characterize component 559-H_2 from soybean bacteroids and investigate its ability to couple to and form a complex with Rhizobial hydrogenase; and 4. purify and characterize the hydrogenase of Azotobacter vinelandii. The proposed research will provide insight into the mechanism of hydrogen uptake by Rhizobial and Azotobacter hydrogenase which, in turn, will lead to a better understanding of H_2 cycling in these organisms. This information is important to genetic engineering of new strains and may lead to improved techniques for selecting the best strains of Rhizobium for inoculation. The results may lead to new assays for hydrogenase activity and may provide new information about the structural organization of the bacteroid membrane. Our knowledge and understanding of hydrogenases in particular, and electron transferring proteins in general, should be significantly enhanced by these studies.

6. UNIVERSITY OF CALIFORNIA
Los Angeles, California 90024

ENERGY CAPTURE AND USE IN PLANTS AND BACTERIA — $87,000

Paul D. Boyer
Department of Chemistry and Biochemistry and
Molecular Biology Institute

This study focuses on what is probably, quantitatively, the most important biological energy transduction in nature, namely the formation of adenosine triphosphate (ATP) by plants and bacteria. The formation of ATP by the membrane-bound ATP synthase complex is coupled to energy provided by light or by oxidation of metabolites. Our laboratory has had a leading role in developing an energy-linked binding change mechanism that appears to offer the best present model for how the ATP synthase functions.

Current hypotheses that are being explored are: 1) that energy serves to promote binding of ADP and P_i and release of ATP by the synthase, 2) that multiple catalytic subunits on the enyzyme participate in cooperative alternation, and 3) that during catalysis there is a rotational change in position of the catalytic subunits in relation to the noncatalytic core. In addition, we have proposed a new hypothesis for how energy transmission from electron-transport enzymes to the ATP synthase might occur.

We are undertaking studies with the chloroplast system on: The heterogeneity of conformation of the catalytic subunits; the nature of the Ca^{2+} as contrasted to Mg^{2+} activation; the spatial locations and reactivity of subunits during catalysis; which subunits are derivatized by inhibitors and substrate analogs; whether two or three catalytic sites operate in alternation; and finally, subunit positional interchanges by assessment of derivatization with fluorescent and related probes. With the E. coli ATP synthase we are studying the catalytic properties of mutant forms of the enzyme, to ascertain the function of various subunits by dissociation and reconstitution, and to develop methods for specific cross-links between subunits as tests for possible subunit spatial relationships during catalysis.

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 and tonoplast 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 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 will use 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 mechanisms by which certain polysaccharide fragments trigger the synthesis by the plant of defensive compounds against fungal attack.

8. UNIVERSITY OF CALIFORNIA
Berkeley, CA 94720

THE REGULATION OF ENZYME SECRETION IN PLANT CELLS — $85,000

Russell L. Jones
Department of Botany

The aim of this project is to achieve an understanding of the events in cereal aleurone which are controlled by Ca^{2+} and to determine how this ion is metabolized. Our investigation of the control of hydrolytic enzyme synthesis and secretion focuses on α-amylase. α-Amylase is present in barley as four distinct isoenzymes, and we have shown that the de novo synthesis of two of these isoenzymes is controlled in part by Ca^{2+} and the plant hormone gibberellic acid (GA). We have prepared a cDNA clone from poly(A^+)RNA isolated from aleurone layers treated with GA plus Ca^{2+} for 12h. We have characterized this clone and shown that it is complementary to RNA which codes for one of the α-amylase polypeptides whose synthesis is controlled by GA plus Ca^{2+}. We will use this cDNA clone to investigate the role of GA and Ca^{2+} in α-amylase gene expression. A project is also underway to prepare monoclonal antibodies to all four α-amylase isoenzymes. Although polyclonal antibodies have been raised to α-amylase, they cannot distinguish between closely related isoenzymes. Monoclonal antibodies will also be used to study the synthesis of barley α-amylase. We will isolate mRNA from aleurone layers incubated in the presence or absence of GA or Ca^{2+} and translate these mRNAs in vitro. The translation products will be identified using monoclonal antibodies. These experiments should pinpoint the site(s) where GA and Ca^{2+} control the de novo synthesis of α-amylase. Experiments are also in progress to study the metabolism of Ca^{2+}. We have shown that aleurone layers contain appreciable levels of Ca^{2+}(0.08% of aleurone dry weight). Eighty percent of this Ca^{2+} is bound to Ca^{2+}-binding ligands, but 20% is readily exchangeable. We are studying the metabolism of this pool with emphasis on its intracellular localization. The control of the transport of Ca^{2+} into and out of this pool is also under study.

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

BIOENERGETICS OF SALT TOLERANCE — $142,000

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

We are investigating the bioenergetics of salt tolerance with respect to ion transport across membranes, redirection of metabolic pathways for increased synthesis of osmoregulatory compounds, replacement of some salt sensitive components with salt resistant ones, and the signals which turn these processes on and off during salt stress. We have developed and adapted methods to study these phenomena mainly in two systems, each suited to answer specific questions. In the 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. Particular attention is paid to sodium transport via a sodium/proton antiporter in the cyanobacterium, as related to adaptation to growth at low and high salinities. In the Synechococcus we explore also the cytoplasmic events which occur during short-term adaptation to salt. In the thylakoids of this organism we are attempting to describe features of the photosynthetic apparatus which are changed by salt stress. Results with these two systems so far have yielded interesting and promising results. Using this multi-organism approach, we intend to develop the conceptual basis for a refined description of the physiology of cellular and membrane adaptation to salt.

10. UNIVERSITY OF CALIFORNIA
Berkeley, CA 94720

THE RELATION OF ALLOZYME HETEROZYGOSITY TO GROWTH UNDER INBREEDING AND OUTCROSSING IN PINUS ATTENUATA — $56,170

William J. Libby
Department of Forestry & Resource Management

The proposed study will investigate the significance of heterozygosity to components of fitness. It will study the relation of heterozygosity to growth in 10-year-old trees growing in plantations. Allozyme heterozygosity of selfed, outbred, and naturally pollinated trees will be related to their vegetative and reproductive rate of development. About 800 trees will be studied; their genotypes will be characterized for about 25 polymorphic enzyme loci. The project will test the hypothesis that heterozygosity promotes growth, fecundity, and developmental stability.

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

METHANOGENESIS FROM ACETATE, A KEY INTERMEDIATE IN NATURE — $74,000

Robert A. Mah
Division of Environmental & Occupational Health Sciences
School of Public Health

The objective of this project is to study the H_2-oxidizing and methylotrophic methane-producing bacteria, especially aceticlastic methanogens from various naturally occurring anaerobic habitats. Enrichments for both H_2-oxidizing and methylotrophic methanogens were initiated by inoculating samples from widely varying environmental conditions such as sediments from the sulfuretums at Wadi el Natrun, Egypt and a solar saltern in Chula Vista, Ca. Substrates used in these enrichments included H_2/CO_2, methanol, acetate, and trimethylamine. The pH of the media corresponded to that of the inocula. For the Wadi el Natrun samples, the pH ranged from 7.3 to 9.5 and the salt concentration from < 1.0 to > 27%. The pH of samples from the saltern was ca. 7.3, and the salinity was near saturation. Vessels containing H_2/CO_2 as substrate produced methane more readily and more often than those with methylotrophic substrates. Methanogenic cocci, rods, and filamentous cells were presumptively identified by fluorescence at ca. 340 nm in both H_2/CO_2 and methylotrophic media. Isolation of these methanogens is now underway, and at least one of the Egypt samples will yield an axenic culture soon. Another strain of halophilic methanogen from the solar saltern is currently in co-culture and will also be obtained in axenic culture. Because of the unusual environmental conditions of the samples (pH, salinity), we expect to describe some new species of methanogens.

12. UNIVERSITY OF CALIFORNIA, SANTA CRUZ
Santa Cruz, California 95064

TONOPLAST TRANSPORT AND SALT-TOLERANCE IN PLANTS — $60,000

Lincoln Taiz
Biology Department

Salinity (excess sodium chloride) is the most widespread limitation on crop productivity in the world today, yet many wild halophytic species, such as Salicornia and Distichlis, thrive under saline conditions. One of the distinguishing features of halophytes is their ability to take up sodium chloride into vacuoles, thus avoiding toxic effects on the cytoplasm. We are hypothesizing that the tonoplasts of halophytes may be specialized for transporting sodium, and that this property may reside in the tonoplast ATPase or in a specific membrane carrier protein. Our goal is to study the transport properties of purified isolated tonoplast vesicles from halophytic species, such as the grass, Distichlis, and to compare these properties with those of a nonhalophytic crop plant, Zea mays. Our studies indicate that the primary transport mechanism on the tonoplasts of corn is an electrogenic H^+-ATPase. Chloride ions are taken up by corn tonoplast vesicles in response to an inside-positive electrical potential generated by the proton pump. Detailed characterization of the transport properties of corn tonoplast vesicles will establish a basis from which comparisons with halophytes can be made. We are also purifying the H^+-ATPase for a detailed analysis of its structure and enzymatic properties. Preliminary results suggest that it is a multi-subunit enzyme having two major subunits with molecular weights of 70,000 and 60,000 daltons. Antibodies to these two subunits are being prepared, which will aid in the further purification of the enzyme. It is hoped that understanding the mechanism of ion transport across halophyte tonoplasts may one day allow salt tolerance to be introduced into crop plants by recombinant DNA techniques.

13. THE UNIVERSITY OF COLORADO
Boulder, Colorado 80309

STUDIES ON OLIGOSACCHARINS, CARBOHYDRATES POSSESSING BIOLOGICAL REGULATORY ACTIVITIES — $116,690

Alan G. Darvill and Peter Albersheim
Department of Chemistry

This project entails identifying, isolating, and characterizing naturally occurring carbohydrates that possess biological regulatory activities. Such carbohydrates are called oligosaccharins. We observe that oligosaccharides, when released from the confines of the plant cell wall, regulate a variety of physiological functions in plants. More specifically, we are studying the purification and identification of (i) an oligosaccharin of plant cell wall origin that elicits phytoalexin (antibiotic) accumulation in plant tissues and that acts synergistically with a hepta-α-glucoside phytoalexin elicitor of fungal mycelial wall origin; (ii) an oligosaccharin, and enzymes involved in its metabolism, that inhibits 2,4-D-promoted growth in pea epicotyls and that may be involved in feedback control of auxin-stimulated plant growth; (iii) an oligosaccharin, hypothesized to be a trigger of the hypersensitive resistance response, that has been isolated from both monocot and dicot cell walls and that inhibits the incorporation of [^{14}C]leucine into proteins in both monocot and dicot cells; (iv) an oligosaccharin that inhibits flowering in duckweed (Lemna gibba G3) and tobacco cultures; (v) oligosaccharins that induce flowers, roots and vegetative bud primordia in isolated tobacco epidermal strips; and (vi) polysaccharides found in maple sap that indicate the presence of oligosaccharin-type molecules in the major conducting fluids of plants, molecules that, themselves, may have regulatory properties within plant tissues.

14. THE UNIVERSITY OF COLORADO
Boulder, Colorado 80309

STRUCTURAL STUDIES OF COMPLEX CARBOHYDRATES AND PLANT CELL WALLS — $145,850

Michael McNeil, Alan G. Darvill and Peter Albersheim
Department of Chemistry

The cell wall is not only a determinant of structure and morphology of plants, but also a source of carbohydrates with biological regulatory functions and an important organelle in host-pathogen interactions. This grant funds our efforts to isolate and structurally characterize the complex carbohydrates that constitute about 90% of the walls of growing plant cells. Our research includes developing methods to purify and structurally characterize complex carbohydrates. Sources of the polysaccharides for this study include cell walls isolated from dicot, monocot and gymnosperm suspension-cultured cells and the extracellular polysaccharides secreted by these cells. The current foci of our research are a xyloglucan hemicellulose and two pectic polysaccharides: rhamnogalacturonans I and II. We have shown that these polysaccharides possess unexpectedly complex structures. RG-II, for example, contains at least 12 different glycosyl residues that include apiosyl, 2-O-methyl fucosyl, 2-O-methyl xylosyl, aceric acid (3-C-carboxy-5-deoxy-L-xylosyl) and KDO (2-keto-3-deoxy-manno-octonic acid). The structures of these polysaccharides are further complicated by the occurrence of O-acetyl esters on some of the glycosyl residues. We are developing methods to structurally characterize complex carbohydrates that include techniques to identify the exact locations of such O-acetyl and other base-labile substituents. We are also developing a procedure that employs lithium/ethylenediamine to degrade uronosyl residues in such a way that the neutral glycosyl residues of the polysaccharides remain intact. This method is particularly applicable to furthering the structural characterization RG-I and RG-II.

15. UNIVERSITY OF COLORADO
Boulder, CO 80309

THE GENETICS OF PATHOGENICITY OF THE PYRICULARIA — $87,570
Barbara Valent and Forrest G. Chumley
Department of Chemistry

The Pyricularia are important fungal pathogens of cultivated and wild grasses. Individual field isolates of Pyricularia infect one or a few different species of grasses. Rice-infecting isolates exist as many races, defined by the variety of rice cultivars they can attack. A goal of this project is to conduct a genetic analysis of the differences in host specificity exhibited by various Pyricularia isolates. We have observed that field isolates of Pyricularia vary in fertility according to host range. Isolates pathogenic to goosegrass (Eleusine indica) have moderate fertility while those pathogenic to rice have very low fertility. We have increased the fertility of isolates that infect goosegrass by a systematic program of inbreeding and selection. These fertile laboratory strains are now being used in a program to develop fertile rice-infecting strains. Our program for improving fertility in Pyricularia field isolates has already led to the identification of Pyricularia genes that play a role in determining host specificity. For example, analysis of sexual crosses conducted between an isolate that infects goosegrass but not weeping lovegrass (Eragrostis curvula) and another isolate that infects weeping lovegrass but not goosegrass indicates that single gene differences control pathogenicity to each host species. In contrast, sexual crosses between rice-infecting strains and goosegrass-infecting strains reveal differences at several loci that determine the ability to infect rice. Among the rice-infecting progeny, we have detected a single gene that controls the ability to infect a rice cultivar that carries the resistance gene, Pi-ta. Molecular genetic analysis of these pathogen genes and others will lead us to an understanding of mechanisms determining host specificity of an important plant pathogen.

16. CORNELL UNIVERSITY
Ithaca, New York 14853

STUDIES OF PHOTOSYNTHETIC ENERGY CONVERSION — $35,927
Andre T. Jagendorf
Section of Plant Biology

Several species of unicellular marine diatoms are known to display considerable physiological plasticity with respect to their photosynthetic antenna size and composition in response to variations in growth irradiance. We propose to investigate the effects of ambient light intensity on the two major functions of antenna pigment-protein complexes: (1) efficient light harvesting and transfer of excitation energy to the photochemical reaction centers, and (2) control of the distribution of excitation energy between the two types of reaction centers. The light harvesting pigment-protein complexes of the diatoms Phaeodactylum tricornutum and Skeletonema costatum are being isolated by mild detergent treatment. The composition of these complexes is being analyzed with emphasis on the accessory pigments chlorophyll c_1 and c_2, and the carotenoids fucoxanthin, diadinoxanthin, and diatoxanthin. The relative antenna size of and efficiency of energy transfer to PSI and PSII are being estimated from the kinetics of P700 photobleaching and fluorescence induction, respectively. The kinetics of fluorescence induction and the reduction of the primary quinone acceptor of PSII (ΔA_{320}) are being measured simultaneously to assess the heterogeneity of PSII reaction centers. The control of excitation energy distribution between PSI and PSII is being investigated by observing slow changes in fluorescence yield and rate of oxygen evolution (State I - State II transitions) as a function of excitation wavelength. Through analysis of the effects of enhancement, the action spectra of PSI and PSII are being individually determined. In combination with measurements of pigment and reaction center concentrations, this allows the calculation of the number of each type of pigment molecule participating in PSI or PSII light harvesting. The influence of growth irradiance on chloroplast ultrastructure is also being investigated.

17. CORNELL UNIVERSITY
Ithaca, New York 14853

EFFECTS OF FREEZING AND COLD ACCLIMATION ON THE PLASMA MEMBRANE OF ISOLATED CEREAL PROTOPLASTS — $63,100

Peter L. Steponkus
Department of Agronomy

The ultimate goal of this project is to develop a mechanistic understanding of cold acclimation and freezing injury to provide a basis for genetic engineering and traditional breeding studies for the improvement of plant cold hardiness and to provide for the formulation of effective protocol for the cryopreservation of plant germplasm. Cryomicroscopic observations reveal that destabilization of the plasma membrane can occur at various times during a freeze-thaw cycle and may be manifested as any one of several symptoms. In non-acclimated protoplasts, the predominant form of injury is expansion-induced lysis due to irreversible endocytotic vesiculation of the plasma membrane. Current studies of the mechanical properties and ultrastructural characteristics of the plasma membrane are directed to the formulation of a quantitative theory to explain membrane behavior during freeze-induced osmotic contraction/expansion and the influence of cold acclimation. A second form of injury is due to the loss of osmotic responsiveness following cooling and is associated with dehydration-induced lamellar to hexagonal$_{II}$ phase transitions in the plasma membrane. Current studies are directed to the influence of cold acclimation and carbohydrates on the incidence of these phase transitions. Both non-acclimated and acclimated protoplasts are subject to intracellular ice formation when cooled rapidly because mechanical failure of the plasma membrane results in seeding of the cytoplasm by extracellular ice. During rapid cooling, plasma membrane failure is associated with the generation of electrical fields during the freezing of the suspending medium. Current studies are directed to measurement of the spatial and temporal characteristics of the electrical field and the electrical properties of the plasma membrane. Isolation of purified plasma membrane vesicles has been completed. Compositional analyses are in progress.

18. CORNELL UNIVERSITY
Ithaca, New York 14853

THE IMPORTANCE OF PHYTOALEXIN TOLERANCE AND DETOXIFICATION FOR PATHOGENICITY. — $59,297

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

The ability of plants to produce antimicrobial compounds (phytoalexins) in response to infection is believed to play an important role in preventing many microorganisms from causing disease. However, phytoalexin-producing plants are nonetheless susceptible to some diseases. Our research has demonstrated that one means by which successful pathogens overcome this potential resistance mechanism is by detoxifying their hosts' phytoalexins. The fungus Nectria haematococca can cause a root and stem rot of pea (Pisum sativum) even though the infected tissue accumulates high concentrations of the phytoalexin pisatin. Virulent isolates of this fungus detoxify pisatin by a demethylation reaction. In progeny of crosses between appropriate fungal isolates, pisatin demethylation segregates as an essential trait for virulence on this host. We have recently found that N. haematococca possesses at least 4 independently acting genes for pisatin demethylation. Two of these genes confer only low rates of demethylation, and do not enhance virulence toward pea. We are currently working to determine the relationship between these genes and the enzyme pisatin demethylase. The enzyme activity in one highly virulent, rapidly demethylating isolate of the fungus has been characterized as a substrate-inducible microsomal cytochrome P-450 monooxygenase. The two required components, NADPH-cytochrome c reductase and cytochrome P-450, have been solubilized and resolved chromatographically. Further purification is underway. Data on the substrate specificity and inhibitor sensitivity of this enzyme are also being collected. Comparison of the physical and catalytic properties of this enzyme with the properties of pisatin demethylase preparations from other isolates of known genotype will help test the hypothesis that N. haematococca possesses multiple structural genes for pisatin detoxifying enzymes.

19. CORNELL UNIVERSITY
Ithaca, New York 14853

STUDIES OF THE GENETIC REGULATION OF THE THERMOMONOSPORA CELLULASE COMPLEX — $60,277

David B. Wilson
Department of Biochemistry, Molecular and Cell Biology

The goal of this project is to construct recombinant organisms which overproduce the most active cellulases produced by the thermophilic bacterium, Thermomonospora YX. Enzymatic screening of E. coli cells transformed by two different Thermomonospora gene banks has identified two genes which produce proteins with CMCase activity. Neither of these genes codes for a major Thermomonospora cellulase. E. coli clone banks will be screened using an antiserum against Thermomonospora culture supernatant that reacts with the major cellulases. Any clones which are positive in this screening will be tested using the specific antisera prepared against each purified cellulase that has been made. If this method fails, then gram positive organisms will be used to construct a Thermomonospora gene bank, which will be screened for the ability to make cellulase and the ability to make proteins which react with the antibody. The originally isolated cellulase gene is also being subcloned to construct E. coli strains which overproduce this enyme. Once the best method for doing this is found, it will be used to construct strains which overproduce any major cellulase genes which are isolated. In addition to constructing strains which overproduce cellulases, attempts will be made to construct strains which can grow on cellulose. If these are constructed, they will be used to try to isolate mutant cellulases which are resistant to inhibition by cellobiose by direct selection of mutagenized cells on plates containing cellulose and cellobiose.

20. CORNELL UNIVERSITY
Ithaca, New York 14853

MICROBIAL ECOLOGY OF THERMOPHILIC ANAEROBIC DIGESTION — $68,083

Stephen H. Zinder
Department of Microbiology

The objective of this project is to provide an integrated understanding of the ecology of the microbial populations in a thermophilic (58°C) laboratory-scale digestor being fed a lignocellulosic waste. Among the methods being used to study these organisms are: 1) viable counts and culture studies using habitat and niche simulating media; 2) direct microscopic observation of populations using phase-contrast, epifluorescence, and electron microscopy; and 3) ^{14}C-radiotracer methods to study carbon flow to methane and physiological ecology of the digestor populations. Results obtained thus far include: 1) the apparent displacement of Methanosarcina by a previously undescribed thermophilic Methanothrix as the dominant aceticlastic methanogen in the digestor. A culture of this Methanothrix is now isolated and is currently being characterized; 2) the co-isolation of a two-membered coculture which converts acetate to methane using interspecies hydrogen transfer; 3) the demonstration that the overall temperature responses of the digestor are greatly dependent on the temperature responses of the dominant aceticlastic methanogen; 4) the demonstration that when Methanothrix was numerous in the digestor that methanogenesis from acetate was the process most easily saturated; and 5) the development of a method to enumerate autofluorescent methanogens in the digestor sludge. Current research centers on studying carbon flow to methane using an HPLC system to fractionate ^{14}C-label, and studies on improving digestor startup by adding specific methanogenic cultures.

21. DESERT RESEARCH INSTITUTE, UNIVERSITY OF NEVADA
P.O. Box 60220, Reno, Nevada 89506

GAS EXCHANGE CHARACTERISTICS OF LEAVES AND THE BIOCHEMICAL REACTIONS LIMITING PHOTOSYNTHESIS — $42,100

Thomas D. Sharkey
Biological Sciences Center

The objective of this research is to characterize the outward manifestations of various limitations on the instantaneous rate of photosynthesis in intact leaves. A specially constructed high speed freeze clamp-gas exchange chamber will be used to take samples of leaves whose gas exchange characteristics have been determined. These experiments will test predictions made by recent models of photosynthesis and identify those areas where our understanding of photosynthesis is incomplete. Particular attention will be paid to the biochemical limitation that occurs in plants growing under optimal conditions. Current indications are that the capacity of plants for triose phosphate utilization (TPU) can impose a limit on the rate of photosynthesis. TPU limited leaves are being studied by gas exchange analysis (especially for oxygen sensitivity), by metabolite analysis of rapidly frozen leaves, and by room temperature steady state fluorescence. The pool size of orthophosphate (P_i) inside the chloroplast will be measured and the P_i metabolism modeled to see how the P_i level changes during the TPU limitation and whether modification of P_i metabolism would result in plants with a higher capacity for photosynthesis. These studies are intended to determine the importance of the TPU limitation to photosynthesis rates and explore the interface between photosynthesis in chloroplasts and the physiology of the rest of the plant.

22. FLORIDA STATE UNIVERSITY
Tallahassee, Florida 32306

GUARD CELL BIOCHEMISTRY — $85,000

William H. Outlaw Jr.
Department of Biological Science

Most H_2O and CO_2 exchange between a leaf and the atmosphere is controlled by stomatal aperture size. Thus, when the surrounding guard cell pair accumulates potassium salts, these cells become more turgid and the pore enlarges. The current aperture size is a compromise between the opposing priorities of preventing dessication and satisfying the demand for CO_2. Typically, many environmental factors are integrated to effect this compromise. The immediate goal of this project is to identify correlations between metabolic events in guard cells and environmental pertubations that affect gas exchange. Because guard cells are sprinkled about in the leaf epidermis, special techniques for their study are necessary. We take three approaches for obtaining pure samples of Vicia faba guard cells: (1) protoplast isolation, (2) dissection of guard cells from frozen-dried leaves and (3) dissection of live guard cells from epidermal peels. In brief, our current work is in the following areas: (1) quantitation of [ABA]* in guard cells and other leaf cells (in collaboration with J. Zeevaart) using physico-chemical analysis; our preliminary results indicate that stressed guard cells accumulate ABA; (2) quantitation of [ABA] in guard cells by ELISA. Unfortunately, our monoclonal lines against ABA-protein are not stable. Thus, we are immunizing rat and rabbit in an attempt to develop polyclonal antibodies; (3) development of techniques for real-time, direct assay of enzyme activity in single cells using natural substrates. This project is virtually completed and its potential impact in many areas of biological research is significant; (4) in vivo, quantitative assay of NR in guard cells. In vivo assay, though more difficult, is considered necessary because of the lability of extracted NR. Preliminary results with small tissue slices (∿100 cells) indicates that necessary miniaturization is feasible.

*ABA - abscisic acid

23. UNIVERSITY OF FLORIDA
Gainesville, Florida 32610

INVESTIGATION OF THE TRANSPOSITION OF MITOCHONDRIAL DNA AND ITS RELATIONSHIP TO FERTILITY IN ZEA MAYS — $58,245 (FY 83)

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 number 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 pBR322 for use as templates in an in vitro transcription system. We have purified an alpha-amanitin sensitive RNA polymerase from maize seedlings that transcribes inserted maize mitochondrial DNA sequences more efficiently than those of the vehicle. We have prepared polyclonal antibody to the RNA polymerase preparation as a tool for recognizing and isolating a putative maize DNA recognition factor in the polymerase preparation. We are attempting to couple the catalytic activity of a pure maize poly A polymerase with that of maize RNA polymerase using the defined templates. We are characterizing the products that accumulate and that hybridize with the maize DNA inserts to ascertain the specific "start" and "stop" signals recognized by the maize polymerases. These results will facilitate the use of portions on the mitochondrial genome as putative vehicles for genetic engineering of this agronomically significant crop.

24. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

ROLE OF CA^{2+} AND CALMODULIN IN PHOSPHORYLATION OF PROTEINS IN PLANTS — $65,000

Milton J. Cormier and Alice C. Harmon
Department of Biochemistry

The long-range goal of this project is to understand the molecular basis by which plant cells respond to external stimuli such as light, temperature changes, growth regulators, etc. The evidence to date suggest that many regulatory processes in plant cells are mediated through stimuli-induced changes in the intracellular levels of free Ca^{2+}. One of the cellular targets for Ca^{2+} has been identified by us as the Ca^{2+}-binding protein, calmodulin. Furthermore, we have shown that the calmodulin-Ca^{2+} complex is required for the activation of plant NAD kinase which regulates the NADP/NAD ratio. We are currently focusing on regulatory processes which are mediated via Ca^{2+}-dependent protein phosphorylation. We have observed Ca^{2+}-dependent protein kinase activity in the supernatant and membrane fractions obtained from a soybean cell line grown in suspension culture. A number of endogenous protein substrates were phosphorylated in the presence of gamma labelled ^{32}P-ATP. Following SDS-PAGE of the precipitated proteins, autoradiograms of the stained gels revealed the presence of about 20 phosphorylated polypeptides of which about half were phosphorylated in a Ca^{2+}-dependent manner. The purification and characterization of these enzymes and some of their substrates are in progress and represent a first step in understanding the role of protein phosphorylation in plants.

25. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

BIOSYNTHESIS OF A MICROBIAL BIOSURFACTANT — $83,790
W. R. Finnerty and Mary E. Singer
Department of Microbiology

The goals of this project are to determine the genetic and biosynthetic mechanism(s) of glycolipid synthesis by a gram-positive microorganism. This product is an extracellular biosurfactant effective in the reduction of viscosity of heavy crude oils and displacement of oil from inert matrices. We are isolating mutants defective in fatty acid degradation, alkane utilization and glycolipid biosynthesis to determine the genetic relationship between glycolipid synthesis and alkane oxidation. Growth of the organism on alkane represents an obligate prerequisite for the synthesis of biosurfactant. We have determined that arsenate-resistance is plasmid-encoded in this organism. This trait is being used to cure the organism of 55-, 14.5- and 11.5 Mdal plasmids and to determine the relationship of plasmid-localized genes to alkane oxidation. Several arsenate-sensitive, hexadecane-negative strains have been recovered following sodium dodedecyl sulfate curing procedures. Enzyme studies have demonstrated the presence of cell-localized esterase and lipase plus a cellular and extracellular acyl transferase. The relationship of these enzymes to glycolipid synthesis, modification or release to the growth medium is being investigated. Current results indicate an excess carbon/limiting nitrogen imbalance stimulates extracellular glycolipid synthesis with maximum extracellular glycolipid synthesis correlating to the stationary growth phase and an external nitrogen concentration of 4 mM. Current studies are directed to determining specific enzymes induced by growth on alkane and the precursor relationship between cellular glycolipid and extracellular glycolipid with respect to biosynthesis, modification and release from the cells.

26. 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. This research has shown how valuable germplasm can be masked on certain genomes by other genomes and 'stored' in a perennial or vegetatively propagated wild species such as Pennisetum purpureum. We have been able to transfer this 'hidden' or 'stored' germplasm to cultivated pearl millet and are in the process of evaluating it. Partially fertile and apomictic BC_2 pearl millet x P. squamulatum hybrids have been produced showing the potential of transferring apomixis from the wild to cultivated species to fix hybrid vigor. Diverse cytoplasm from the wild species have been transferred to pearl millet and are in the initial stages of evaluation. The overall impact would be on increased, more efficient and more reliable production of food, fiber, and forage.

27. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

ENVIRONMENTAL STRESS MEDIATED CHANGES IN TRANSCRIPTIONAL AND TRANSLATIONAL REGULATION OF PROTEIN SYNTHESIS IN CROP PLANTS — $86,520

Joe L. Key
Department of Botany

When soybean seedlings are subjected to an immediate shift from 30° to 40° or to a gradual (e.g. 3° per hr) increase to 40° to 45° there is a dramatic and rapid shift in the pattern of protein synthesis. Most normal protein synthesis rapidly declines at a temperature of about 40° and a new set of proteins (hsps) is rapidly induced. The synthesis of hsps correlates with the rapid induction, detectable within 3 to 5 minutes, of hs-specific RNAs encoding a complex set of 30 to 40 polypeptides. These sequences subsequently accumulate to as many as 20,000 copies per cell. The system appears to be self-regulated since it "turns off" after 6 to 8 hr of continuous hs, indicated by the depletion of hs mRNAs, cessation of hsp synthesis, and the resumption of normal protein synthesis. This "turn off" occurs more slowly during prolonged high temperature treatment than when the seedlings are returned to 30° after a short, 2 to 4 hr hs at 40°.

One putative function of hs proteins is a role in the development of tolerance to high temperatures through a conditioning treatment at a lower, hsp inducing temperature. An initial hs treatment (e.g. 2 hr at 40° or 5 to 10 min at 45° followed by 2 hr at 30°) provides thermoprotection to a subsequent, otherwise lethal temperature (e.g. 2 hr at 45°). This short-term thermal adaptation correlates with the accumulation of hsps and their selective localization during hs. A wide range of plant species are known to synthesize a complex group of hsps. There is considerable variation in the electrophoretic pattern of the 15 to 27 kD hsps, while the high molecular weight hsps are highly conserved. Although many stress agents induce low levels of hs mRNAs based on Northern hybridization analyses using hs cDNA clones to probe poly(A)RNAs from tissues stressed by other agents, only arsenite treatment closely mimics hs, as evidenced by intense radiolabeling of hs proteins. Cadmium also induces a small subset of the hs proteins and hs mRNAs. The hs genes comprise several multigene families, probably with over-lap among these families, as analyzed by Southern hybridization, 2D gel analyses of hybrid-selected translation products, and DNA sequence analysis of cDNA and genomic clones.

28. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

SOYBEAN RIBULOSE BISPHOSPHATE CARBOXYLASE SMALL SUBUNIT GENE FAMILY: GENE STRUCTURE AND REGULATION OF GENE EXPRESSION — $73,000

Richard B. Meagher
Department of Genetics

The goal of this research has been to determine the mechanism of the regulation controlling the transcription of the nuclear-encoded ribulose bisphosphate carboxylase small-subunit (RuBPCss) gene family in soybean. Although the soybean gene family contains 8-12 members, we have focused our attention on two unlinked but closely related pairs of genes, SRS1 and SRS2. The steady state levels of poly A+ transcipts from both genes are very high in light-grown leaves and shoots and at least 50 to 100 fold lower in dark-grown leaves and shoots. We estimate that message levels may range from 0.2% in the dark to 2% of the poly A+ mRNA in the light. To determine the manner in which the mRNA levels were controlled, we assayed the transcription rates for these genes in isolated nuclei under a variety of light and dark conditions. In summary, these genes can show a slight (2-4 fold) transcriptional induction in as little as 30 minutes after dark-grown seedlings are exposed to light. Maximum transcriptional induction takes between 24 and 48 hours of light. In light pulse experiments, where plants are exposed to only short periods of light and then returned to the dark before assaying for transcriptional levels, 10 minutes of white light can lead to a 10 fold induction of transcription five hours later. If white light is followed by 10 minutes of far red light, little or no transcriptional induction is observed. These results confirm that phytochrome is involved in transcriptional control of these genes. We will continue to analyze the transcriptional regulation of the members of the small subunit gene family in soybeans. We have just begun to use these strong control signals to direct the transcription of foreign genes in plants.

29. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

THE MICROBIOLOGY AND PHYSIOLOGY OF ANAEROBIC FERMENTATIONS OF CELLULOSE — $220,000

Harry D. Peck, Jr. and Lars 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 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.

30. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

PHYTOCHROME PROPERTIES AND FUNCTION IN PHOTOSYNTHETICALLY COMPETENT PLANTS — $50,319

Lee H. Pratt
Department of Botany

Plants respond, via the chromoprotein phytochrome, to the wavelength distribution of incident radiant energy in ways that alter both photosynthetic productivity and the partitioning of photosynthetically fixed carbon within the plant. In part because of the low abundance of phytochrome in green plants, however, almost nothing is known about the molecule as it exists in them. As a first step towards understanding how phytochrome modulates primary plant productivity, therefore, the properties of this pigment as it exists in green, photosynthetically competent tissues is being investigated, with a focus on two plants of agronomic importance: oats (Avena sativa L.) and peas (Pisum sativum L.). Polyclonal and monoclonal antibodies to oat and pea phytochromes, both of which were purified from etiolated tissues, are being produced and used to quantitate, visualize, and characterize phytochrome from green plant tissues. While developing and using an enzyme-linked immunosorbent assay that is sensitive to picogram (subfemtomol) levels of phytochrome, it became evident that phytochrome from green plants is antigenically distinct from phytochrome isolated from etiolated, but otherwise identical, plants. This antigenically unique phytochrome is being characterized, not only to determine whether it might arise from some artifact, but also to learn how it differs from the pigment as isolated from etiolated tissue. To achieve these goals, all currently available monoclonal antibodies to phytochrome (about 40 at present) are being screened to determine which of them bind to phytochrome from green plants and to which part of the phytochrome molecule they bind. Monoclonal antibodies that are specific to epitopes on phytochrome from green plants will, if possible, be made in the near future to permit its selective immunopurification, immunoquantitation, and immunovisualization.

31. THE UNIVERSITY OF GEORGIA
Athens, Georgia 30602

NITROGEN CONTROL OF PHOTOSYNTHETIC PROTEIN SYNTHESIS — $65,000
Gregory W. Schmidt
Department of Botany

The detrimental effect of nitrogen deficiency on photosynthetic carbon metabolism is a major limitation to plant growth and productivity. Photorespiratory assimilation of oxygen by ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBPCase) results in a severe loss of reduced carbon when ammonium is not readily available. Nitrogen also influences photosynthetic efficiency by affecting the levels of chloroplast proteins. Our research concerns the molecular basis of the nitrogen regulatory process in photosynthetic cells. As a model system, we employ the unicellular alga Chlamydomonas reinhardtii grown in a continuous culture system in which nitrogen provision is precisely controlled. This enables high resolution analyses of the status of the photosynthetic apparatus in nitrogen limited cells and the molecular consequences of nutrient repletion. The major proteins whose levels are regulated by nitrogen are RuBPCase and the apoproteins of thylakoid membrane light-harvesting complexes. Immunochemical analyses show these proteins are preferentially reduced when the cells are grown in nitrogen-limiting conditions. With antibodies against seven other proteins we have determined that nitrogen differentially regulates the accumulation of the alpha and beta subunits of the photosynthetic ATP synthetase, the apoproteins of Photosystem I and II reaction centers, a core protein of PS II, a protein of the water oxidation complex and plastocyanin. In vivo pulse-chase labeling studies establish the effects of nitrogen on the rates of synthesis and turnover of these and other cellular proteins. Assays of mRNA abundance have revealed that some nitrogen-regulated proteins are strongly regulated at a transcriptional level. Detailed studies are in progress to resolve the means by which nitrogen profoundly affects expression of both nuclear and chloroplast genes that encode some, but not all, photosynthetic proteins.

32. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

THEORY ON EXTREME THERMOPHILIC ANAEROBES GROWING OVER TEMPERATURE SPANS OF 40°C OR MORE. — $40,000
Juergen K. W. Wiegel
Department of Microbiology

Bacteria that grow over 70°C are considered extreme thermophiles. Among these, there are species able to grow over an extended temperature span of 40°C or more. Examples for this group are the anaerobic eubacteria Clostridium thermohydrosulfuricum, Thermoanaerobium brockii, Thermoanaerobacter ethanolicus, the anaerobic archaebacterium Methanobacterium thermoautotrophicum as well as the aerobic Bacillus stearothermophilus. They all grow in the temperature range of approximately 35 to 78°C. For these species we have shown that they exhibit a biphasic dependence of the growth rate (or other growth parameters) and the growth temperature. The corresponding Arrhenius graph has a curve with an intermediary plateau, which, depending on the organism, can be very distinct or only minor. The plateau is found between 55 and 60°C. This biphasic growth response will be investigated on the enzyme (protein) level by examining the protein pattern of the organisms grown at various temperatures including T_{min}, T_{opt}, and T_{max}, below, at, and above the plateau. Initially we will use two dimensional gel electrophoresis (O'Farrell's gel) to obtain the differences in protein pattern related to growth temperatures. These experiments will be followed by temperature shifts with the employment of radioactive labeled amino acids. These studies will reveal whether a synthesis of new proteins occurs in bacteria when shifting the growth temperature from below to above that of the plateau. This is done to evaluate the forwarded hypothesis: that these organisms inherit, for some critical enzymes/proteins, two sets of proteins: one for the lower range of 35 to approximately 65°C and one for the upper temperature range from around 50 to 78°C.

33. GRAY FRESHWATER BIOLOGICAL INSTITUTE
Navarre, Minnesota 55392

PHYSIOLOGY AND GENETICS OF METHANOTROPHIC BACTERIA — $44,761
R.S. Hanson
Gray Freshwater Biological Institute/University of Minnesota

The objectives of this project are to develop techniques for genetic studies of the regulation one-carbon metabolism in bacteria that utilize one carbon compounds. Because these bacteria are resistant to many antibiotics used as markers on plasmids employed for genetic engineering of gram negative bacteria and are not transformed by these plasmid DNA's we have constructed new cloning vectors with the following properties. The vectors contain resistance markers for kanamycin and tetracycline and can be transferred between Pseudomonas aeruginosa, Escherichia coli and several methylotrophs by conjugation. The vectors contain a cos gene that permits recombinant (vector/DNA) molecules to be packaged into bacteriophage lambda. The bacteriophage, constructed in vitro, is used to transfer hybrid DNA into E. coli. Clones containing genes encoding seven enzymes for the catabolism of methanol assimilation of formaldehyde have been identified in clones by complementation of mutations in methylotrophs after mating with clones in the E. coli genomic library. These genes have been mapped on five different 50 kbp DNA fragments. The gene encoding the large molecular weight (60,000) subunit of methane monooxygenase from a type II methylotroph has also been cloned. The clones carrying this gene have been identified using antibodies prepared using the pure protein as an antigen. Current studies are directed at precisely mapping genes involved in C_1 metabolism and cloning the promoter for the gene encoding the methane monooxygenase. This protein represents about 20% of the total cellular protein and the gene appears to be present in a single copy in the cells. We have also isolated and completed a restriction map of a 125 kbp plasmid from a type II methylotroph. This plasmid is transferred by conjugation to Ps. aeruginosa and is capable of mobilizing chromosomal genes from Organism SB1.

34. HARVARD UNIVERSITY
Cambridge, Massachusetts 02138

UNRAVELING PHOTOSYSTEM II — $80,000
Lawrence Bogorad
Department of Cellular and Developmental Biology

The prokayotic cyanobacteria and eukaryotic higher green plants carry out basically the same type of oxygen evolving photosynthesis. Since some species and strains of cyanobacteria can be transformed with foreign DNA, they are suitable organisms for molecular biological analyses of the photosynthetic apparatus. The current focus of this project is on using cloned characterized maize chloroplast genes for components of the photosynthetic apparatus to identify and isolate the comparable genes from banks of clones of DNA prepared from two species of cyanobacteria that can be transformed. The maize chloroplast genes we have tested hybridize to the cyanobacterial DNA and permit us to identify the corresponding cyanobacterial genes. These genes, in variously modified forms, are to be reintroduced into the cyanobacterium in order to identify particular photosynthetic functions with segments of the protein for which the gene codes.

35. HARVARD UNIVERSITY
Petersham, Massachusetts 01366

STRUCTURE AND FUNCTION OF FRANKIA VESICLES IN DINITROGEN FIXATION BY ACTINORHIZAL PLANTS — $72,852
John G. Torrey
Cabot Foundation, Harvard Forest

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

36. UNIVERSITY OF IDAHO
Moscow, Idaho 83843

GENETICS AND CHEMISTRY OF LIGNIN DEGRADATION BY STREPTOMYCES — $73,000
Don L. Crawford
Department of Bacteriology and Biochemistry

The objectives of this research are to chemically characterize the intermediates of lignin degradation by Streptomyces, and to develop genetic techniques for manipulating and characterizing the lignin-degrading enzyme systems of these microorganisms. We are isolating and quantifying important lignin degradation intermediates, particularly water soluble, modified lignin polymers produced by Streptomyces strains. We are also examining and comparing the chemistry of modified lignin polymers produced by different Streptomyces as they decompose lignins. Enhanced lignin degrading strains are being isolated using ultraviolet irradiation mutagenesis, and by gene amplification using the recombinant DNA technique protoplast fusion. Protoplast fusions include interspecies crosses between different ligninolytic Streptomyces and intraspecies self fusions between protoplasts from a single species. Stable strains which overproduce polymeric intermediates have now been obtained using both mutagenesis and fusion. The chemistry of the intermediates generated by mutants and recombinants is now being examined and compared with that of intermediates generated by wildtype cultures. An enzyme system involved in lignin degradation has been discovered in our Streptomyces. Mutants and recombinants which are enhanced in lignin degrading ability also produce higher levels of this enzyme complex, which catalyzes the cleavage of β-ether linkages in lignin. The studies are aimed at developing an understanding of the pathways and genetics of lignin catabolism in Streptomyces. The application of our research findings will be the development of bioconversions for the production of chemicals from lignin, a renewable underutilized resource. Genetically manipulated strains already show promise for use in bioconversions to produce useful modified lignin polymers.

37. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

FATTY AND AROMATIC ACID CATABOLIZING BACTERIA IN METHANOGENIC ECOSYSTEMS — $65,000

Marvin P. Bryant
Department of Dairy Science

The goals of this project are to isolate and describe the biochemical ecology and systematics of syntrophic cocultures of proton-reducing (H_2-forming) acetate-forming bacteria or pure cultures of bacteria that catabolize fatty acids and benzenoids in the complex food web of bacteria degrading organic matter to CO_2 and CH_4. Species PA-1 and P-2 from digester sludge degrade phenylacetate (PA), phenol (P), benzoate and phenylpropionate in coculture with H_2-using Wolinella (W) which reduces fumarate to succinate. PA-1 + W produces mainly acetate, propionate, succinate and CO_2 from PA and fumarate and catabolizes many other lowly substituted benzenoids and other compounds not used by W or, so far, by PA-1 alone. P-2 + W produces mainly acetate, succinate and CO_2 from P and fumarate. Two new rumen species catabolize methoxybenzenoids to hydroxy-benzenoids and produce acetate or butyrate. A third new rumen species catabolizes trihydroxy-benzenoids such as gallate, with equimolar H_2 or formate required, to acetate, butyrate and caproate and catabolizes crotonate but not many other possible energy sources to acetate and butyrate in the presence or absence of formate. Peptostreptococcus productus from sludge and intestinal tract of man is shown to be the most rapid CO and H_2-CO_2-using mesophilic acetogen as yet documented. Our theory that syntrophy with H_2-using bacteria is necessary for anaerobic catabolism of saturated fatty acids other than formate and acetate and benzenoids containing less than three hydroxyls on the benzene ring to non-benzenoid products in methanogenic ecosystems is so far supported and little or none of this usually occurs in gastro-intestinal tract methanogenic ecosystems. Biochemical, physiologic and systematics research in these areas is continuing.

38. UNIVERSITY OF ILLINOIS
Urbana, IL 61801

PHOTOSYNTHESIS IN INTACT PLANTS — $106,000

A.R. Crofts
Department of Physiology and Biophysics

Information on functional aspects of photosynthesis in intact plants, especially on the operation of electron transfer and energy conversion processes, has been limited by the availability of suitable instrumentation. We are developing several items of apparatus for measuring these reactions on intact plants in the field. The instruments are being developed, and used in in vivo studies, in parallel with studies in the laboratory using similar instruments for measurement of reactions in vitro with isolated chloroplasts. Two portable instruments have been built, and a third is in development. These measure the kinetics of electron transfer through the two-electron gate (the herbicide binding site) using a double flash technique, fluorescence induction kinetics (continuous illumination), and spectrophotometric changes, respectively. The micro-computer based instruments weigh 10-20 lbs., are battery powered, and completely portable. We have been using the first of these instruments to study the kinetics of reduction of plastoquinone in herbicide-resistant weeds, and have identified a characteristic inhibition of this reaction in all atrazine-resistant biotypes so far studied. In vitro studies suggest that this symptom is associated with a change in affinity of the binding site for plastoquinone on PS II, and a much greater change in affinity for the herbicide. Our studies account for the lowered growth-yields of the herbicide-resistant plants in terms of an increase in photochemical "misses" due to the altered equilibrium constant for electron transfer in this reaction. We are extending our studies to the characterization of other environmental effects, and continuing a program of basic research into electron transfer on the donor and acceptor sides of PS II.

39. UNIVERSITY OF ILLINOIS
Urbana, IL 61801

MECHANISM OF PROTON PUMPING IN BACTERIORHODOPSIN — $72,618

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. We have also determined that the number of protons pumped also depends on the presence of the C-terminal tail. Most recently we have discovered that bacteriorhodopsin tightly binds divalent cations and this binding partially depends on the presence of the C-terminal tail. We are continuing to investigate the role of the cations and the tail on proton pumping.

40. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

DEVELOPMENT OF A GENETIC SYSTEM FOR BACTEROIDES — $50,000

Jeffrey F. Gardner and Abigail A. Salyers
Department of Microbiology

The goal of this project is to develop a method for introducing foreign DNA into Bacteroides so that polysaccharide catabolism by these bacteria can be studied at the genetic level. We have constructed several chimeric plasmids which contain DNA from E. coli plasmids and DNA from pBF4, the conjugal Bacteroides plasmid which codes for clindamycin resistance. We have demonstrated that R751 can mobilize one of these chimeric plasmids, pE52, from E. coli to Bacteroides uniformis and Bacteroides thetaiotaomicron. pE52, which contains only 3.8 kb of pBF4 DNA, is stable in E. coli and in Bacteroides. By contrast, some of our chimeric plasmids which contain larger segments of pBF4 DNA are highly unstable and participate in genetic rearrangements in E. coli. We have preliminary evidence that this may be due to the presence of a transposable element which carries the gene for clindamycin resistance. We are now attempting to demonstrate that there is a transposable element and that it can transpose in Bacteroides. If so, we will determine whether it can be used for transposon mutagenesis of Bacteroides. In addition to our work on construction of chimeric plasmids we have cloned DNA fragments from Bacteroides thetaiotaomicron which appear to carry genes for two polysaccharide-degrading enzymes. We will use these clones to investigate the regulation of the enzymes in Bacteroides.

41. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

BIOCHEMICAL AND BIOPHYSICAL STUDIES ON E.COLI AEROBIC RESPIRATORY CHAIN — $85,000
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 from. 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 apprent 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. The enzyme has also recently been purified to homogeneity and mutant strains lacking this enzyme have been isolated. The combined use of genetics and biochemical characterization will result in a detailed understanding of this system in the near future.

42. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

GENETICS OF THE METHANOGENIC BACTERIUM, METHANOCOCCUS VOLTAE WITH ATTENTION TO GENETIC CONTROL MECHANISMS — $50,000
Jordan Konisky
Department of Microbiology

The objectives of this project are to both develop a genetic system and to characterize gene expression in the methanogen, Methanococcus voltae. We have previously reported the cloning of methanogen arginine and histidine biosynthetic genes. These genes are being characterized with particular emphasis on the elucidation of promoter and other DNA sequences involved in the regulation of gene expression. We are also investigating the molecular basis of our finding that methanogen genes which are expressed weakly in E. coli can be activated by the insertion of E. coli DNA into methanogen sequences. Controlled methods are being developed for the isolation of methanogen mutants which are resistant to antibiotics and other agents which inhibit methanogen growth. Such mutants will be examined in an attempt to define the molecular basis for such resistance. These studies serve as a useful approach to probe methanogen metabolism and surface structure. In an attempt to develop gene vectors for gene transfer into methanogens, we are screening new Methanococcus isolates for the presence of plasmids. Such plasmids would be engineered to contain appropriate genes for selection of transferred DNA. This approach complements other attempts to develop a gene transfer system such as direct transformation of protoplasts or protoplast fusion.

43. 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 — $80,810

John R. Laughnan
Department of Genetics and Development

These investigations involve coordinated field and laboratory studies on cytoplasmic male sterility (CMS) in maize, with principal focus on genetic changes in nuclear and mitochondrial DNA (mtDNA) that accompany reversions from CMS to male fertility at both the nuclear and cytoplasmic levels. They also aim at a better understanding of the organization of the main mitochondrial chromosome and of so-called satellite DNA molecules that co-exist as plasmid like elements in the mitochondrion. A first approach involves studies at the molecular level on nuclear reversion to male fertility and will employ maize transposable controlling elements to identify nuclear restorer genes at different chromosomal sites, and to isolate and characterize them at the molecular level. We are also investigating the molecular characteristics of a variety of cytoplasmic male fertile revertents; major characteristics of these revertents appear to be determined by the nuclear genotype. We are extending the studies indicating that plasmid disappearance upon cytoplasmic reversion is dependent on nuclear genotype and are also investigating whether the overall organization of the mitochondrial genome is governed by nuclear genotype, as preliminary results suggest. Developmental aspects of the event will be studied further; it is now possible to relate left and right halves of the tassle to corresponding halves of the stem and leaf portions of the plant, and therefore to correlate ear performance with the positions of tassle reversion events. Recent genetic studies indicate that the nuclear restorer elements of CMS are transposable, and further studies are now under way to determine how this behavior correlates with that of the known controlling elements in maize, and to identify special characteristics of the transposition event in this case.

44. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

ACETOCLASTIC METHANOGENESIS — $70,777

Ralph S. Wolfe
Department of Microbiology

We propose to define the enzymology of methane formation from acetate by aetophilic methanogens. Knowledge of the biochemistry of this area of methanogenesis lags far behind that of methanogenesis from CO_2 and H_2 largely because of the difficulty in mass culturing cells on acetate. However, in nature about 70% of the methane formed is derived from the methyl group of acetate, an indication of the importance of this process in anaerobic biodegradation. We propose to follow the strategy that we have used successfully to study the biochemistry of methanogenesis in other methanogens. This involves mass culture of the organism, development of a fractionation procedure to resolve the enzymes and coenzymes, development of procedures for purification of the components to the homogeneous state, and definition of the role of each component. These basic studies are prerequisite to an understanding of the acetophilic methanogens and their role in anaerobic degradation.

45. THE INSTITUTE OF PAPER CHEMISTRY
Appleton, Wisconsin 54912

RAMAN MICROPROBE INVESTIGATION OF MOLECULAR STRUCTURE AND ORGANIZATION IN THE NATIVE STATE OF WOODY TISSUE — $46,340
Rajai H. Atalla
Chemical Sciences Division

The objective of this project is to explore molecular structure and organization in the cell walls of native woody tissue. The primary investigative tool is the Raman microprobe which has made possible spectroscopic exploration of domains of the order of 1 μm in sections of native tissue. Preliminary explorations on tissues from loblolly pine (Pinus taeda L) and from black spruce (Picea mariana) revealed evidence of variations in composition within the secondary walls of individual cells as well as between adjacent cells in the same annual ring. They also provided indications of molecular orientation relative to the plane of the cell walls. More recent studies focussing on tissue from Picea mariana have shown that, in a majority of locations in the secondary wall, the phenyl propane units of lignin are preferentially oriented in the plane of the cell wall. The spectral features associated with cellulose are consistent with alignment of the anhydroglucose rings perpendicular to the plane of the cell cross section. The continuing effort is directed at a mapping of the patterns of variation of both composition and molecular orientation. The results further our fundamental understanding of cell wall architecture and can be of use in analysis and design of industrial processes which use biomass as a primary resource.

46. IOWA STATE UNIVERSITY
Ames, Iowa 50011

PROLINE METABOLISM IN PLANTS UNDER ENVIRONMENTAL STRESSES — $62,000
Cecil R. Stewart
Department of Botany

The goal of this project is to understand the subcellular mechanism which causes proline to accumulate under environmental stresses. Since one of the known causes is inhibition of proline oxidation, we have continued our studies of this process in mitochondria. Mitochondrial proline transport has been measured by osmotically induced size changes in combination with oxygen uptake. Uptake is absolutely stereospecific and partially dependent on the energy of an electrochemical gradient. Based on the effects of FCCP and valinomycin, it has been shown that uptake is gradient dependent only at high proline concentrations and it is the pH component of the gradient that drives uptake rather than the electrical component.
Steady state proline levels in stressed leaves have been determined to find the relationship between proline levels and salt content. A linear relationship between salt and proline content above a threshold salt level is consistent with proline functioning as a compatible osmoticum and excess salt being accumulated in the vacuole. Changes in proline levels during recovery from wilting have been contrasted with changes after removal from salt and are consistent with proline levels responding to reversible changes in salt concentration during dehydration and rehydration. Current studies are aimed at testing these interpretations directly using isolated protoplasts and vacuoles.
These results contribute to our understanding of plant adaptions at the molecular level and provide information on which to base possible plant manipulations using new molecular techniques.

47. UNIVERSITY OF KENTUCKY
Lexington, Kentucky 40546-0091

PHYSIOLOGY/BIOCHEMISTRY OF PHOTOACTIVATION OF OXYGEN EVOLUTION: PROBES FOR THE S-STATE PROTEIN — $28,328 (FY 83)

George M. Cheniae
Agronomy Department

This project is focused towards: 1) elucidation of the biochemical/physiological reactions underlying the multiquantum process required for the photoactivation of O_2 evolving centers; and 2) studies of the multimeric O_2 evolving center (OEC) with emphasis on the subunit compositional effects on O_2 evolution and the reactivity of the topography of the OEC complex to chemical analogs of the H_2O molecule. A number of different studies (developmental; recovery from inactivation of active OEC by heat/cold stress and chemical analogs (NH_2OH) of water) indicate that the photoconversion of inactive OEC to active OEC by PSII during photoactivation is a fundamental process in the oxygenic photosynthetic organisms. We study the photoconversion of inactive to active OEC under two conditions: 1) following a photoinhibition of PSII, which saturates at weak light intensity ($t_{\frac{1}{2}} \sim 2$-3min) and thereby confers requirement for light dependent synthesis of two specific PSII polypeptides on chloroplast 70S ribosomes for reappearance of active OEC; and 2) photoactivation per se, a process independent of protein synthesis, in which Mn^{+2} and specific PSII polypeptides of the OEC complex are assembled into thylakoids to yield active OEC centers. The other focus of this project involves selective extraction of subunits of the OEC, subsequent reconstitutions and determination of effects on O_2 evolution, PSII reactions, and modifications of reactivity of OEC to chemical analogs of H_2O. These studies include effects of specific protein-modifying reagents and specific affinity/photoaffinity reagents containing groups known to react rather specifically with OEC.

48. LEHIGH UNIVERSITY
Bethlehem, Pennsylvania 18015

A GENETIC APPROACH TO SECRETION AND HYPERPRODUCTION OF CELLULASE BY TRICHODERMA — $82,900

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

Cellulases are important glycoprotein enzymes of great potential industrial importance in the recycle and use of cellulosic biomass. Production of cellulases by fungi involves protein synthesis at the level of the endoplasmic reticulum, glycosylation in the endoplasmic reticulum and presumably the Golgi complex followed by packaging into secretory vesicles and finally secretion to the external milieu. Little is known of the precise sequence of events involved in the production of extracellular glycoprotein enzymes by lower eukaryotic microorganisms. In an effort to elucidate these steps, we are employing the cellulase system of Trichoderma reesei as a model. We have isolated a range of auxotrophic, drug resistant and temperature sensitive mutants from both the wild type, QM6a, and a number of the high yielding cellulase mutants which we have previously isolated (e.g., RL-37 and Rut-C30). We are developing a recombination system by heterokaryon formation and protoplast fusion to identify the various steps in secretion. In addition, we are studying the effects of glycosylation inhibitors and membrane perturbing agents on the biochemical nature of the enzymes and the secretory pathway. A better understanding of cellulase biosynthesis at the molecular level should result in more selective and sensitive systems for isolating improved mutants and contribute toward fermentation optimization.

49. MARTIN MARIETTA LABORATORIES
1450 South Rolling Road
Baltimore, Maryland 21227-3898

STUDIES OF PHOTOSYSTEM II USING ARTIFICIAL DONORS — $95,370
Dr. Richard Radmer
Biosciences Department

The goal of this project is to study aspects of photosynthetic oxygen evolution using specialized mass spectrometric and spectrophotometric techniques. We are using a mass spectrometer system constructed in-house to monitor the amount and isotopic composition of the gas exchange elicited by short saturating flashes, e.g., O_2, N_2 from NH_2OH oxidation, $^{18}O_2$ from $H_2{}^{18}O$ oxidation, etc. Studies using $H_2{}^{18}O$ are being carried out to determine whether there are bound non-exchangeable (partially oxidized?) intermediates generated during the course of H_2O oxidation to O_2. We are also using "H_2O analogs," i.e., hydroxylamine and hydrazine and their methyl-substituted derivatives, to attempt to characterize various modified TSF2 System-II preparations. (These studies are based on our earlier work using broken chloroplasts.) A spectrophotometric TMPD assay, developed earlier in our laboratory, is being used to ascertain the population of active and inactive O_2-evolving centers in a given preparation. The aim of these studies is to correlate the topography of the O_2-evolving site (as determined by the reactions of the H_2O analogs) with the protein complement and the photochemical activity of the modified TSF2 preparations.

50. UNIVERSITY OF MARYLAND
College Park, Maryland 20742

ENERGY-DEPENDENT CALCIUM TRANSPORT MECHANISMS IN PLANT MEMBRANES — $63,558
Heven Sze
Department of Botany

The objectives of this project are (i) to identify and characterize active calcium (Ca) transport systems in higher plant tissues and (ii) to understand the mechanisms that regulate Ca fluxes. Plant tissues include tobacco callus (Nicotiana tabacum), oat (Avena sativa) and corn (Zea mays) roots. Subcellular membranes are isolated and purified by differential and density centrifugation, and ATP-dependent 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 ionophores: one requires a pH gradient and one does not. The Ca/H antiport is anion-sensitive and vanadate-resistant suggesting that Ca uptake is driven by a pH gradient generated by a tonoplast-type H-pumping ATPase. Another Ca pump (probably a Ca-pumping ATPase) is vanadate-sensitive and experiments are underway to determine the membrane identity. To understand how Ca fluxes are regulated, the effect of phytohormones and toxins on the various Ca transport systems will be studied. For example, we have found that a fungal toxin (Helminthosporium maydis T) decreases active Ca transport into isolated mitochondria from susceptible corn but not resistant corn. We are conducting studies to determine more specifically the mode of toxin action. Since Ca levels are important in regulating various physiological and biochemical processes, these studies of Ca transport are central to our understanding of not only the mechanism and regulation of solute transport but also the regulation of plant growth and development.

51. MASSACHUSETTS INSTITUTE OF TECHNOLOGY
Cambridge, Massachusetts 02139

ANTIBODY ANALYSIS OF THE RHIZOBIUM MELILOTI SURFACE — $80,000
Ethan R. Signer
Department of Biology

Rhizobium meliloti forms nitrogen-fixing nodules on the roots of alfalfa. Both polyclonal sera and monoclonal antibodies (MAb) to the bacterial surface will be used to identify components essential for the initial nodulation step(s). Immunoprecipitation analysis with polyclonal sera will be used to identify components differing in isogenic Nod^+ and Nod^- pairs. MAb will be produced and used to isolate Ab-insensitive nodulation-defective (Nod^-) mutants by methods we have developed. Surface changes as a function of growth conditions and phases will be studied. Several methods will be tried to circumvent immunodominant antigens and reveal weaker ones, particular immunosuppression in combination with in vitro immunization. Two defects in later nodulation stages identified in earlier work will also be pursued.

52. UNIVERSITY OF MASSACHUSETTS
Amherst, Massachusetts 01003

CONVERSION OF CELLULOSE TO ETHANOL BY MESOPHILIC BACTERIA — $77,000
Ercole Canale-Parola, Clifton E. Dowell, and
Susan B. Leschine
Department of Microbiology

The main objective of the project is to study the genetics of mesophilic anaerobic bacteria that ferment cellulose to ethanol. The bacteria used in this investigation are strains of Clostridium that i) convert to ethanol not only cellulose, but also components of the hemicellulosic portion of biomass, and ii) are relatively ethanol tolerant. The studies include plasmid analysis, as well as fine-structure mapping of genes involved in the breakdown of cellulose to soluble sugars. These genes code for cellulase system enzymes such as endo-1,4-β-glucanases, exo-1,4-β-glucanases, β-glucosidases, and cellobiose phosphorylase. The research includes characterization of cellulase system enzymes present in ethanol-producing mesophilic clostridia, as well as of enzymes utilized by these bacteria in the breakdown of hemicellulosic components. The work will provide fundamental information on the genetics of cellulose-fermenting, ethanol-producing clostridia. This information will be valuable for the understanding of metabolic processes utilized by anaerobic bacteria to degrade cellulose, and for the development of clostridial strains that can be used in the industrial conversion of biomass to ethanol. Finally, the research will elucidate basic aspects of the genetics of clostridia. At present, very little is known about the genetic systems of this large group of anaerobic bacteria.

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

CONTROLLED MEDIA FOR PLANT TISSUE CULTURE — $55,000 (FY 83)
Norman E. Good
Department of Botany and Plant Pathology

We are very disturbed by the fact that commonly used plant tissue culture media are uncontrolled, with constantly changing levels of hormones, vitamins, nutrients and pH. Consequently, we are exploring ways of stabilizing most of these factors. The techniques used are: 1) To buffer substances by having a reservoir of bound substance in equilibrium with the free solution. Thus we are exploring the use of H^+ buffers to regulate pH and the use of charcoal or ion-exchange resins pre-equilibrated with hormones and vitamins to maintain their concentrations; 2) To provide reservoirs in the form of conjugates of hormones which are in themselves metabolically inactive but break down slowly to give reasonably constant levels of the free active form of the substance. This technique is especially useful for maintaining a variety of constant auxin levels, the level depending on which conjugate is used; 3) To provide hormones which are chemically and metabolically stable, for instance the auxin analogue 2,4-D. This approach has been used widely but at a great penalty since by so doing one tends to frustrate any attempt by the tissue to regulate messages being sent, the same hormonal message being read over and over, probably at the cost of differentiation. Using these techniques we are looking again at recalcitrant tissue culture systems, where there have been problems of producing or maintaining callus from organized tissue, of inducing the development of callus from protoplasts, and of inducing organogenesis from callus.

54. UNIVERSITY OF MINNESOTA
St. Paul, Minnesota 55108

CORN STORAGE PROTEIN - A MOLECULAR GENETIC MODEL — $103,950
Joachim Messing
Department of Biochemistry

The current work is an extensive study of the nucleotide sequences of the genes in maize encoding the most abundant class of storage proteins in the endosperm tissue, the zein protein family. These studies emphasize the use of a recently developed tool in recombinant DNA technology, the M13 cloning system to produce the data and the aid of a computer in analyzing the data. We assume that the efforts in improving the methodology of the M13 system are justified because of their impact on research of gene structure and function in many laboratories. We have used these techniques to study differential gene expression in an economically important plant, corn. To understand the control mechanism of gene expression during tissue development, we have chosen the zein multigene family as a molecular genetic model. These genes represent a particularly interesting case because zein proteins accumulate in large quantities, are synthesized exclusively in the endosperm tissue, consist of a large closely related family of proteins, and are synthesized during a brief period of development. They make up to 50% - 60% of the total protein in the corn kernel (Nelson, 1969) and, therefore, represent a major component in the feed of livestock. Ultimately, the physical basis of the control of the expression of these genes much reside in their nucleotide sequence.

55. UNIVERSITY OF MINNESOTA
Minneapolis, Minnesota 55455

THE MECHANISM OF SWITCHING FROM AN ACIDOGENIC TO A BUTANOL-ACETONE FERMENTATION BY CLOSTRIDIUM ACETOBUTYLICUM — $74,000

Palmer Rogers
Department of Microbiology, Medical School

The obligate anaerobic bacteria, Clostridium acetobutylicum, ferments sugars to form acetic and butyric acids during early growth and then later produces entirely ethanol, acetone, and butanol. Experiments are designed to investigate the molecular mechanisms used by this bacteria to regulate the shifting of the enzyme activities catalyzing the four-branched fermentation pathways.
Following mutagenesis with N-nitrosoguanidine or ethylmethane sulfonate, we are screening for three different classes of mutants. Initially we have isolated rifampicin-resistant and streptomycin-resistant mutants as a rapid screen for the development of protoplast fusion. Secondly, amino acid auxotrophs will be selected, since these bacteria grow slowly without amino acids in the presence of vitamins. Thirdly, using plates with indicator dyes, fermentation mutants producing limited acid or limited alcohols will be isolated.
Protoplast fusion of Clostridium acetobutylicum is under study. Protoplast formation and regeneration techniques are now combined with protoplast fusion methods to produce double-mutants from single drug resistant parents. Protoplast fusion will be used in conjunction with a panel of acid-non-producer mutants to determine the number of factors regulating the fermentation program of this bacteria.
Enzyme activities were monitored from cell-free extracts for butanol and butyric acid formation. We find a rapid 100 fold rise in butanol forming enzymes coordinate with production of butanol followed by a rapid decay of activity when butanol synthesis stops. Experiments are designed to determine the role of enzyme synthesis and activity modification during these changes.

56. UNIVERSITY OF MINNESOTA
St. Paul, Minnesota 55108

MOLECULAR APPROACHES TO GENOMIC ORGANIZATION — $58,780

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

We propose to develop physical methods to determine the chromosomal location of isolated maize genomic segments. The first procedure uses a modified dot hybridization technique combined with the use of DNAs from a series of trisomic, disomic and monosomic maize plants. We have been able to demonstrate (in simulation experiments) the capacity to distinguish the presence of one versus two copies of unique genomic segments. This experiment is now being repeated with a number of genomic probes. The second procedure uses ^{3}H or ^{125}I-labeled RNA probes for in situ hybridization to determine the chromosomal location of genomic segments present at low reiteration frequencies. Highly labeled probes have been produced from genomic clones transferred to a transcription vector that contains a bacteriophage SP6-specific RNA polymerase promoter. These RNA probes give a high detection sensitivity and a low grain background.

57. UNIVERSITY OF MISSOURI
Columbia, MO 65211

PHOTOSYNTHESIS, CLONING, AND BIOCONVERSION OF SOLAR ENERGY IN CYANOBACTERIA — $66,764

Louis A. Sherman
Division of Biological Sciences

The goal of this proposal is to identify and clone the genes coding for membrane proteins involved in cyanobacterial photosynthesis. Cyanobacteria represent an excellent organism for such studies since they perform an aerobic photosynthesis nearly identical to green plants, and they are suitable for genetic, biochemical, and biophysical studies in photosynthesis. The organisms used for these studies, Anacystis nidulans R2 and Aphanocapsa 6714, are both transformable strains, and can thus be used for experiments involving genetic manipulation. We are using three types of experimentation to identify and clone specific genes. The first procedure utilizes heterologous probes to detect and clone related genes from A. nidulans. This procedure has enabled us to clone the genes coding for a Ca^{+2}-binding protein homologous to calmodulin, the rapidly-turned over, 32kDa, atrazine-binding protein (and the gene from a DCMU-resistant mutant that gives rise to the resistant-phenotype), the P700 Chl a-binding protein, and subunit IV of the cytochrome b_6/f complex. We have also isolated DCMU and Cd-resistant mutants in A. nidulans, characterized the specific lesion, and used the transformation system to isolate the appropriate gene. Interesting, both mutants appear to affect (at least in part) the same PSII protein; this is a 34kDa species that is also rapidly-turned over. In the DCMU-resistant mutant, the protein is altered to a 35.5 kDa polypeptide, whereas in the Cd-resistant mutant the turnover appears to be affected. The Cd-resistant mutant demonstrates a low fluorescence phenotype, implying that the 34kDa protein functions on the oxidizing side of PSII. Finally, we are using transposon mutagenesis in the heterotrophic strain Aphanocapsa 6714. This technique will generate mutants affecting oxygen-evolution and PSII activity; we will then use the antibiotic marker on the transposon to clone the specific genes.

58. MOUNT SINAI SCHOOL OF MEDICINE OF CUNY
New York, New York 10029

THE RESPIRATORY CHAIN OF ALKALOPHILIC BACTERIA — $72,828

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 redox carriers distinguishable by 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 conservation. This, in turn, helps meet the special energy costs of life at very high pH. Initial studies of H^+ translocation support this hypothesis. More detailed studies of H^+ translocation will be conducted using whole cells, protoplasts and/or vesicles, and reconstituted systems. The characteristics of the respiratory chain complex III and cytochrome oxidase will be examined further, the latter complex has been purified to homogeneity from Bacillus firmus RAB. The regulation of cytochrome levels and arrays are of particular interest in this system because of the observation that non-alkalophilic mutants have quantitatively and qualitatively fewer cytochrome species. Protocols for study of cytochrome expression, as a function of pH, have been developed.

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

GENBANK (TM), THE GENETIC SEQUENCE DATA BANK — $40,000
Christine K. 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 April 1, 1984, GenBank contained 2.94 million bases, comprising 3576 sequences. The data bank is operated under contract to Bolt Beranek and Newman Inc. (BBN) of Cambridge, MA. Data collection, verification, entry and annotation are performed under the direction of Dr. Walter Goad 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, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, and Division of Research Resources (of NIH), the National Science Foundation, the Department of Energy and the Department of Defense, 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, including Telenet access, is also available to anyone, but only a limited number of users can be accommodated at any one time. The first hard-copy edition of the database was available in May 1984 as a 2-volume supplement to Nucleic Acids Research and contained the combined databases of GenBank and the European Molecular Biology Laboratory's Nucleotide Sequence Library. Curators have been appointed to oversee selected portions of the database and more are to be appointed this year. Work is currently in progress to provide GenBank users with a directory of information about the availability of sequence analysis and manipulation software that can be used with GenBank.

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

BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERIZATION OF HIGHER PLANTS WITH REDUCED PHOTORESPIRATION — $40,000
Raymond Chollet
Department of Agricultural Biochemistry

The ultimate goal of this project is to elucidate mechanisms which reduce photorespiration and the associated O_2 inhibition of photosynthesis in terrestrial higher plants. Only two such mechanisms for reducing this seemingly wasteful metabolic process are known in nature, both of which involve a biochemical CO_2-concentrating system to provide the bifunctional enzyme ribulosebisphosphate carboxylase/oxygenase (RuBisCO) with an elevated pCO_2/pO_2 ratio. However, recent findings in this laboratory [Holaday & Chollet (1983) Plant Physiol. 73:740-745] and elsewhere indicate that neither of these two known mechanisms is operative in Moricandia arvensis and Panicum milioides. Yet, it is firmly established that bundle-sheath ultrastructure and photorespiratory activity in intact leaves of both species are intermediate between those features of C_3 and C_4 plants [Holaday et al. (1981) Biochim. Biophys. Acta 637: 334-341, (1982) Plant Sci. Lett. 27: 181-189]. Present studies indicate that: (i) the RuBisCO enzyme is neither altered kinetically [CO_2/O_2 specificity factor, $Km(CO_2)$] nor compartmented intercellularly between the mesophyll and bundle-sheath chloroplasts in both C_3-C_4 intermediate species; and (ii) M. arvensis and P. milioides may reduce photorespiration, at least in part, by internally refixing photorespiratory CO_2 via RuBisCO more efficiently than closely related C_3 species (M. foetida, P. laxum). Future investigations will pursue this latter aspect in greater detail with isolated leaf cell-types and organelles and evaluate whether the recently discovered C_3-C_4 intermediate Flaveria species [Holaday et al. (1984) Planta 160: 25-32] possess a limited C_4-like CO_2-concentrating system or some other mechanism(s) for reducing photorespiration.

61. UNIVERSITY OF NEBRASKA
Lincoln, Nebraska 68583-0722

VIRUSES OF EUKARYOTIC GREEN ALGAE $65,000
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. 300 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.

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

METHANE PRODUCING BACTERIA: IMMUNOLOGICAL CHARACTERIZATION $55,000
Conway de Macario, E.,* Macario, A.J.L.,* and Wolin, M.J.°
*Laboratory of Immunology
°Laboratory of Environmental Biology and Field Services

The long-term goals of this research is to standardize methods for immunologic examination of microbial communities containing methanogens and to determine the antigenic fingerprint of these bacteria. Procedures for sampling microbial communities of practical importance, e.g. digesters, are being developed to obtain specimens amenable to serologic analysis. The antigenic fingerprints of methanogens in these specimens are being determined at various time intervals. The presence of antigenic markers of methanogens in the fluid phase is also being investigated, not only in digesters, but also in bacterial cultures and suspensions of various kinds. Specific objectives are to develop methods for rapid, direct identification of "serotypes" by assaying the bacteria themselves and/or their markers released into the fluid phase, and to monitor the methanogenic population during operation of digesters. Correlations serotype(s)/digester's performance are being sought for. These studies should yield new methodology applicable to the analysis of a variety of complex ecosystems, and should help improve biotechnology concerned with elimination of organic wastes and production of methane. The methods and the data should also be useful in genetic engineering involving methanogens.

63. 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 and Biochemistry

Our long-term objectives are to establish in a higher plant an experimental system that is firmly defined by biochemical, genetic, and physiological criteria. Since somatic cells can be cultured essentially as microbial populations, even to the extent of obtaining recessive mutations that can be expressed in haploid cells, the ability of appropriately manipulated cells to regenerate to the organismal state allows exceptional opportunities to study development. Nicotiana silvestris and/or N. plumbaginifolia are the organisms of choice, and the pathway of aromatic amino acid biosynthesis is the focal point for study of gene-enzyme relationships. Highlights of our multiphasic approach are the identification and characterization of the biochemical steps associated with the synthesis of aromatic amino acids, the subcellular partitioning of these enzymes between different intracellular compartments, and the characterization of key regulatory enzymes. We will isolate both regulatory mutants and structural-gene mutants, and will seek to regenerate plant organisms which express such mutations. Completion of the latter objective may require temperature-sensitive mutations. Molecular-genetic approaches will be taken to identify and isolate genes, to use them as probes to study gene expression, and to establish gene locations. We seek to understand physiological, biochemical, developmental, and genetic interrelationships that are associated with an important biosynthetic pathway in a single experimental system. The aromatic amino acid pathway is of central importance in plant metabolism. Analysis of this pathway could have profound impact on the understanding of the biology of the plant because the pathway leads to protein precursors, growth regulators, a diverse range of secondary metabolites, and to medically significant drugs.

64. UNIVERSITY OF NORTH CAROLINA
Chapel Hill, North Carolina 27514

CHARACTERIZATION OF TRICHODERMA REESEI CELLULASE GENES — $87,000

Darrel W. Stafford
Department of Biology

Our laboratory is interested in the structure function relationships of the two major cellobiohydrolases of Trichoderma reesei. One of the goals is to achieve expression of high levels of these proteins in E. coli and in a fungal system. Questions about the relative activities of glyscosylated vs non glycosylated and mutant proteins will be examined. To this end we have cloned cellobiohydrolase I and cellobiohydrolase II into E. coli. The gene for cellobiohydrolase I was identified by synthetic oligonucleotides and shown to be correct by sequence analysis. The gene for CBH II was identified by screening an expression library with an antibody provided by Dr. Ross Brown and Dr. M. Gritzaldi. At present we are still characterizing both clones by sequence analysis.

65. NORTHWESTERN UNIVERSITY
Evanston, Illinois 60201

GENETICS OF THERMOPHILIC BACTERIA — $60,104
Neil E. Welker
Department of Biochemistry, Molecular Biology
and Cell Biology

The goal of this project is to develop reliable genetic exchange systems in thermophilic bacilli. We have demonstrated chromosomal and plasmid DNA transformation in competent cells, transfection with thermophilic phage TP-19, TP-25 and TP-36 DNA, and PEG-induced plasmid DNA transformation of protoplasts in several prototrophic strains of thermophilic bacilli. This group is composed of strains of Bacillus stearothermophilus, Bacillus coagulans, Bacillus licheniformis, Bacillus sphaericus, and Bacillus subtilis. The temperature range of growth of these strains overlaps in a continuous manner between 20°C and 90°C. We will determine optimal conditions for preparing competent cells and the regeneration of protoplasts of each strain. Plasmids will be isolated or constructed that are stably maintained in each of these strains. We intend to construct a shuttle plasmid(s) that can be transferred between strains whose combined temperature ranges for growth can be as great as 70 degreees. These techniques will facilitate genetic analysis of thermophilic bacilli; detect intraspecific transfer of genetic information; and clone desired genetic trails (e.g. cellulose hydolysis, ethanol production) from other thermophiles which lack a genetic exchange system and/or possess growth characteristics which make them unsuitable for research. Insights gained from studies with thermophilic bacilli may be used to devise genetic exchange systems in other thermophilic bacteria.

66. OHIO STATE UNIVERSITY
Columbus, Ohio 43210

DEVELOPMENT OF GENETIC SYSTEMS FOR ANALYSIS OF THE OBLIGATE ANAEROBE METHANOBACTERIUM RUMINATIUM PS — $67,000
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 using a variety of mutagens to isolate drug resistant and auxotrophic mutants of Methanococcus vannielli. A plasmid, pME2001, isolated form Methanobacterium thermoautotrophicum is being used to construct shuttle vectors. Plasmids capable of replication in the methanogen and in Escherichia coli, Bacillus subtilis and/or Saccharomyces cerevisiae are now available. A number of genes capable of complementing auxotrophic mutations of E. coli and, in one case (argA) also a B. subtilis mutation, have been cloned from methanogenic species. These genes are being incorporated into the shuttle vectors to provide selectable traits. Several of the cloned genes are being sequenced. The complete DNA sequences of a gene, isolated from Methanobrevibacter smithii, which complements E. coli proC mutations has been determined. Regions of DNA used to initiate and terminate transcription of this gene, in both E. coli and M. smithii, will be characterized. An insertion sequence which originated in M. smithii (ISM1) has been isolated and sequenced. We are determining the extent to which this element transposes in M. smithii. The insertion sequence will be modified by in vitro enzymatic procedures to create an element capable of introducing DNA into the genome of methanogens. Transformation procedures which are currently used to introduce DNA into bacterial species are being tested, under anaerobic conditions, with M. vannielli to determine if transformation of this species can be obtained using standardized protocols. Shuttle vector DNAs are used as donor DNAs. Successful transformation will be detected by DNA:DNA hybridization using nick-translated plasmid DNAs as the radioactive probes.

67. UNIVERSITY OF OKLAHOMA
Norman, OK 73019

METABOLISM OF FATTY ACIDS BY SYNTROPHOMONAS WOLFEI $47,011
Michael J. McInerney
Department of Botany and Microbiology

The degradation of fatty acids is often the rate-limiting step in the conversion of organic matter to methane and carbon dioxide. Recently, a new metabolic group of bacteria called the hydrogen-producing acetogenic bacteria has been discovered that anaerobically degrades fatty acids with hydrogen production. The slow growth rate of these bacteria and the fact that they can only grow in coculture with hydrogen-using bacteria is a hindrance to the further physiological characterization of this important group of bacteria.
We have developed methods to grow 10-liter batches of Syntrophomonas wolfei - Methanospirillum hungatei coculture. About 1.5 grams wet weight of cells of the coculture is obtained within 2 weeks. Cell-free extracts of S. wolfei are obtained by selectively lysing the S. wolfei cells with lysozyme-ethylene diamine tetraacetate and by removing the unlyzed methanogen cells by centrifugation. Quantitation of Factor-420 (a cofactor unique to methanogens) shows that about 98 percent of this material is removed by centrifugation. This indicates that the cell-free extracts of S. wolfei are essentially free of contamination by cellular components of the methanogen. High amounts of activity of 3-hydroxyacyl-CoA dehydrogenase were detected in S. wolfei extracts. Thus, this procedure yields extracts that are active and essentially free from contamination by cellular components of the methanogen. The methods will be used to study the metabolism and physiology of S. wolfei. Particular emphasis will be placed on the mechanisms of energy conservation and hydrogen production.

68. PENNSYLVANIA STATE UNIVERSITY
University Park, Pennsylvania 16802

THE ROLE OF TURGOR PRESSURE IN PLANT GROWTH $61,841
Daniel Cosgrove
Department of Biology

This project is investigating the dependence of plant growth on cell turgor pressure, especially as influenced by conditions of water stress. The growth of pea stems is being measured using marking techniques and high-resolution position transducers. Pea seedlings (Pisum sativum L.) are grown under various regimes of water stress. As less water is available to the plant, stem elongation is reduced. We are measuring each of the parameters governing cell growth to elucidate the relationships between water availability, cell turgor pressure and cell growth. Turgor pressure (hydrostatic pressure) in individual cells is directly measured with the micro-pressure probe, a device which also enables us to measure the membrane hydraulic conductivity and the cell volumetric elastic modulus - two physical properties of cells which influence the rate of water absorption. These measurements will be improved by use of a computer-assisted version of the pressure probe which is being constructed. The total solute concentration (osmolality) of sub-nanoliter droplets (approx. the volume of one large cell from the pea stem cortex) is being measured by cryoscopic osmometry. Current studies are aimed at determining whether cell turgor and the osmolalities of the intracellular and extracellular solutions are changed by mild water stress and, if so, how such changes relate to the reduction in growth. Future experiments will determine whether extracellular solutes serve as part of a turgor-regulating mechanism. A novel method for measuring the yielding properties of the cell wall (that is, wall extensibility and the yield threshold, or minimum turgor for cell expansion) by in vivo stress relaxation is being developed and tested. This method will be used to determine whether water stress alters cell wall properties in such a way as to inhibit growth.

69. UNIVERSITY OF PENNSYLVANIA
Philadelphia, PA 19104

FACTORS GOVERNING LIGHT DRIVEN ELECTRON AND PROTON TRANSLOCATION IN PROTEINS ACROSS MEMBRANES — $82,000

P. Leslie Dutton
Department of Biochemistry & Biophysics

Obligatory to respiratory and photosynthetic energy supply to the cell and tissue is the stage in which electron transfer and, coupled to the electron transfer, proton transfer is directed across the cytoplasmic membrane. In most membrane proteins little is known about these charge separations while in others, particularly the photosynthetic reaction center, rough details of the structural and energetic arrangements are starting to emerge. We are studying planar monolayers or proteins deposited using a Langmuir-Blodgett trough, on planar glass/electrode coated supports; these provide 10^2-10^3 times the surface density of protein encountered in the native membranes. We are measuring and manipulating electric reactions directly, with simultaneous spectrophotometric assay of the oxidation-reduction reactions. Using Langmuir-Blodgett techniques we are determining guidelines to prevent denaturation of proteins on the trough air-water interface, to obtain two-dimensional order, and to generate a greater degree of vectorial asymmetry (and therefore activity) of the protein in the films, as well as exploring different electrode materials and surfaces. We are now working mainly with the reaction centers from Rps. sphaeroides which transfer electrons (only) across the monolayers. With the native system at the current level of development, we have revealed that specific flash kinetics of the primary photosynthetic charge separative and recombination reactions can be modulated by electric fields. We are preparing to explore systems in which the native ubiquinone is replaced by quinones with systematically varying electrochemical properties, anticipating field effects descriptive of the charge separation and recombination dictated by the properties of the quinone. Fundamental insights to the function of these molecular electronic devices are anticipated. We are planning electrode design and film production for the proton pumping ubiquinol-cytochrome c oxidoreductase.

70. 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 — $79,934

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

The objective of this project is to identify and characterize proteins and corresponding genes associated with NaCl tolerance in cultured tobacco cells. We have identified several polypeptides associated with NaCl tolerance, and the levels of some of these (58 Kd, 37 Kd, 35.5 Kd, 34 Kd, 26 Kd, 19.5 Kd and 18 Kd) increase as cells become more adapted. A 26 Kd polypeptide comprises about 10% of the total cell protein in cells adapted to 25 g/l NaCl, and is virtually absent in unadapted cells. The 26 Kd protein is synthesized in response to exogenous abscisic acid (ABA) in the presence or absence of NaCl. When exposed to NaCl, unadapted cells begin sooner to synthesize the 26 Kd protein and grow in the presence of ABA than in the absence of ABA. Both the synthesis of 26 Kd protein and enhanced tolerance to NaCl is stable for several generations when cells adapted to 25 g/L NaCl are transferred to medium without NaCl. Quantitation of the 26 Kd protein is being carried out using antibody raised against 26 Kd protein isolated and purified from NaCl adapted cells. Antibody also will be used to isolate mRNA from polysomes in an in vitro translation system. The 26 Kd protein from adapted cells is a basic protein with a PI greater than 8.0. It is a proline rich hydrophobic protein. We have determined the N-terminal amino acid sequences (first 50 amino acids) of the 26 Kd protein and a synthetic nucleotide probe will be synthesized to isolate the 26 Kd protein gene(s) from a genomic library of tobacco. Subcellular localization of the 26 Kd protein is being determined and it appears not to be associated with the mitochondria or chloroplast. The major In vitro translation products of mRNA from unadapted and NaCl adapted cells do not appear different using the rabbit reticulocyte or wheat germ system. To help identify genes whose expression is associated with adaptation, cDNA libraries from mRNA from adapted and unadapted cells were prepared and are being screened.

71. PURDUE UNIVERSITY
West Lafayette, Indiana 47907

PRIMARY GENE PRODUCTS AS PLANT DEFENSES — $80,000
Donald E. Foard and Larry L. Murdock
Departments of Botany and Plant Pathology and of Entomology

The long-term objective of this research is an understanding of the biological interaction between insect proteinases and plant proteinase-inhibitors. Our model system is the cowpea weevil (Callosobruchus maculatus) on cowpea (Vigna unguiculata). Our studies are aimed at understanding in detail: (1) the vulnerable system in the insect, i.e., the digestive proteinase; (2) the method of recruitment of proteinase activity in the feeding cycle and the effects of various inhibitors on levels of proteinase activity; (3) the chemical nature and comparative biochemistry of proteinase inhibitors; (4) the effects on growth and development of individuals and populations of C. maculatus when it eats food containing inhibitors of its digestive proteinase. These experiments can best be performed with the seed-feeding C. maculatus, which is very amenable to studies in the laboratory. In the future, we shall attempt to adapt this model system to studies of leaf and root-feeding insects representative of pests of standing biomass.

72. THE ROCKEFELLER UNIVERSITY
New York, NY 10021

NUCLEAR GENES FROM NICOTIANA ENCODING THE SMALL SUBUNIT OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE — $100,000
Anthony R. Cashmore
Laboratory of Cell Biology

Two nuclear genes from pea encoding the small subunit of ribulose-1,5-bisphosphate carboxylase have been isolated and characterized. In collaboration with the laboratory of Drs. Schell and Van Montagu in Gent, Belgium, we have used Agrobacterium-mediated transformation to examine regulatory sequences in these genes A hybrid gene was constructed in which a 5' noncoding fragment from the small subunit gene SS36 was joined to the coding region of the bacterial gene encoding chloramphenicol acetyl transferase. This hybrid gene was shown to demonstrate light-inducible expression upon introduction into tobacco cells. Deletions of the 5' noncoding fragment have been prepared to further define the "inducer" sequences responsible for mediating light-induction.

A second type of hybrid genes have been constructed which contain the coding region for the small subunit transit sequence. These hybrid genes are being used in both in vitro and in vivo studies to examine the feasibility of translocating foreign polypeptides into chloroplasts.

We propose to extend the above studies by isolating small subunit genes from Nicotiana plumbaginifolia. The structure and expression of these genes will be characterized in a manner similar to our studies with the pea small subunit genes. Transformation studies with hybrid genes will also be carried out. Here, in contrast to the studies with the pea small subunit genes, we will be examining the effect of DNA regulatory sequences in their homologous cell type.

73. RUTGERS - THE STATE UNIVERSITY
New Brunswick, New Jersey 08903

CELLULASE - A KEY ENZYME IN FERMENTATION $55,230

Douglas E. Eveleigh and James D. Macmillan
Department of Biochemistry and Microbiology, Cook College

Cellulose is a major component of biomass and a potent energy resource. In this regard, the enzyme cellulase can be employed to convert cellulose to glucose, a product that can be fermented to a variety of energy-sparing products. The focus of this project is the selective detection of cellobiohydrolase using monoclonal antibodies. Cellobiohydrolase represents a major component of the cellulolytic complex produced by Trichodera reesei, and currently there are no direct methods to assay for it. Thus we have prepared monoclonal antibodies against purified cellobiohydrolase of Trichoderma reesei RUT C-30 by fusing spleen cells from an immunized mouse with myeloma cells (P3X 63Ag8653). Forty-four groups of hybridoma cells out of 180 fusates yielded a positive antibody to cellobiohydrolase. Three different hybridomas (8B3, 2A2, IC6) were chosen for characterization. These monoclonal antibodies were able to be used to detect cellobiohydrolase in both crude and purified cellulase preparations. In comparison, polyclonal antiserum developed against cellobiohydrolase reacts both with this protein and unfortunately also with several other proteins (other cellulase components?). We are attempting to clarify these differences by in vitro preparation of cellobiohydrolase. Here, autoradiographs utilizing monoclonal antibodies reveal one band migrating as cellobiohydrolase whereas polyclonal antiserum reacts with an additional five proteins. These results using the monoclonal approach appear to yield satisfactory specificity and will permit development of a specific assay for cellobiohydrolase.

74. SMITHSONIAN INSTITUTION
Washington, D.C. 20560

A PRIMARY LIGHT HARVESTING SYSTEM: THE RELATIONSHIP OF PHYCOBILISOMES TO PHOTOSYSTEM I AND II $62,162

Elisabeth Gantt
Environmental Research Center
Rockville, Maryland 20852-1773

It has long been known that energy absorbed by phycobiliproteins is transferred to photosystem II and is then distributed to I. Consequently, it has been predicted that phycobilisomes would be in close association with photosystem II. Our laboratory has succeeded in isolating phycobilisomes which have photosystem II activity, as measured by oxygen evolution, and reduction of dichlorophenol indophenol. In a special medium (0.5 M phosphate buffer, 0.5 M sucrose, 0.3 M citrate, and 10 mM magnesium chloride) the phycobilisomes remain intact, and the PS II activity is preserved. These preparations are deficient in the PS I reaction center pigment P700, as well as in the PS I polypeptide CP 1. Preparations of this system are being further characterized to determine the P680 content, to ascertain the DCMU-binding protein, cytochromes, and other polypeptides which are specific for PS II, and also for PS I. In addition, the sidedness of thylakoid membranes of red algae and cyanobacteria is being explored by immunocytochemistry (EM) and fractionation on acrylamide gels. Attachment of the phycobilisomes, and the location of the terminal emitter(s) of phycobilisomes, are integral parts of these studies. Results from these studies will aid in our understanding of the energy transfer in the photosynthetic apparatus of these groups of organisms.

75. SOUTHERN ILLINOIS UNIVERSITY
Carbondale, Illinios 62901

REGULATION OF ALCOHOL FERMENTATION BY ESCHERICHIA COLI — $62,000
David P. Clark
Department of Microbiology

The purpose of this project is to elucidate the way in which the fermentative synthesis of ethanol is regulated in the facultative anaerobe Escherichia coli. We have isolated a variety of regulatory and structural mutations. In particular we have used suicide substrates of both alcohol dehydrogenase and acetaldehyde CoA dehydrogenase to isolate mutants defective in the interconversion of acetyl CoA and ethanol. Analysis of these mutants together with protein purification has suggested that alcohol dehydrogenase and acetaldehyde CoA dehydrogenase are, in fact, separate enzymatic activities of the same protein. We are presently characterizing the ethanol synthesizing enzyme complex with respect to its metal ion requirements, pH optimum, inhibitors and molecular properties. Expression of the adhE gene which codes for alcohol dehydrogenase and acetaldehyde dehydrogenase is under the control of the nearby adhC control region and also of the newly discovered adhR gene which is distant from the adhCE operon. The regulatory interactions which affect expression of the adh genes are being investigated by means of gene fusions. A collection of gene fusions is being accumulated, in which the gene for β-galactosidase, lacZ, is fused to promoters which are inactive aerobically but active anaerobically. In addition, suicide substrates of alcohol dehydrogenase are being used for the direct selection of inserts of fusion forming λ lacZ phage into the adh gene.

76. STANFORD UNIVERSITY
Stanford, California 94305

GENETIC ENGINEERING OF CORN AND OTHER HIGHER PLANTS — $85,200
Ronald W. Davis
Department of Biochemistry

Our long term goal is to study and modify the expression of genes in maize. We want to understand the molecular basis for the exquisite specificity of gene expression with regard to cell type, timing during growth, pattern in tissue, amount produced, induction by hormones, and changes in response to physical and chemical stress. With this knowledge an exact program of regulation can be built in vitro for any gene to increase or decrease expression in a precise manner. We believe that modification of the expression of existing maize genes, as well as the introduction of novel genetic material, will be of great long term value in crop improvement to increase efficiency and number of usable products.

Our goal requires two research directions. First, we will analyze the structure of genes using existing and newly developed techniques for gene isolation and mapping. Second, we will analyze the function of genes in the homologous organism requiring the development of new techniques for reintroducing genes into maize.

77. STANFORD UNIVERSITY
Stanford, California 94305

DETERMINATION OF THE ROLE THAT THE ALCOHOL DEHYDROGENASE GENES (Adh1 and Adh2) PLAY IN FLOODING TOLERANCE, BY GENETIC ANALYSIS AND IN VIVO NMR SPECTROSCOPY — $60,000

Oleg Jardetzky
Stanford NMR Center

The potential for using the alcohol dehydrogenase genes (Adh) and gene products as biochemical markers for the selection of crop plants with increased flood tolerance will be assessed. This will be done by defining the physiological roles that Adh1 and Adh2 play in maize plants exposed to hypoxic environments; specifically, their role in ethanol synthesis and utilization, and their relationship to flooding tolerance. We will combine a comprehensive genetic analysis with methods, principally in vivo nuclear magnetic resonance spectroscopy, to monitor the metabolic activity of roots and other organs of maize during and after flooding. In the genetic analysis, using mutants, levels of Adh1 and Adh2 gene products will be varied over wide ranges, while keeping all other genes constant. We will quantitate tolerance to flooding as the ability to recover (e.g. growth, and normal metabolic functions) after periods of flooding. ^{31}P- and ^{13}C-NMR will be used to follow critical metabolic parameters (such as intracellular pH, phosphorylation potential and energy charge, and the accumulation of toxic metabolic end products) in intact tissues; both excised tissues and tissues in whole plants will be examined. The effect of varying Adh gene product levels on metabolism during and after flooding, and on flooding tolerance, will be determined. Such an analysis will allow unambiguous assignment of the role of the Adhs gene in flooding tolerance, and so let us determine if these genes and gene products can be used as markers for selection of plants that show increased yield in areas prone to flooding.

78. STANFORD UNIVERSITY
Stanford, California 94305

CLONING AND MAPPING OF EARLY SYMBIOTIC GENES OF RHIZOBIUM MELILOTI — $78,616

Sharon R. Long
Department of Biological Sciences

Biological nitrogen fixation has the potential to replace part of our consumption of energy-costly ammonia fertilizer. Symbiotic associations of Rhizobium and legumes form the most important source of agricultural biological nitrogen fixation. We are using genetics and molecular biology to study the symbiosis between Rhizobium meliloti and its plant host, alfalfa (Medicago sativa). We generated mutants of R. meliloti which cannot establish nodules on plants, and thereby identified the bacterial genes required for infection. We have used transposable elements to create 82 mutations in the nodulation DNA region, and have identified 4 complementation groups. Two of these A and B, are sharply defined and are located at the right side of an 8.7 kb EcoRI DNA fragment. Just to the left of these are two further groups of mutants: C, which is large and may contain more than one operon; and F, which contains two mutants showing a leaky (+/-) phenotype. These 4 groups of genes have been complemented by cloned nod gene DNA of other organisms, demonstrating that they are general or conserved invasion genes, rather than being used in host determination. In addition to the genes of the 8.7 kb EcoRI fragment, we have identified another nodulation gene region by complementation of mutant strain WL131. We have cloned this DNA region and have shown it is located near the other nod genes. To improve our ability to map R. meliloti genes, we have developed a transducing phage system and have demonstrated its use in correlating physical DNA distance with genetic data. We will continue to investigate the position of nodulation genes in R. meliloti during the next year.

79. STANFORD UNIVERSITY
Stanford, California 94305

CARBON DIOXIDE AND THE STOMATAL CONTROL OF WATER BALANCE AND PHOTOSYNTHESIS IN HIGHER PLANTS — $80,000

Eduardo Zeiger
Department of Biological Sciences

Stomatal regulation of gas exchange in leaves has an impact on photosynthesis and plant productivity. Stomatal conductance depends on the turgor relations of guard cells, which are modulated by active ion transport in response to environmental signals. Guard cells are thus multisensory devices transducing stimuli into metabolic events which result in optimal stomatal conductances under continuously changing environmental conditions. This research is aimed at the characterization of the stomatal responses to carbon dioxide and their role in the control of stomatal conductance and its coupling with leaf photosynthesis. Recent findings in our laboratory point to the guard cell chloroplast as a receptor site for the CO_2 signal in guard cells. Immunocytochemical studies with antibodies against the CO_2-fixing enzyme RUBP carboxylase showed that the antibodies bound to guard cell chloroplasts. The discrepancy between these data and previous biochemical studies pointing to a lack of the carboxylase in guard cell chloroplasts could indicate that the enzyme is present in an inactive form or that its activation requires hitherto undefined assay conditions. Current studies with isolated guard cell chloroplasts purified from protoplasts are providing direct evidence for photophosphorylation, a potential source of energy for stomatal opening. A modulation of photophosphorylation by CO_2 is a possible transducing mechanism for the stomatal responses to CO_2. Ongoing gas exchange studies of photosynthesis and stomatal conductance under varying CO_2 concentrations are testing these hypotheses in the intact leaf. Further progress should increase our understanding of the impact of stomatal function on plant productivity and enhance our ability to evaluate possible consequences of atmospheric CO_2 enrichment on the biosphere.

80. TEXAS A&M UNIVERSITY
College Station, Texas 77843

METABOLIC MECHANISMS OF PLANT GROWTH AT LOW WATER POTENTIALS — $33,867

John S. Boyer
Department of Soil and Crop Sciences

The goal of this new project is to understand the mechanisms regulating plant productivity when water is in limited supply, i.e., when plants have low water potentials (low Ψ_w). Our investigations will be both subcellular and organismal and will involve direct measurements of tissue water status that allow comparison with work from other laboratories. We have shown that productivity is frequently regulated by photosynthesis at low Ψ_w. Chloroplasts lose activity and often cause the change in photosynthesis. We will investigate how these losses occur and what chloroplast reactions are inhibited in sunflower (Helianthus annuus L.). First, we will test the effects of varying the cell content of inorganic ions, particularly Mg^{2+}, and the light intensity during dehydration because previous work has implicated these factors in the chloroplast response to low Ψ_w. Second, we will investigate the activity of chloroplast partial reactions in intact leaves using several spectral techniques to determine whether effects previously observed in isolated chloroplasts reflect changes occurring in vivo. Cell enlargement is important for root development and the reproductive process at low Ψ_w and will be studied as well. We will focus on the primary signals that alter growth rates. Particular emphasis will be placed on tissue turgor and water potential gradients as possible candidates for the primary signal because earlier work is not in agreement about the role of turgor of Ψ_w in growth at low Ψ_w. We will use soil-grown plants as well as a seedling culture system for soybean (Glycine max L. Merr.) and maize (Zea mays L.). Growth regulator mutants will be employed to determine whether abscisic acid and gibberellic acid play a role in the primary signals or as subsequent regulators of the growth response.

81. UTAH STATE UNIVERSITY
Logan, Utah 84322

BIOENERGETICS OF THE METHANOGENIC BACTERIA — $70,000
Jack R. Lancaster, Jr.
Department of Chemistry and Biochemistry

The research in our laboratory is directed toward delineating how energy is conserved by the methanogenic bacteria. We are specifically addressing the question of how phosphorylation is coupled to the eight-electron reduction of CO_2 to CH_4 by H_2 and to the conversion of formate to CH_4. We are initially attempting to answer whether the overall mechanism is Substrate-Level Phosphorylation (SLP) or Electron Transport Phosphorylation (ETP). Our efforts presently involve determining whether transmembrane ion gradients play a primary role in methanogenesis-driven ATP synthesis. We are utilizing both whole cells and subcellular preparations of M. voltae and M. thermoautotrophicum and are measuring the effects of dissipation of ion gradients (utilizing ionophorous antibiotics) on ATP synthesis as well as examining for the presence of a membrane-bound electron transfer complex (a requirement for ETP). We are also characterizing a very active ATPase activity in M. thermoautotrophicum, specifically with regard to adenine nucleotide metabolism and energy coupling.

82. Washington University
St. Louis, Missouri 63130

GENETIC ENGINEERING WITH A GENE ENCODING A SOYBEAN STORAGE PROTEIN — $65,000
Roger N. Beachy
Department of Biology

The goal of this project is to identify the nucleotide sequences that regulate the transcriptional expression of a gene encoding the a'-subunit of B-conglycinin, one of the globular storage proteins of soybean (Glycine max). One such gene, GMg 17.1, was isolated and its DNA sequence determined. The exonuclease Bal 31 was used to resect increasing amounts of DNA sequences in front of the gene since similar experiments with other eukaryotic genes demonstrated that these sequences controlled the expression of such genes. Nine mutants containing from 30 to 850 nucleotides 5' of the gene were recovered. Each of these mutant genes, as well as the non-mutant genes, has been ligated into the plasmid pMON 200, a shuttle vector (designed by scientists at Monsanto Company, St. Louis, MO) used to transfer desired genes into the Agrobacterium tumefaciens Ti-plasmid. Petunia and tobacco tumor cells and plants that result from transformation experiments with A. tumefaciens will be used to determine the degree of, and specificity of, expression of the soybean seed protein gene in its foreign environment.

In other experiments the structural region of Gmg 171 was ligated to a transcriptional promoter from cauliflower mosaic virus, and mobilized into petunia cells via the Ti-plasmid transformation system. Transformed cells were regenerated into whole plants. Storage protein mRNA was detected in transformed cells (tumor as well as regenerated plants), and we are currently analyzing protein from tumors, and leaves and seeds of the regenerated plants to determine that the mRNA was translated. These experiments were done to verify that the gene under study is not a pseudogene and that it indeed encodes an a' subunit of B-conglycinin.

83. WASHINGTON UNIVERSITY
St. Louis, MO 63130

HYDROXYPROLINE-RICH GLYCOPROTEINS OF THE PLANT CELL WALL (HPRGPCW) — $50,000

J. E. Varner
Department of Biology

Hydroxyproline-rich glycoproteins in plants apparently have structural roles and roles in cell-cell recognition. We are characterizing the chemical, physical, and biological properties of a wall hydroxyproline-rich glycoprotein that is one third protein and two thirds carbohydrate. The carbohydrate is 3% galactose and 97% arabinose. The protein is 46% hydroxyproline, 14% serine, 12% histidine, 7% lysine, 11% tyrosine and 6% valine and has an isoelectric point of about pH 11. The glycoprotein is 100% in the polyproline II conformation. It is a rigid rod 80 nm long. After secretion into the wall space the glycoprotein is slowly insolubilized apparently by the formation of isodityrosine cross-links. All preliminary evidence indicates that there are several closely related genes that may be presumed to code for several closely related precursors of hydroxyproline-rich proteins and that the bases coding for $serpro_4$ is a common sequence of these genes. The tissue distribution of the HPRGPCW promises clues about its function. All cells may have a small amount of HPRCPCW. The level increases dramatically when cells are 1) put into callus or suspension culture, and 2) when cells are wounded or infected. In seeds seed coats are by far the richest in HPRGPCW. Within the seed coat the outer one or two cell layers have the most HPRGPCW. We are 1) determing the amino acid sequence of this (these) protein(s) by a) sequencing cloned cDNA made against the purified mRNA, b) sequencing DNA from genomic clones selected by the cDNA, 2) characterizing the prolyhydroxydase that hydroxylates selected peptidylprolines, 3) trying to find which tyrosine residues are involved in cross-linking, 4) studying tissue distribution and the control of expression of the gene for this structural protein, and 5) beginning to examine the relatedness of the various hydroxyproline-rich glycoproteins.

84. WASHINGTON STATE UNIVERSITY
Pullman, Washington 99164

REGULATION OF TERPENE METABOLISM — $71,344

Rodney Croteau
Institute of Biological Chemistry, 6340

Oils and resins from plants are increasingly employed as replacements for petroleum-based industrial feedstocks, and the potential for the expanded use of these renewable resources is considerable. To realize this potential, fundamental knowledge of the biochemistry of oil and resin terpenes is needed, particularly as it applies to regulatory mechanisms at the enzyme level. The objective of this research program is to provide such an understanding through the intensive investigation of two model systems (camphor metabolism in Salvia officinalis and menthone metabolism in Mentha piperita) which allow probing the control of both biosynthetic and catabolic processes involved in monoterpene accumulation. The pathways of biosynthesis responsible for the accumulation of both monoterpenes during development are now established. At maturation, menthone produced in the leaves is reduced to neomenthol which is glucosylated and transported to the rhizome. At this site the glucoside is hydrolyzed, the aglycone oxidized back to menthone and the cyclic ketone cleaved by conversion to 3,4-menthone lactone. Modified β-oxidation of the carbon skeleton of the lactone affords acetate which is employed in the biosynthesis of acyl lipids and phytosterols of the rhizome. A lactonization step is also involved in the catabolism of camphor. Monoterpene transport from mature foliage can thus be explained as providing a carbon source to the developing rhizome in which recycling to other lipids via acetate occurs. A major focus of the research is on factors which may mediate developmental changes in the rates of biosynthetic and catabolic processes. The new information gained from this investigation will have important consequences for the yield and composition of terpenoid oils and resins that can be made available for industrial exploitation.

85. WASHINGTON STATE UNIVERSITY
Pullman, Washington 99164

DNA TRANSFORMATION OF PLANT CELLS DEFECTIVE IN NITRATE REDUCTASE ENZYME ACTIVITY — $45,000

Andris Kleinhofs and Paul F. Lurquin
Department of Agronomy and Soils and Program in Genetics and Cell Biology

We are cloning and characterizing the nitrate reductase genes from Escherichia coli and using these genes to transform nitrate reductase-deficient plant mutants. The objectives of this work are to develop plant cell transformation techniques by using the nitrate reduction trait as a selectable marker and to gain insight about the genetic control of nitrate assimilation in higher plants. The work to date has concentrated on the chlA locus. This gene was cloned and subcloned into two small regions each containing a different gene capable of correcting different E. coli chlA mutations. These data indicate that the chlA locus is complex and consists of at least two cistrons. One of the subclones was sequenced in order to permit construction of a hybrid gene for expression in plant cells. These constructs will be tested for transformation of nitrate reductase-deficient plant mutants. These experiments will tell us which plant genes are homologous in function to which E. coli genes. Direct hybridization homology was tested and determined to be negative. Similar work with the other E. coli nitrate reductase-deficient genes will allow us to identify the corresponding genes in higher plants. This relationship between the plant and bacterial genes is important to establish to allow more detailed investigation of these gene functions. Nitrate reductase-deficient mutants are available in dicotyledonous as well as monocotyledonous higher plants. The dicotyledonous plants can be transformed with Agrobacterium based vectors. We will use gene constructs that have been shown to work with dicotyledonous plants to learn how to transform monocotyledonous plant species. These techniques will be important in the genetic engineering of monocotyledonous species which constitute most of our major crop plants.

86. UNIVERSITY OF WASHINGTON
Seattle, Washington 98195

STUDIES ON THE CONTROL OF PLANT CELL ENLARGEMENT BY CELLULAR PARAMETERS — $67,348

Robert E. Cleland
Department of Botany

The goal of this research is to determine how plant cell enlargement is controlled at the cellular level. In many stem and coleoptile cells, the hormone auxin causes the cells to excrete protons, and the resulting lowered wall pH is then responsible for wall loosening and cell enlargement. Auxin is believed to exert this effect by activating a plasma membrane ATPase (PM-ATPase) in some fashion. We have been studying the effect of auxin on isolated PM-enriched fractions of membrane vesicles from pea roots, using fluorescence quenching to follow ATP-driven proton transport. We have demonstrated that auxin causes a significant reduction in the Km for ATP, so that the ATPase is more efficient in utilizing low levels of ATP, and have shown that this is specific for active auxins. To study this more effectively, we have been developing procedures for solubilizing the ATPases and reconstituting them into synthetic vesicles. This system has been used to study the effect of the phytotoxin fusicoccin (FC) on ATP-driven proton transport. After solubilization of membrane proteins with deoxycholate, the proteins are incubated with FC, then reconstituted into vesicles. The FC-pretreated vesicles have a significantly reduced Km for MgATP, with no change in Vmax. Our second project has been to develop a new technique for measuring the yield threshold (Y)--the turgor that must be exceeded if walls are to extend. Tissues are excised, treated for 30 min with FC, then after removal from a water source their turgor (P) is measured with a micro-pressure probe or with a psychrometer. Within 1-2 hours, P falls until it reaches Y, and then stabilizes. The growth-effective turgor (P-Y) can also be measured by this technique.

87. UNIVERSITY OF WASHINGTON
Seattle, Washington 98195

GENETIC ENGINEERING OF YEASTS FOR FERMENTATION OF XYLOSE TO ETHANOL — $70,000

Benjamin D. Hall and Clement Furlong
Department of Genetics

A major constituent of hemicellulose is xylose which occurs in all woody plant tissues. Our main objective is to construct yeast strains that are capable of growing fermentatively on xylose. Both Saccharomyces cerevisiae and Schizosaccharomyces pombe can convert the 2-ketopentose xylulose to ethanol, but are unable to ferment xylose. This is due to a natural inability to convert xylose into xylulose. The introduction of the xylose isomerase gene, which mediates this reaction, into these yeasts should enable them to grow fermentatively on xylose. We have cloned the xylose isomerase structural gene from Escherichia coli and determined its sequence (Schellenberg et al. JBC, in press). Direct evidence that the gene cloned codes for the xylose isomerase protein was provided by a comparison between a partial NH_2-terminal amino acid sequence and the DNA sequence which were found to be in complete agreement. The xylose isomerase gene has been fused to promoter fragments from the ADHI and ADH genes from S. cerevisiae and S. pombe, respectively. Yeast transformants containing the fused ADH-xylose isomerase gene have been isolated and are being studied both for the production of xylose isomerase enzyme and the ability to utilize xylose. The transformants produce detectable amounts of xylose isomerase cross reacting material as determined by Western blot analysis using antibodies raised against purified xylose isomerase protein. Studies on the pentose metabolism of these transformants are now in progress.

88. UNIVERSITY OF WASHINGTON
Seattle, Washington 98195

GENETICS IN METHYLOTROPHIC BACTERIA — $70,000

Mary E. Lidstrom
Department of Microbiology and Immunology

The goal of this project is to determine the organization of genes necessary for growth on one-carbon compounds in a facultative methanol utilizer, Pseudomonas AM1, and an obligate methane-utilizer, Methylosinus sporium. We are isolating C-1 specific genes by complementing P. AM1 methanol mutants with clone banks constructed in broad host range vectors. The complementing clones are characterized by restriction mapping, and C-1 specific genes are defined by a combination of transposon mutagenesis and subcloning. Expression studies are being used to determine gene-polypeptide relationships. DNA-DNA hybridization will be used to compare relatedness between genes encoding similar functions in these two methylotrophs.

89. UNIVERSITY OF WISCONSIN
Madison, WI 53706

ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE — $40,255
Jerry L. Kermicle
Department of Genetics

Alleles of the R gene confer specific patterns of anthocyanin pigmentation to tissues of the corn plant and seed. We seek to identify, map and characterize the interaction between components which govern the presence, distribution, intensity, and timing of pigmentation. Mutational and recombinational analyses of alleles existing in geographically diverse races serve to characterize variation at two levels. The R gene is represented more than once in some alleles due to chromosome segment duplication. Each representation (genic element) functions independently, governing pigmentation of particular plant or seed parts. Individual genic elements are subdivided by recombination into regions which are responsible for the tissue-specific action of that element and other regions which can be substituted between elements of contrasting tissue-specific effects. Spontaneous mutations as well as variants isolated following chemical mutagenesis and insertion of transposable elements are utilized in the analysis. These studies provide a map of R pigmenting components which incorporates variation in the number and kind of genic elements. We are characterizing other phenomenon of R gene regulation, such as paramutation, in similar terms. We want to relate the components for such phenomena to the map of basic pigmenting determiners.

90. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

STARCH SYNTHESIS IN THE MAIZE ENDOSPERM AS AFFECTED BY STARCH-SYNTHESIZING MUTANTS — $56,303
Oliver E. Nelson
Department of Genetics

During the past year, our major effort has been concentrated on an examination of the oligosaccharide synthase activities present in developing maize endosperms. These synthases (one starch granule-bound and the other soluble) utilize glucose-1-PO_4 as a substrate and synthesize maltodextrins of not more than ten glucose units. Our results indicate that the initial products are phosphomaltodextrins. Phosphorylase can use the oligosaccharides formed by these enzymes as primer molecules to which glucose units are added, and it is possible that these enzyme systems supply the primer molecules used by all starch-synthesizing enzymes. A report of these results is now ready for submission. The debranching enzyme deficiency in the sugary maize mutants has been reported in PLANT PHYSIOLOGY (1984, 74, 324-328).

91. UNIVERSITY OF WISCONSIN-MADISON
Madison, Wisconsin 53706

CARBON ISOTOPE FRACTIONATION IN PLANTS — $73,500
Marion H. O'Leary
Department of Chemistry

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 the 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. We also develop other methods for using stable isotopes to study plant metabolism. We have developed an isotopic method to measure the activity of carbonic anhydrase and fumarase in vivo in CAM plants. We have measured the carbon isotope fractionation associated with the diffusion of CO_2 in aqueous solution. We are currently developing methods for measuring carbonic anhydrase and fumarase activity in C4 plants. We are continuing to measure the carbon isotope fractionation associated with CO_2 fixation in CAM plants in order to determine the variation in the fractionation with environment and physiology. We are also constructing a total isotopic carbon balance for CAM plants. We are also beginning studies of isotope fractionation in C4 plants.

92. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

PHYTOCHROME FROM GREEN PLANTS: ASSAY, PURIFICATION AND CHARACTERIZATION — $55,000
Peter H. Quail
Botany Department

This project is directed toward assaying, purifying and characterizing phytochrome from chlorophyllous tissue of both terrestrial and aquatic plants. The approach involves (a) the use of polyethyleneimine (PEI) to remove chlorophyll from phytochrome-containing extracts, thereby permitting spectroscopic characterization; and (b) the use of immunochemical procedures coupled with proteolytic mapping for further characterization. The peak position of Pr from green Avena tissue is shifted some 15 nm to shorter wavelength relative to its etiolated-tissue counterpart. Western blot analysis demonstrates the presence of two species of phytochrome in this tissue: a minor polypeptide of 124 kilodaltons (kD) and a major polypeptide of 118 kD. Immunoprecipitates contain all of the 124 kD species with >95% of the 118 kD species remaining in the supernatant. The 118 kD species is structurally distinct from etiolated-oat phytochrome: peptide maps of 118 kD green-oat phytochrome are substantially different from those of etiolated-oat phytochrome and the two phytochromes exhibit differential antigenicity toward monoclonal antibodies. The significance of these data is that they indicate that green-oat tissue contains two distinct molecular species of phytochrome - a minor species resembling the 124 kD molecular from etiolated tissue and a major species previously uncharacterized.

93. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

MOLECULAR MECHANISM OF ENERGY TRANSDUCTION BY PLANT MEMBRANE PROTEINS — $58,401

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 which is essential for the uptake of minerals and nutrients. The 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 and bacterial cells. 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. In addition to its active site involved in ATP hydrolysis, a previously uncharacterized ion binding site is being probed, using the irreversible carboxyl-modifying reagent, dicyclohexylcarbodiimide. A procedure has been developed to purify the hydrophobic ATPase and its cleaved fragments by reverse-phase high pressure liquid chromatography. By solid- and gas-phase protein sequencing techniques, the amino acid sequence surrounding this site will be determined. Current studies focus on plasma membrane H^+-ATPase purified from two species: the mycelial fungus, Neurospora crassa, and the higher plant, Avena sativa. We find that the two enzymes share similar reactivity to active-site directed reagents. Work is in progress to determine which amino acids are conserved in the sequence in the active sites of the enzyme from these two species. Results from this study will be used to define a molecular mechanism by which this protein converts chemical energy into electrical energy.

94. UNIVERSITY OF WISCONSIN-MADISON
Madison, Wisconsin 53706

ONE CARBON METABOLISM IN ANAEROBIC BACTERIA: ORGANIC ACID AND METHANE PRODUCTION — $88,000

J. G. Zeikus
Department of Bacteriology

We are engaged in understanding one and multi-carbon metabolism by acetogenic and methanogenic bacteria. Our studies with the acetogen, Butyribacterium methylotrophicum, have focused on the elucidation of the enzymes, carbon flow, and the electron and carbon carriers of the pathways of acetate and butyrate synthesis during growth on substrates such as glucose, pyruvate, methanol, formate, carbon monoxide, and hydrogen-carbon dioxide. Specifically, enzymes dealing with one carbon oxidation/reduction reactions, glycolysis, and electron transfer are being assayed in the organism after growth on various substrates. Electron carriers are being identified using spectral and chromatographic techniques. Carbon flow into end products is traced using ^{13}C nuclear magnetic resonance spectroscopy. In our studies with the methanogen, Methanosarcina barkeri, we are investigating the catabolism of acetate with a soluble in vitro system for methane production from this substrate. Using inhibitors, ^{14}C tracers, and extract fractionation, we are indentifying possible enzymes and coenzymes involved in the pathway. This work has led to a model of ATP synthesis coupled to methanogenesis from acetate. Electron flow and hydrogenase function during growth on non-gaseous substrates is also being investigated.

95. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

CHLOROPHYLL-PROTEIN COMPLEXES: PHOTOREGULATION OF TRANSCRIPTION, STABILITY AND PHOSPHORYLATION — $130,000

John Bennett
Biology Department

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

96. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

PLANT MOLECULAR GENETICS — $210,000

Benjamin Burr and Frances A. Burr
Biology Department

The primary focus of this laboratory is an investigation of the phenomena associated with the activity of transposable genetic elements in maize. These phenomena are also known in other crop plants and are of economic importance. When plants are regenerated from tissue culture, heritable variation is observed in the progeny. Mutations at the shrunken locus occur about ten times more frequently than in control plants. Although we have demonstrated that endogenous regulatory elements can be activated by this process, the majority of mutant alleles contain no detectable rearrangements at the DNA level. We conclude that in this system some mechanism other than transposable elements is responsible for most of the mutations. If this finding can be generalized, it means that the use of tissue culture to generate variability is much less efficient than chemical mutagenesis. Studies are continuing to determine the mechanism of mutagenesis. A high degree of variability is known to persist in some commercially imporant cultivars after many generations of inbreeding. Because the biological basis for this phenomenon is unknown, this problem leads to difficulties for seed certification. We are attempting to see if transposable elements are involved in these instabilities. Shrunken and waxy mutants have been selected in these stocks and mutant alleles are being examined at the DNA level to determine whether insertional mutations have been obtained. A mutator line has been identified by Professor D. S. Robertson at Iowa State University. Four new shrunken mutations generated in this stock have four different insertional elements, one of which is strain specific.

97. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

MECHANISMS OF ENERGY CONVERSION IN PHOTOSYNTHESIS — $280,000
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 in 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. In cyclic flow, electrons return to the cytochrome complex via ferredoxin-$NADP^+$ reductase, which is non-covalently bound to its stromal surface. The structures of the complexes and binding of reductase are studied using cross-linking reagents. The protein kinase activity of reductase is being evaluated for its possible in vivo role in state transitions, which control apportionment of excitation energy, from light-harvesting chlorophyll, between photosystems. 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. 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.

98. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

THE CHARACTERIZATION OF MODIFIED T-DNA GENES AND HOW THEY ARE EXPRESSED IN TRANSFORMED PLANT TISSUE — $150,000
Daniela Sciaky
Biology Department

The T-DNA of Agrobacterium tumefaciens provides an excellent model system for the study of how genes are expressed in plants. We are studying two spontaneous mutations in the tms locus of the T-DNA, both which result in the formation of tumors that make shoots. One spontaneous mutation has occurred in the T-DNA after transformation of Nicotiana otophora by pTiB6806 and has resulted in the deletion of a region of the tms locus of the T-DNA. The second mutation is due to the insertion of IS66, a 2.5 kb insertion sequence, in the T-DNA of pTiA66. The site of insertion of IS66 has been localized to within the structural region of the gene for tms transcript 2. Sequencing of IS66 has shown that it contains several open reading frames, including one frame that is 1600 nucleotides in length. Studies are now underway to determine whether IS66 is transcribed in plants and whether it can act as a transposable element in plants.

99. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

THE PHYSIOLOGY AND BIOCHEMISTRY OF CYANOBACTERIA — $200,000
Harold W. Siegelman
Biology Department

Cyanobacteria are cosmopolitan organisms of soils, marine, and freshwaters. They are frequently responsible for water blooms and can cause serious water management problems in rivers, lakes, and reservoirs. The success of cyanobacteria in nature is probably associated with an efficient photosynthetic mechanism, frequent possession of gas vacuoles permitting vertical migration in the water column, and ready adaptability to varying environmental factors. The molecular structure and composition of the photosynthetic energy collection systems including phycobilisomes, chlorophyll proteins, and carotenoids are being characterized in several cyanobacterial species. Phycobilisomes from cells grown at several light intensities and spectral qualities are purified by hydrophobic-interaction chromatography, salt precipitation, and gel chromatography. Their apparent mass and structure are being characterized by electron microscopy, gel permeation, and absorbance spectroscopy. HPLC and rapid scanning spectrophotometry will be used to determine the phycobiliprotein stoichiometry of the phycobilisomes. Three-dimensional space-filling models of phycobilisomes are being constructed by computer. Antibodies to highly purified phycobiliproteins are being raised to initiate a molecular biology examination of the control mechanisms regulating phycobilisome composition. Cyanobacteria grown at several light intensities and qualities show profound morphological and fine structure changes which are being examined by electron microscopy. HPLC methods are used to purify the peptide toxins of Microcystis aeruginosa. They are also being used to develop a quantitative analytical assay for the toxins. The pathological and hematological effects of the toxins are being examined with mice.

100. LAWRENCE BERKELEY LABORATORY
Berkeley, California 94720

LIGHT REGULATION OF NUCLEAR AND CHLOROPLAST GENE EXPRESSION IN Euglena gracilis — $55,000
James C. Bartholomew
Laboratory of Chemical Biodynamics

Even though the chloroplasts of photosynthetic organisms contain their own genome, many of the functions carried out in the organelle are encoded in the nucleus. Some of the proteins which function in chloroplast are encoded partially in the chloroplast and partially in the nucleus. The expression of both these genomes is regulated by light. Light stimulates mRNA synthesis as well as DNA synthesis in Euglena gracilis. Presumably, this light response is mediated by photopigments in the cells which receive the light and convert it into a signal which controls nucleic acid activity in both the genome compartments. We have a series of bleached mutants of Euglena which lack various photopigments as well as have different levels of chloroplast DNA. We are using these mutants to study the involvement of the photopigments and chloroplast DNA in the stimulation of nuclear DNA and RNA synthesis. First, we are characterizing the cell cycle response of these various mutants by flow cytometry. Wild type Euglena conditioned in the resting medium in the dark can be stimulated to reenter the cell cycle by light. We are comparing the kinetics of reentry into the cell cycle of the bleached mutants with that of the wild type to discern the role of the chloroplast supplied functions in regulating the DNA replication response in the nucleus. We are also comparing the light stimulated expression of nuclear encoded chloroplast functions in the wild type vs bleached mutants to determine if the chloroplast plays a role in the regulation of the nuclear genes. We have begun to map the nuclear genes for the light harvesting chlorophyll a/b proteins in Euglena to determine if polymorphisms exist for this/these polypeptide(s). All of these studies are designed to help us understand the factors involved in the coordinate regulation of the genes in the two physically distinct compartments.

101. LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA, Berkeley, CA 94720

PLANT BIOCHEMISTRY: REGULATION OF PHOTOSYNTHETIC METABOLISM AND SECONDARY BIOSYNTHESIS — $352,000

James A. Bassham, Henry Rapoport
Chemical Biodynamics Division, Lawrence Berkeley Laboratory

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. Gene expressions resulting in syntheses of pyruvate phosphate dikinase (PPDK) and of isoforms of phosphoenolpyruvate carboxylase (PEPC) are examined as a function of C_4 and C_3 metabolism, tissue type (leaf and seed), and conditions of cells in tissue culture (whether differentiating or non-differentiating). In the case of PPDK, in vitro translation of mRNA from plant tissues and determination of molecular weight of the resulting polypeptide is used to determine whether chloroplast or cytoplasmic type PPDK is being expressed. 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.

102. LAWRENCE BERKELEY LABORATORY
University of California
Berkeley, California 94720

MOLECULAR GENETICS OF THE PHOTOSYNTHETIC GENES OF RHODOPSEUDOMONAS CAPSULATA — $133,000

John E. Hearst
Chemical Biodynamics Division

The goal of this project is a detailed molecular understanding of the primary chemical events in the storage of light energy by the photosynthetic purple non-sulfur bacterium, Rhodopseudomonas capsulata. In our initial efforts to achieve this goal we have sequenced the genes for the reaction center subunit polypeptides H, M, and L. Comparison of these sequences with those of the psbA and psbD loci of the chloroplast genomes of Chlamydomonas reinhardii and of these same loci in higher plants such as spinach and pea has established that the D1 and D2 chloroplast proteins which are coded by these loci contain sequences of 31 amino acids about 60% in from the amino termini of these proteins which are similar to the sequences at the same positions which we determined in the L and M subunits of the bacterial reaction centers. We have postulated that these sequences, which have been conserved during the three billion years of evolution since the divergence of the purple non-sulfur bacteria and higher plants, are functionally related to quinone binding in the reaction centers of these bacteria as well as the reaction centers of photosystem II in photosynthetic eukaryotes. We have synthesized nucleic acid oligonucleotides which code for these two highly conserved sequences and we intend to probe for equivalent genes in a number of other photosynthetic species, including the thermophilic cyanobacterium Synechococcus sp. and perhaps the primitive organism Chloroflexus aurantiacus. The objective here is establishing the phylogenetic relationships between the many photosynthetic organisms. We are engaged in a study of quinone binding and electron transport in the isolated R. capsulata reaction center complexes of wild type and mutant strains. We are continuing to generate photosynthesis mutants which we typically generate by Tn5.7 transposon mutagenesis. The physical characterization of these mutants is being worked on in collaboration with the laboratory of Professor Kenneth Sauer.

103. LAWRENCE BERKELEY LABORATORY
Berkeley, California 94720

CHARGE SEPARATION IN THE PHOTOSYNTHETIC LIGHT REACTIONS — $290,000
Kenneth Sauer , Melvin Klein
Chemical Biodynamics Division

The photosynthetic light reactions begin with the absorption of photons by chlorophyll and other pigment molecules followed by transfer of excitation to reaction centers where primary charge separation takes place. We are measuring the time course of these processes in the picosecond to microsecond time domain using fluorescence decay kinetics and time-resolved electron paramagnetic resonance (EPR) spectroscopy. The fluorescence decay from intact photosynthetic membranes consists of several exponential components that fall into two classes: those yields that are sensitive to the open or closed state of the reaction centers and those that are not. The insensitive components, which are typically faster (50-500 ps) reflect excitation loss during the process of transfer from the pigments that absorbed the light toward the reaction centers. Under favorable circumstances we have also been able to detect the rise or induction of this fluorescence, which is a direct indication of these transfer times. We are seeking to understand the mechanism of this excitation transfer process. By combining the observations of reaction center-sensitive fluorescence with measurements of the kinetics of appearance and decay of characteristic EPR signals, we can monitor the initial stages of the charge separation process. In this manner we have identified several of the molecular components involved in the reaction center complexes. Through analysis of the amino acid sequence of the reaction center polypeptides from the photosynthetic bacterium Rps. capsulata and comparison with that from a chloroplast thylakoid protein, we have identified a distinctive oligopeptide segment that we believe is the binding site for quinone electron acceptors. We hope to determine the important aspects of intermolecular interactions and geometric relations that enable these photosynthetic components to function with very high energy conversion efficiency.

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

PHOTOCHEMICAL CONVERSION OF SOLAR ENERGY — $117,000
Lester Packer, Rolf J. Mehlhorn, Alexandre T. Quintanilha
Membrane Bioenergetics Group, Applied Sciences Division

The goal of this research is to identify the molecular mechanism of the photochemical activity of bacteriorhodopsin, an extremely stable and simple energy-transducing membrane protein which directly couples absorption of visible light to the production of proton and electrical gradients across membranes of halobacteria. Bacteriorhodopsin is studied in its natural membrane, in isolated purple membrane sheets and after reconstitution into liposomes. In the next phase of research, the role of light-induced surface potentials will be further defined and compared by using an ESR spin probe method and laser Doppler velocimetry (electrophoretic light scattering). In native and chemically modified purple membranes, carboxyl groups will be cross-linked to lysine residues under conditions favoring either intra- or intermolecular bonds to study the effect of conformational constraints on function and the relationship of molecular segments to one another. Chemical modification and cross-linking studies of the hydrophilic tail of bacteriorhodopsin will be undertaken to determine its involvement in function. If there is a precise stoichiometry for proton release and uptake by bacteriorhodopsin, correlating with the photocycle, then it will be possible to formulate molecular mechanisms. Results to date indicate variable stoichiometry for purple membranes in suspension. We have recently shown, by quasi-elastic light scattering and electron microscopy, that purple membranes are rarely in suspension as free flattened sheets, but more often exist as stacks of membranes. A thorough investigation of factors which effect the uniformity of purple membranes in suspension will allow more accurate measurement of the stoichiometry.

105. LAWRENCE BERKELEY LABORATORY
University of California

RESONANCE STUDIES IN PHOTOSYNTHESIS — $64,000
Alan Bearden
Division of Biology and Medicine

This research is aimed at understanding the biophysical processes important to both primary reactions in green-plant photosynthesis and electron-transfer in subsequent "dark" bioenergetic electron-transport. Techniques include electron paramagnetic resonance and optical spectroscopy. Continuing experiments using laser-initiated photochemical conversion and oriented chloroplast membrane fragments and reaction-center mutants of Chlamydomonas are underway in an effort to gain further information about the order of photochemical reactants (donors and acceptors) and their inter-relation. Tunneling as a method of electron-transfer has long been implied in bioenergetic reactions in both photosynthesis and other pathways. Following a successful demonstration of a Hopfield charge-transfer band in Chromatium (Proceedings of the National Academy of Sciences 81, 135-139 (1984)) we are continuing both theoretical and experimental studies of the role of multicomponent vibrational systems in biophysical processes. These more physical studies may have a range of applicability in such seemingly disparate topics as enzyme specificity, fundamental limits on energy-transduction processes, and the role of coherence in light absorption by important macromolecules.

106. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

ANALYSES OF PHOTOSYNTHETIC MEMBRANES — $215,000
Charles J. Arntzen
MSU-DOE Plant Research Laboratory

The goal of our research is to identify cellular factors which limit biomass productivity by limiting the quantum efficiency of photosynthesis (especially in plants growing in non-optimal environments). Our approach is to use biophysical and biochemical analyses for characterization of isolated photosynthetic membranes from green plants, algae, and photosynthetic bacteria. We have devised protocols which allow us to subfractionate these membranes so that individual pigment-proteins (chlorophyll and carotenoids non-covalently associated with unique polypeptides) and enzymatic complexes can be purified and characterized. We are particularly interested in the genetic controls which regulate synthesis, turnover, and post-translational modification of these membrane components. Our current research emphasis is to characterize cellular controls of the stoichiometry of pigment-proteins and reaction center components of chloroplast membranes, and to define mechanisms which regulate the assembly of supramolecular protein complexes in these membranes. The long range goal of these efforts is to identify genetic or cultural means by which photosynthetic efficiency can be increased, especially in plants suffering from environmental stress. Our shorter term emphasis is in three specific areas. We are investigating: a) the polypeptides of the photosystem II complex of higher plant chloroplasts which participate in the water oxidizing reactions of photosynthesis; b) the assembly and post-translational modifications of the chlorophyll b-containing light-harvesting pigment proteins; and c) the properties of quinone binding proteins of photosynthetic membranes with special emphasis on the molecular characterization of quinone or quinone analog "target sites".

107. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

DIFFERENTIAL GENE EXPRESSION IN RHIZOBIUM — $165,000

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 on the R. japonicum/soybean system. Specific genes whose expression is regulated during nodule development or by other physiological changes and genes involved in the regulation of these expression changes have been isolated. These genes have been isolated either by hybridization screening procedures utilizing analogous genes from other organisms as hybridization probes or by complementation of genetically defective bacterial strains by cloned R. japonicum DNA. The genes isolated thus far include those encoding the nitrogenase subunits, glutamine synthetase I, the large subunit of ribulose bisphosphate carboxylase, delta-aminolevulinate synthetase, adenylate cyclase, and a nif regulatory gene termed nifA. 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. In addition, R. japonicum strains carrying mutations in these genes are being constructed by site-directed mutagenesis procedures in order to more precisely define their roles in nodule differentiation, nitrogen fixation and physiological adaptation as a whole. These further understandings of these processes should enlighten the viewpoint from which the symbiotic relationship might be further optimized and manipulated.

108. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

RESISTANCE OF CROP PLANTS TO ENVIRONMENTAL STRESS — $160,000

Andrew D. Hanson
MSU-DOE Plant Research Laboratory

This project concerns three metabolic pathways that are activated by stresses: betaine synthesis, promoted by water-and salt-stress: lactate synthesis, induced by O_2-deficit; and gramine synthesis, elicited by high temperature stress. These stress-activated pathways may be metabolic adaptations for coping with stress; if we knew this to be true, and if the pathways were understood genetically and biochemically, natural or induced genetic variation for such pathways could be useful in breeding stress-resistant crops. (1) Betaine. To evaluate the adaptive worth of betaine accumulation, barley isopopulations high and low in betaine are being developed, in order to test their performance under salt-and water-stress. We are also screening an M_2 population of barley for betaine nulls. The biosynthesis of betaine is being studied by supplying the precursor [^{14}C]choline to spinach leaf protoplasts and subcellular fractions therefrom. We have found that chloroplasts oxidize [^{14}C]choline to betaine, and that this oxidation is promoted by light. We are now attempting to isolate the choline-oxiding system from chloroplasts. (2) Lactate. We have shown that anaerobic barley roots can accumulate lactate, and that these roots have anaerobically-inducible lactate dehydrogenase (LDH) activity which can be resolved on native gels into a set of isozymes. We will produce polyclonal antibodies against LDH, in order to investigate synthesis of LDH peptides during anaerobiosis, and to help in the future isolation of plant Ldh genes. (3) Gramine. The indole alkaloid gramine is a defensive compound found in barley leaves; it is derived from tryptophan via a pathway that includes two N-methylation steps. Heat stress increases N-methyltransferase (NMT) activity specific for these steps. We are attempting to purify the NMT activity, firstly to find whether there are separate NMT's for the two methylation reactions and secondly in order to produce antibodies.

109. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

ACTION AND SYNTHESIS OF PLANT HORMONES — $210,000
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 conversion of ACC to ethylene. We were able to show that this reaction proceeds in isolated vacuoles. We are now trying to define the nature of the ethylene-forming system and we are trying to reconstitute it from homogenates. Ethylene action is being studied in rice where ethylene induces rapid growth.

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. Ultimately, we hope to find out how cytokinins delay senescence and couteract symptoms of plant stress.

110. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

CELL WALL PROTEIN — $125,000
Derek T.A. Lamport
MSU-DOE Plant Research Laboratory

Our major goal is elucidation of the role played by the hydroxyproline-rich cell wall glycoprotein extensin. Recently we demonstrated in muro pools of two monomeric extensin precursors (designated P_1 and P_2), and a new crosslinked amino acid, isodityrosine (IDT) present in extensin, but absent from the precursors. This led to the "warp-weft" model of the primary cell wall which postulates a highly crosslinked extensin network ("weft") penetrated by a cellulose microfibrillar "warp".
We propose to characterize the "warp-weft" model by sequencing the monomeric extensin precursor to determine probable crosslink domains and their frequency, hence defining network porosity.
Other approaches involve attempts to establish a precursor crosslink-generating system in vitro so that, by subtractive peptide mapping, we can compare this with partially crosslinked extensin extracted from growing cell walls. We shall also attempt direct electron microscopic and in muro electron microscopic observation of extensin precursor and crosslinked extensin, moving towards the ultimate question: Do cellulose microfibrils indeed penetrate the pores of a highly crosslinked protein network?

111. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

REGULATION OF FLOWERING — $90,000
Anton Lang
MSU-DOE Plant Research Laboratory

This program deals with regulation of flower formation by hormone-like (i.e. translocatable) substances, the current main focus being on inhibitors of flower formation ("antiflorigen"). The existence of antiflorigen has been conclusively demonstrated, in previous research under this program, by grafting experiments. 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) conditions. This physiological evidence strongly indicates that both antiflorigen and florigen are ubiquitous in flowering plants, at least monocarpic herbaceous ones. The next logical step would be isolation and chemical identification of these compounds. 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. In addition, work has been initiated with several inhibitors of gibberellin biosynthesis (growth retardants), which have recently become available and are considerably more effective than those previously used, in order to determine to what extent gibberellins are essential in the regulation of flower formation.

112. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

INTERACTION OF NUCLEAR AND ORGANELLE GENOMES — $165,000
Lee McIntosh
MSU-DOE Plant Research Laboratory

In the development and maintenance of a photosynthetic apparatus in higher plants and other photosynthetic organisms there is an ordered series of events which lead to the ability to form complex carbon compounds from gaseous carbon dioxide and light energy: photosynthesis. The scope of the research involved in this task is to understand the underlying mechanisms of gene regulation, especially those genes whose products are involved in photosynthetic reactions, and to develop methods which make it possible to approach the future improvement of heritable characteristics in higher plants. This research is designed to delineate: 1) The molecular mechanisms by which the genomes of the nucleus and chloroplast control the development and maintenance of photosynthetic competence in higher plants. 2) The molecular/genetic basis for the relation of structure to function in photosynthetic polypeptides involved in electron transport, initially Photosystem II (PS II). 3) The structural basis for catalysis of the primary "dark-reaction" enzyme Ribulose 1,5-bisphosphate carboxylase/oxygenase. 4) Development of a photosynthetically assayable system of site-specific gene modification on higher plant and cyanobacterial genes involved in photosynthesis.

113. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

ACQUISITION OF ENVIRONMENTAL INFORMATION THROUGH LIGHT PERCEPTION — $125,000

Kenneth L. Poff
MSU-DOE Plant Research Laboratory

The ultimate goal of this work is an understanding at the molecular level of sensory transduction in plants from stimulus to response. Our emphasis is on non-visual light perception and temperature perception. We are studying the "blue light" photoreceptor system which controls numerous light responses such as phototropism in plants and the phytochrome photoreceptor system which regulates plant photomorphogenesis by using specific inhibitors and genetic mutants as probes blocking specific initial steps in the transudction sequence. Light direction is measured in plants through a photochemical measurement of light intensity on the two sides of the plant. Understanding this aspect requires knowing the internal light intensities. Since direct measurement is intrusive, and models difficult due to the absorption and scattering complexities of the tissue, we are developing techniques for using an internal physiological pigment such as phytochrome as a physiological quantum counter which can in turn be asayed using microspectrophotometry. Isolation and analysis of photoreceptor pigment mutants which will permit dissection of the transduction sequence are in progress for Arabidopsis, corn, and Dictyostelium.
Thermotaxis by Dictyostelium is being used as a model system for the study of temperature perception because of four unique characteristics: (1) the extreme sensitivity to temperature, (2) the narrow temperature range over which temperature is measured (3) the dependence of the thermotaxis range on the previous growth temperature, and (4) the fact that thermotaxis can be observed in individual cells.

114. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

PHYSIOLOGICAL AND MOLECULAR GENETICS OF HIGHER PLANTS — $175,000

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 higher plants. We are using this conceptual framwork to investigate several distinct aspects 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 net photosynthesis we are attempting to create useful or informative variation in the enzyme RuBP carboxylase/oxygenase by in vitro modification of the genes which encode the enzyme. We are also attempting to resolve several uncertainties in our model of photorespiration by the isolation of specific classes of mutants in Arabidopsis thaliana which are theoretically possible but have eluded discovery.
B) Although genetic variation in disease resistance is available in many species, it is not known what the genes for resistance encode. The concept that plant cell-surface receptors are an important component of some classes of host defense mechanisms is well established. In the context of this theory, we are exploring the possibility that hybridoma techniques can be used to define and characterize the biochemical differences between disease-resistant and suceptible cultivars of barley.
C) As part of a study of several mutants of Arabidopsis with altered chilling sensitivity, we have isolated a novel series of mutants of this species which appear to specifically lack certain fatty acid desaturases. We are attempting to determine the precise biochemical defect, and are examining the effect of the resulting changes in lipid composition on membrane associated functions and on the growth and vigor of the mutants in various environmental conditions.

115. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

DEVELOPMENTAL BIOLOGY OF NITROGEN-FIXING ALGAE — $160,000
C.P. Wolk
MSU-DOE Plant Research Laboratory

Certain cyanobacteria (blue-green algae) trap solar energy via photosynthesis and utilize the resulting chemical reducing power to fix atmospheric nitrogen gas (N_2). They thereby produce ammonia, which then is utilized as the nitrogen source for cellular growth. The initial steps of N_2 assimilation take place in specialized cells called heterocysts. The objective of this project is principally to understand the supply of energy and electrons to, and the oxygen protection of, nitrogen fixation within heterocysts. Our current approach makes use of our recently developed methodology for introducing cloned genes into nitrogen-fixing cyanobacteria by conjugation from Escherichia coli, and is primarily genetic. That is, we are developing methods, using site-specific mutagenesis and transposons, for isolating mutants of Anabaena and Nostoc defective in cellular differentiation, nitrogen fixation and photosynthesis. Additional, simpler, more rapid means for introducing genes into Anabaena are being sought, as are means for visualizing transcriptional differences between cells cooperating in N_2 fixation. With partial support from N.S.F., we are also constructing a physical and genetic map of the Anabaena genome using the technique of cosmid walking. Thus, we have made substantial progress toward developing tools for genetic analysis 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.

116. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

ENVIRONMENTAL CONTROL OF PLANT DEVELOPMENT AND ITS RELATION TO PLANT HORMONES — $160,000
Jan A. D. Zeevaart
MSU-DOE Plant Research Laboratory

Environmental factors such as daylength, temperature and moisture have pronounced effects on growth and development of plants. Plant hormones often function as chemical messenger between the perception of an environmental factor and the morphological expression. The objectives of this task are to characterize the hormones involved, how their synthesis and catabolism are regulated by the environment, and how these hormones act. Topics under investigation are: (1) Stem growth and flowering in rosette plants, the so-called "physiological dwarfs", as regulated by gibberellins (GAs). We know that in spinach the conversion of GA_{19} to GA_{20} is under photoperiodic control. The immediate objective is to prepare a cell-free system that catalyzes this step, and then to determine how the activity of the enzyme is regulated by the daylength. (2) In single-gene dwarf mutants of tomato and Arabidopsis we will determine which steps in the gibberellin biosynthetic pathway are blocked. This will be done by feeding various precursors and GAs. Furthermore, endogenous GAs of the wild type will be identified and compared with the GAs in the mutants, if detectable at all. (3) Regulation of abscisic acid (ABA) synthesis, catabolism, and distribution. We are measuring early changes in apoplastic ABA in Xanthium strumarium leaves to see if this parameter is more closely related to stomatal aperture than bulk leaf ABA during the onset of water stress. Since our earlier work suggests that stress-induced ABA is not directly produced from mevalonic acid, we are investigating the possibility that xanthoxin, a breakdown product of xanthophylls, is the precursor of ABA.

117. OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37831

ENERGY AND NUTRIENT UTILIZATION EFFICIENCY IN INTENSIVE FOREST BIOMASS PRODUCTION — $200,000

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

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 tulipifera), loblolly pine (Pinus taeda), sweet gum (Liquidambar styriciflua), and black locust (Robinea pseudoacacia) are being studied in a 20 ha managed plantation. 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. Biochemical analysis of tissues from 147 trees sampled during the 1983 growing season is in progress. Quantification of seasonal changes in sugars, lipids, starch, cell wall, and lignin in tissues will be used to compare energy allocation patterns between species and treatments. In studies of nitrogen kinetics, principal effects of intensive nitrogen fertilization have been found on soil nitrification potential but little effect on nitrogen mineralization rates has been noted to date.

118. SOLAR ENERGY RESEARCH INSTITUTE
Golden, CO 80401

BASIC PHOTOBIOLOGY RESEARCH — $105,000

Michael Seibert, Paul F. Weaver, and Stephen C.T. Lien
Photoconversion Research Branch

Three areas associated with photobiological H_2 metabolism will be studied. Task 1 will determine the degree of integration among H_2 metabolism, N_2 fixation, photosynthesis, and the multiple energy-conversion mechanisms present in photosynthetic bacteria, particularly Rhodopseudomonas and Rhodospirillum species. A broad spectrum of mutants is being examined with defective redox components operative in autotrophic or heterotrophic, anaerobic or aerobic, and light or dark growth modes. These defects are being characterized by pleiotropic losses of other growth modes, spectral and enzymatic properties, and protein separation and quantitation techniques. The data will be used to amplify H_2 evolution in organisms which utilize two distinct evolving hydrogenases as well as nitrogenase. Task 2 has lead to the first purification of hydrogenase from a eucaryotic source (Chlamydomonas reinhardtii). Kinetic studies of the isolated enzyme will examine the role of positively charged groups on the interaction between hydrogenase and its electron mediators. New efforts will elucidate the mechanism of anaerobic activation of the enzyme. The role of iron in the activation and catalysis reactions of algal hydrogenase will be examined. Task 3 will investigate physicochemical and structural properties of Photosystem II (PS II) in algae, chloroplasts, and detergent-fractionated PS II preparations that maintain O_2-evolving capacity. Work will emphasize extraction of several extrinsic proteins associated with O_2-evolution and examination of the properties of the extracted membranes. Antibodies to the proteins will be produced for structural studies. New research will be initiated to examine a recently recognized intrinsic protein that may be part of the O_2-evolving complex.

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