DOE/ER-0147

Annual Report and Summaries of FY 1982 Activities Supported by the Division of Biological Energy Research

October 1982

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

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

In the growth of the BER program since its inception in 1979 efforts have been made, within the limitations of dependence on unsolicited proposals, to encourage proposals in certain research areas which have not received appreciable federal research support and which have been identified as having considerable significance to future biotechnology developments. Some examples of the research priority areas identified include: 1) definition of stress effects in plants at the biochemical and biophysical levels, and including adaptive mechanisms, 2) the biosynthesis and biodegradation mechanisms of the key natural polymers, cellulose, lignin and other polysaccharides, 3) the development of better understanding of the genetics of those microbes responsible for degradations and fermentations, especially anaerobic microorganisms, 4) the physiological genetics and biochemistry of important processes in both plants and microbial species, and 5) interactive relationships between different species of organisms as with plant host-pathogens, microbial consortia, and plant-soil microbes with emphasis on recognition events, and reciprocal information and substrate flows. In addition to these areas the BER program maintains a significant stake in photosynthesis (the driving force of biological processes), and other energetics research, plant growth and development mechanisms, genetics of plant systems, microbiological studies on metabolic regulation, thermophily and other areas as will be seen in the following pages of the report. The rationale for pursuing many of these studies was presented in a recent paper (R. Rabson and P. Rogers, The Role of Fundamental Biological Research in Developing Future Biomass Technologies, Biomass 1, 17-37, 1981). BER program priorities are dynamic in respect to the evolution of science with the changing nature of problems, the availability of new techniques and as insights about approaches become clearer.

It is unnecessary to indicate the considerable future expectations for what is commonly called "biotechnology". Genetic engineering is sometimes mistakenly equated to biotechnology. Our conception is that a whole constellation of techniques and studies, besides genetic ones, constitute "biotechnology" and will be required for substantial progress. The powerful procedures of genetic engineering can be applied most effectively only when there is adequate understanding of the process or trait which would be changed by genetic manipulation. In this context the BER program emphasizes the need to achieve a better understanding of genetic systems, the expression of genetic information *and* the basic physiology and biochemistry of processes that they control. Like all good scientific endeavors there is growing convergence between disciplines, in this case genetics, physiology and biochemistry. The newer genetic techniques, in fact, provide tools of immense versatility and power to understand a number of previously refractive phenomena including development and differentiation. In a way the advent of these genetic techniques is akin to the opportunities for the solution of many biochemical problems offered by the advent of radioisotopes on the research scene in the late 1940's and early 1950's.

Fiscal Year 1982 witnessed continued steady growth of the Biological Energy Research (BER) program. The BER activity reflects the increasing attention being paid to long-term fundamental studies. The program areas on which the BER focuses, the plant and microbial sciences, are ones in which excitement and enthusiasm are building as part of the current revolution in biological research approaches. Research areas are experiencing a renaissance of interest even among researchers not occupied previously with problems in such areas as plant genetics, fermentations and other bioconversions. This is important for two reasons. First, there is room for considerably more high quality researchers in many of these problem areas which have been underpopulated for years. Secondly, the entry of researchers with different backgrounds serves to cross-fertilize the research areas and bring to bear additional experience and expertise. Yet there is a potential danger inherent in the movement of researchers untrained in plant and microbial sciences into these areas by virtue of a possible lack of understanding of the specialized attributes of the systems on the part of the new entrants. This suggests the need for new types of training and/or collaboration for crossover researchers and for the encouragement of more young investigators to train for these rapidly developing areas. One of the earlier deterrents to career development in these areas was the absence of reasonably stable research support. That seems to be changing and DOE's BER program along with the U. S. Department of Agriculture (USDA) Competitive Grants Program, the National Science Foundation's involvement plus industrial interest should be indicative that these previously less appreciated research areas do have a promising future.

As an indication of the kinds of results the BER program generates a few examples of recent achievements include: 1) the discovery that inorganic pyrophosphate may serve as an energy substrate for a variety of bacteria (summary #20); 2) ethylene stimulated growth can account for the adaptation of certain plants to flooding conditions (summary #91); 3) a newly discovered intermediate water soluble breakdown product of Streptomyces degradation of lignin may have use commercially (summary #26); 4) using immunological procedures a new system for recognizing methanogenic bacteria has been developed (summary #50); 5) a number of new instruments designed for measuring parameters of photosynthesis in the field have been built and are ready for testing (summary #28).

In FY 1982, 82 new proposals were received and reviewed with the peer review systems. Of these, 16 were funded. With regret some very good proposals were not able to be funded.

The distribution of BER funds in FY 1982 is illustrated below:

	No. of Projects	FY 82 Funding (in thousands of $)	% of Total Funds
University Contracts	66	4331	51
Michigan State University Plant Research Laboratory	13	1550	18
National Laboratories Brookhaven National Lab Lawrence Berkeley Lab Oak Ridge National Lab Solar Energy Research Inst.	12	1940	23
Other Research Institutions (federal, state, industrial, non-profit)	9	534	6
Conference and miscellaneous	3	65	1
TOTAL	103	8420	

Complementing the major research component of the BER program is the occasional provision of support to activities which are designed to further research efforts. Three examples of this kind of support during FY 82 are as follows:

1. Partial support for the symposium entitled, "The Biological Basis of New Developments in Biotechnology" which was held at the University of Minnesota May 25-28, 1982. The proceedings of this meeting are now in press (edited by A. Hollaender, A.I. Laskin, P. Rogers, S. Dagley, R. Hanson, L. McKay, J. Messing and C. Wilson, Plenum Press, New York, 1982).

2. Partial funding for the research/training program at the Marine Biological Laboratory, Woods Hole, Massachusetts, entitled "A Microbial Ecology Summer Research Program with Special Emphasis on Studies with Anaerobic Microorganisms" held from June 14 to August 20, 1982.

3. Along with other federal agencies the BER program contributed to the establishment of a nucleic acid sequence data bank. The project is spear-headed by the National Institute of General Medical Sciences. The goal is to collect the rapidly growing number of sequences into a single data base and ultimately provide the information to the scientific community for comparative and other analyses (see summary #46).

No year goes by in which the BER program can be managed without the assistance of others. This includes the many reviewers in the U.S. and abroad who were kind enough to contribute some of their valuable time to evaluate proposals sent to them by post and the researchers who were generous enough to fit in time to serve on ad hoc review panels. Dr. Oliver Smith of the Department of Biology of Marquette University also provided most capable assistance to us during this year as an IPA (Intergovernmental Personnel Act) appointee. To all of these persons we acknowledge their efforts with much appreciation.

On the following pages research project summaries are provided for each of the research tasks supported by BER during FY 1982 plus several which have been active this year, but funded from monies from previous fiscal years. Each project is normally supported on an annual basis with incremental funding each year of the period designated when the project is initiated. Generally projects are peer reviewed on three-year rotations. Projects are eligible for continuing support provided they receive strong endorsement in the peer review process.

Funding levels are indicated for projects, however, most of the projects which are carried on in the national laboratories are represented by level of effort in scientific man years rather than funding levels.

Any reader with questions about the details of an individual project may contact the particular investigator involved. 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. BATTELLE COLUMBUS LABORATORIES
Columbus, Ohio 43201

COMPLEXES OF CHLOROPHYLL DERIVATIVES WITH HEME APOPROTEINS — $98,000
Robert M. Pearlstein
Organic and Polymer Chemistry Section

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

2. BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH AT CORNELL
Ithaca, NY 14853

CARBON METABOLISM IN LEGUME NODULES — $41,345
Thomas A. LaRue
Nitrogen and Crop Yields

The goal of the project is to understand how the legume plant provides energy to support symbiotic nitrogen fixation. Our immediate interest is to determine the pathways of carbon metabolism in the nodule and discover which carbon compounds the bacteroid obtains from the plant cytosol. We concentrate on the soybean (Glycine max) but important observations are confirmed with pea (Pisum sativum) and sweet clover (Melilotus alba). Because the nodule cytosol is microaerobic, it is possible to metabolize carbon compounds by fermentative pathways. We have documented the production of alcohol and acetaldehyde by the host cytosol, and the metabolism of these compounds by the bacteroid. Anaerobic metabolism yields less energy than does aerobic metabolism, and this might contribute to the high metabolic cost of symbiotic nitrogen fixation.

3. BRANDEIS UNIVERSITY
Waltham, Massachusetts 02254

EFFECT OF LIGHT ON RESPIRATION AND DEVELOPMENT OF PHOTOSYNTHETIC CELLS — $42,535
Martin Gibbs
Institute of Photobiology for Cells and Organelles

The objective is to identify the pathways of hydrogen production in anaerobically adapted green algae, primarily Chlamydomonas reinhardii and Chlorella pyrenoidosa. The approach utilizes enzymic, manometric and chromatographic techniques in following the fermentation products formed in the light and in the dark. Enzymic profile studies will be carried out to determine the cellular site(s) of hydrogen production. Isolated chloroplast preparations (the site of starch, the source of hydrogen production in the dark) will be used to identify the pathways involved and the regulatory properties of the enzymes will be determined. The site and appearance of hydrogenase during "adaptation" will be investigated. The "hydrogenase-containing" photosynthetic cell may be a potential means of converting carbon dioxide to fermentation products.

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

HYDROGENASES, THEIR CLONING, PREPARATION, CHARACTERIZATION AND MODIFICATION FOR USE IN PHOTOBIOREACTORS — $75,000
Robert G. Bartsch, Martin D. Kamen and Nathan O. Kaplan
Department of Chemistry, A-002

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

5. UNIVERSITY OF CALIFORNIA
Santa Cruz, California 95064

RESPIRATION OF ROOTS: RESPONSE TO LOW O_2 STRESS — $46,266
Harry Beevers — (FY 81 funds)
Department of Biology

This work concerns the unusual ability of rice grain to germinate (though producing only a coleoptile) under completely anaerobic conditions. In 1% O_2 a normal, but slower growing, seedling develops. Thus, rice is a prime example of a plant that can withstand low O_2 environments and we are examining the reasons for this ability. A previous hypothesis (Crawford) has suggested that such plants survive by producing less toxic products than ethanol under anaerobic conditions but from our evidence and from work by ap Rees with other species, this does not appear to be so. We are investigating the ability of rice tissues to withstand ethanol accumulation and the offsetting effects of ethanol leakage to the medium. In complete anaerobiosis the development of several enzymes, particularly catalase, is drastically curtailed; normal levels are attained in 1% O_2. We are investigating growth, respiration and enzyme development in rice in low O_2 and comparing the results with those from wheat which fails to germinate in complete anaerobiosis.

6. UNIVERSITY OF CALIFORNIA
Los Angeles, California 90024

ENERGY CAPTURE AND USE IN PLANTS AND BACTERIA — $75,000
Paul D. Boyer
Department of Chemistry and Biochemistry

Our studies focus on how sunlight energy is used to synthesize adenosine triphosphate (ATP) in plants. This is quantitatively the most prominent chemical reaction in the world. In particular we are studying the ATP synthase complex in chloroplast thylakoid membranes from spinach, and the catalytic steps by which the energy-requiring formation of ATP is coupled to the energy-yielding translocation of protons driven by the electrochemical proton gradient generated in light. Our laboratory has developed the energy-linked binding change hypothesis for ATP synthesis, in which identical catalytic sites on the enzyme participate in coordinated alternation. Experimental evidence favors the view that energy promotes competent binding of inorganic phosphate and adenosine diphosphate (ADP) at one catalytic site and the release of ATP from another. The use of energy input from transmembrane proton gradients to drive changes in binding affinities of reactants is a new concept in enzymology, and needs critical evaluation from many approaches. Studies are underway using ^{18}O-labeling probes of reversal of bound ATP cleavage with the membrane-bound ATP synthase complex and the isolated CF_1-ATPase portion, and of the nature and extent of bound intermediates with ^{3}H and ^{32}P-labeled substrates. Results indicate previously unrecognized modes of control, including a reversibly deactivated state of the synthase that retains ATP bound to a catalytic site. Labeling patterns of bound ADP on CF_1-ATPase are being assessed to determine if divergent results from other laboratories are supportive of the binding-change mechanism. Other experiments check the nucleotide specificity for modulations of reaction steps by measurements of ^{18}O retention and distribution in products formed. Plans are being developed for use of E. coli mutants defective in the ATP synthase complex as further probes of energy coupling mechanisms.

7. UNIVERSITY OF CALIFORNIA
Davis, California 95616

FLUORESCENCE PHOTOBLEACHING MEASUREMENTS OF PLANT MEMBRANE VISCOSITY: EFFECTS OF ENVIRONMENTAL STRESS — $20,000 (6 months)

R. W. Breidenbach and D. W. Rains
Plant Growth Lab/Agronomy and Range Science

The purpose of this project is to determine the role of the plasma membrane in determining the responses of different crop species to temperature and salinity: two factors strongly limiting plant productivity. The experiments utilize fluorescence photobleaching recovery to compare the lateral diffusion rates of proteins and lipids in the plasma membranes of plants different 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.

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

BIOENERGETICS OF SALT TOLERANCE — $125,000

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

This work is to identify pathways of ion transport responsible for salt tolerance in different organisms. Energy transducers which accomplish active transport of sodium and chloride ions are to be purified and characterized as to their structure and mechanism of action. Halobacterium halobium, which grows at several molar salt concentrations, contains two distinct light-driven transport systems: one for protons (bacteriorhodopsin), and another for chloride ions (halorhodopsin). The former, functioning in concert with a proton/sodium antiporter, causes rapid sodium efflux from cells and cell membrane vesicles upon illumination. The latter will cause rapid chloride influx. Both systems cause the development of inside negative membrane potential. We are engaged in the description of membrane potential, ionic movements and volume changes which occur when these transport systems are activated, singly or together. We are also studying the molecular architecture of these ionic pumps. Bacteriorhodopsin, the better described pigment, is chemically modified in ways which alter its function, and models are developed for how protonation and deprotonation of specific groups can accomplish the ordered translocation of protons across the width of this transmembrane protein. As to halorhodopsin, we are now able to purify this pigment and are in the process of comparing its primary structure to that of bacteriorhodopsin. To this end, we are developing the methodology for handling hydrophobic peptide fragments. This part of the project will result in an appreciation of how apparently similar proteins will transport very different ions. The second major area involves salt tolerant and salt sensitive plants, such as sugar beet varieties, tomatoes, and Atriplex. Membranes of spheroplasts, tonoplasts and chloroplasts are examined for transport properties, volume changes and membrane potential in order to identify energy dependent ion movements.

9. UNIVERSITY OF CALIFORNIA, Los Angeles
Los Angeles, California 90024

METHANOGENESIS FROM ACETATE, A KEY INTERMEDIATE IN NATURE — $70,000

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

The objectives of this project were three-fold: 1. to isolate and characterize new strains/species of aceticlastic methanogens. 2. to examine the role of H_2 in the conversion of acetate to methane. 3. to isolate genetic mutants of methanosarcina. We previously reported on objective nos. 2 and 3 and have concentrated our efforts in the second year on objective no. 1. Three methanogens have been isolated, one from an anaerobic filter (Yu and Mah, ASM Abstracts, March, 1982) and two from a brine pond containing 25-30% salt. The anaerobic filter isolate is morphologically unlike other methanogens, occurring in irregular forms comprised of distinctive subunits each surrounded by its own cell wall material. The other two methanogenic isolates are extreme halophiles and are still being characterized.

10. UNIVERSITY OF CALIFORNIA, SAN DIEGO
La Jolla, California 92093

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

Christopher Wills
Department of Biology, C-016

We began this project by examining functional mutants of yeast alcohol dehydrogenase, selected according to techniques developed in this laboratory, with a view to isolating strains that show a higher rate of ethanol production. In the course of investigation of the regulation of isozymes of this enzyme, we have discovered a new class of mutants in yeast marked by alteration of a more fundamental cellular process, the transfer of reducing equivalents across the mitochondrial membrane. Most of the mutants we have examined so far show an increased activity of the malate-aspartate shuttle, and in consequence their rate of uptake of metabolites and production of ethanol are markedly increased. We are intensively investigating the genetics and biochemistry of these mutants, and seeking other selective processes for altering different mitochondrial transport systems such as dicarboxylate transport.

11. UNIVERSITY OF COLORADO
Campus Box 215
Boulder, Colorado 80309

STUDIES OF PLANT CELL WALLS AND OF PLANT-MICROBE INTERACTIONS $270,000
Peter Albersheim, Alan G. Darvill, Michael McNeil, and
Barbara S. Valent
Department of Chemistry

There are five major projects in this laboratory. They are: identifying, isolating, and characterizing naturally occurring carbohydrates with biological regulatory functions; developing new methods for the purification and structural characterization of complex carbohydrates; delineating the structures of the polysaccharides present in the walls of growing plant cells; studying the structure and function of polysaccharides secreted by Rhizobium species; and developing a genetic system for Pyricularia oryzae, the fungus that causes the rice blast disease, in order to identify the genes and gene products responsible for race and cultivar specificity in this host-pathogen system. The first three projects listed are interrelated and are all involved with the primary focus of the laboratory, the study of plant cell wall oligosaccharides that appear to regulate a variety of physiological functions in plants, such as flowering, growth rate, phytoalexin synthesis, and hypersensitive resistance. The value of these biologically active oligosaccharides to the forest products, ornamental horticulture, and agricultural chemical industries is being assessed through collaborations with industries. Many different varieties of higher plants and microbes are used in these studies.

12. CORNELL UNIVERSITY
Ithaca, NY 14853

STUDIES OF PHOTOSYNTHETIC ENERGY CONVERSION $70,000
Roderick K. Clayton
Division of Biological Sciences

Bacteriochlorophyll (bchl) in natural pigment-protein complexes is non-covalent. This results in strong bathochromic absorption bands shifts, at the far-red from 770nm in vitro to 800-890 nm in vivo. The nature of the pigment binding to the protein and the basis of the band shifts are little known. With the success in developing a technique for reversible dissociation of bchl from an antenna complex of Rhodospirillum sphaeroides (B850), it is now possible to probe the nature of the binding. The approaches are as follows:

1. Competitive binding studies with bchl a and analogs.
2. Raman spectroscopy of different reconstituted complexes including antenna complex B875, B850 with and without bchl 800 and others. These studies may also be expected to reveal differences in carotenoid isomeric configurations between the B850 and B875 complexes.

With another technique of random photo-oxidative destruction of dimeric bchl in the carotenoidless mutant of Rp. spheroides, it has been possible to produce monomeric bchl in vivo for studying bathochromic shifts as a function of bchl-bchl interactions. The same strategy of progressive photodegradation of the components of RC's will be used to probe the function of the six chromophores (4 bchl and 2 bacteriopheophytin) in the photochemistry of RC's. Still other efforts are planned in attempting to reconstruct photosynthetic membranes starting with isolated antenna complexes, RC's and phospholipid vesicles. These will be combined in varying proportion, and at various surface densities while monitoring for capabilities of excitation energy transfer. The prospect of spontaneous assembly into structures resembling native photosynthetic membranes will be examined.

13. CORNELL UNIVERSITY
Ithaca, NY 14853-0144

EFFECTS OF FREEZING AND COLD ACCLIMATION ON THE PLASMA MEMBRANE OF ISOLATED CEREAL PROTOPLASTS — $82,236 (two years)

Peter L. Steponkus
Department of Agronomy

The primary objective of this project is to characterize the area stress-strain relationship of the plasma membrane of isolated protoplasts (Secale cereale L. cv. Puma) to provide a quantitative and mechanistic explanation for expansion-induced lysis during thawing, one form of freeze/thaw injury. The SSR of the plasma membrane may be described in terms of an elastic law and a surface energy law. For small area deformations over short periods of time (seconds), the amount of material in the plane of the membrane is conserved and deformations follow a simple elastic relation. For large area deformations over longer periods of time (minutes), material is introduced into, or lost from, the plane of the membrane and the tension equilibrates to a constant resting value (γ_r), i.e. the membrane follows a surface energy alw. The elastic component (intrinsic) is similar for protoplasts isolated from either non-acclimated or acclimated tissues, i.e. maximum elastic expansion is limited to about 3%. The area modulus of elasticity, k_A, is 152 $mN \cdot m^{-1}$ for non-acclimiated protoplasts and 141 $mN \cdot m^{-1}$ for acclimated protoplasts. Lysis occurs at similar tensions in both, i.e. 4-6 $mN \cdot m^{-1}$. There are, however, significant differences between acclimated and non-acclimated protoplasts in the characteristics which affect extrinsic membrane behavior. While large surface area contractions are irreversible in non-acclimated protoplasts, they are largely reversible in acclimated protoplasts. Contraction of non-acclimated protoplasts results in endocytotic vesiculation of the plasma membrane. In contrast, contraction of acclimated protoplasts results in exocytotic extrusion of membrane components. Staining characteristics suggest that there is a preferential extrusion of membrane lipids. Current experiments are directed to quantification of the extensive changes in area as a function of tension and time, δA_o (γ,t).

14. CORNELL UNIVERSITY
Ithaca, NY 14853

MICROBIAL ECOLOGY OF THERMOPHILIC ANAEROBIC DIGESTION — $60,549

Stephen H. Zinder
Department of Microbiology

Microbial conversion of biomass to methane is an important process which is presently used in sewage treatment, and which is being seriously considered for the conversion of other biomass substrates. Our understanding of the microorganisms involved in this process and the interactions among these microbes is still rudimentary. 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 digester 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, epifluorescence, and electron microscopy; (3) ^{14}C-radiotracer methods to study carbon flow to methane and physiological ecology of the digester populations. Results obtained thus far include: (1) the discovery and isolation of a two-membered microbial consortium which converts acetate to methane using interspecies-hydrogen-transfer rather than acetate-splitting. This mechanism has been proposed previously but never demonstrated; (2) the demonstration that acetate-splitting methanogens appear to be the population in the digester most sensitive to an upward temperature shift; (3) the development of a method for observing bacteria attached to cellulose particles. The cells are stained with DAPI, a DNA-specific fluorescent dye, and then are observed by epifluorescence microscopy; (4) the discovery of a new thermophilic acetate-splitting methanogen. Several other new and potentially useful thermophilic anaerobes may also be isolated from the digester.

15. FLORIDA STATE UNIVERSITY
Tallahassee, Florida 32306

GUARD CELL BIOCHEMISTRY: RESPONSE TO ENVIRONMENTAL STIMULI CAUSING CHANGES IN GAS EXCHANGE — $116,500

William H. Outlaw Jr.
Department of Biological Science

The objective of this project is to improve our understanding of how plants regulate water loss. Such an understanding is important: about 85% of the water consumed in the U.S. is for irrigation. There are several consequences: (a) increased competition for water by energy-yielding processes (e.g. oil from shale), (b) intrinsic energy demands, (c) aquifer depletion, (d) reduction of fertility by soil salinity. Virtually all water loss from plants occurs through stomata on the leaf surfaces. Each stomate is flanked by two parallel, sausage-shaped cells (each about 0.001" long), joined at the ends. The aperture size between these cells is under physiological and environmental control. Atmospheric CO_2, used for photosynthesis, also enters the leaf through stomata. Thus the plant must "decide" the optimum aperture sizes required to admit sufficient CO_2 while preventing desiccation. Study of guard cell biochemistry poses formidable technical challenges because of their small size and interspersion among other cell types. We are meeting these challenges in two ways: (a) direct analysis of guard cell metabolites and enzymes using microfluorometry capable of sub-pmol quantitation. As this approach is almost untried in biology, much effort is being expended on development of sound protocols and instrumentation. (b) direct analysis of guard cell protoplasts enzymically digested from leaf tissue. Like the previous one, this approach is not well developed, but we have made several significant improvements in protocol. These micro-level approaches are complemented by our design and construction (presently in mid-stage) of a sophisticated control system, which will be used, in part, to produce suitably pre-treated leaves for analysis. Submission of two manuscripts for publication marks our progress during the past year. One shows a profile of the major carbon pathways in guard cells and other leaf cells. The other details a generally applicable method for improving protoplast stability during aqueous two-phase purifications.

16. UNIVERSITY OF FLORIDA
Gainesville, FL 32610

INVESTIGATION OF THE TRANSPOSITION OF MITOCHONDRIAL DNA AND ITS RELATIONSHIP TO FERTILITY IN *ZEA MAYS* — $58,055

Rusty Jay Mans
Department of Biochemistry and Molecular Biology

We want to define, at a molecular level, the transposition event in maize mitochondrial DNA detected in fertile cytoplasmic revertants derived from cytoplasmic male sterile (*cms*) S-type maize. This reversion appears to involve the integration of linear S-1 and S-2 DNA molecules into larger DNA molecules. We will attempt to establish an *in vitro* assay for detection and characterization of components involved in regulating expression of the mitochondrial genome, e.g., a suspected nuclear gene product that restores *cms* plants to fertility. Thus far by measuring hybridization with *Sau* 1 restriction fragments of S-1 and S-2 DNAs cloned into plasmid pBR322 we have evidence for transposition of both S-1 and S-2 into higher molecular weight DNA components of the maize mitochondrion. Use of the cloned probes has shown linear, multiple molecules (concatemers) of S-1 and S-2 in sterile lines and the possibility that these are replicative intermediates will be examined. S-1 sequences have been detected among restriction fragments of nuclear DNAs from several maize lines. These sequences are unlikely to be due to mitochondrial contamination based on reconstruction experiments. Transcripts homologous to S-1 have been detected after microinjection of cloned segments of S-1 into the nuclei of frog oocytes. Cloned segments of both S-1 and S-2 are transcribed *in vitro* by maize RNA polymerase II. This evidence supports the view that S-1 or S-2 may prove suitable as vehicles for the introduction of desired genetic sequences into the genome of an agronomically and industrially important crop.

17. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

MICROBIAL DESULFURIZATION AND DENITROGENATION OF FOSSIL FUELS — $80,000

William R. Finnerty
Department of Microbiology

The objectives of this project are the isolation and study of bacteria which oxidize and render water-soluble sulfur and nitrogen-containing polycyclic aromatic constituents of liquid fossil fuels. Such organisms, one with the ability to oxidize dibenzythiophene (DPT) (S) and another which attacks carbazole (N) have been isolated. Characterization of the bacteria and their reactions on these compounds have continued. Metabolism of these compounds leads to a variety of products and the metabolic pathways involved are still under investigation. The genes encoding at least some of these functions appear to reside on plasmids in most of the strains of bacteria studied so far. The plasmids have been characterized as regards size and digestion of them with restriction enzymes. Subsequent cloning of the restriction fragments in a broad host-range plasmid vehicle which transforms Pseudomonas-like organisms is planned. These oxidative processes are specific for the compounds metabolized and may serve as models for bioreactors which could remove the bulk of undesirable hydrocarbon constituents in petroleum which yield pollutants when combusted.

18. UNIVERSITY OF GEORGIA
Tifton, Georgia 31793

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

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

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

19. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

ENVIRONMENTAL STRESS MEDIATED CHANGES IN TRANSCRIPTIONAL AND TRANSLATIONAL REGULATION OF PROTEIN SYNTHESIS IN CROP PLANTS — $76,000

Joe L. Key
Department of Botany

The objectives of this project relate to gaining an understanding of the mechanisms operative in environmental stress-mediated changes in patterns of transcription and translation in crop plants, in particular the soybean. Cloned cDNA-northern blot hybridization analysis and in vivo labeling/2D gel fractionation of the labeled proteins have been used in these studies. When seedlings of soybean and other crop plants are shifted from the normal growth temperature to some higher temperature (heat shock) there is a rapid shut-off of normal protein synthesis and rapid induction of a new set of proteins (hsp's). These hsp's seem to afford some type of protection or acclimation to still higher temperatures which normally are lethal. This "physiological protection" seems to relate to selective and preferential localization of some of the hsp's. The ability to synthesize hs mRNAs and hsp's at otherwise non-permissive temperatures appears to be a part of the "protective" mechanism. Other stresses (e.g. water, salt, energy, high abscisic acid, high 2,4-D) induce some hs mRNAs to levels of the hs response, but the responses to these stresses seem to be generally different from heat shock. There is tight control exerted by heat shock at both the level of transcription and the level of translation. Most normal mRNAs (i.e. 28°) persist for several hours during a 40° hs; some loss in mRNA complexity occurs during hs at 42.5°.

20. UNIVERSITY OF GEORGIA
Athens, GA 30602

THE MICROBIOLOGY AND PHYSIOLOGY OF ANAEROBIC FERMENTATIONS OF CELLULOSE — $237,000

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

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

21. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

PHYTOCHROME PROPERTIES AND FUNCTION IN PHOTOSYNTHETICALLY COMPETENT PLANTS — $45,063

Lee H. Pratt
Department of Botany

Phytochrome is a chromoprotein that senses the wavelength distribution of incident light energy and modulates the growth and development of green plants, presumably to maximize the efficiency with which this incident radiant energy is converted to chemical energy via photosynthesis. The overall goal of this project is to initiate a characterization of phytochrome and its reactions in green, photosynthetically competent tissues. This work depends heavily on recently developed methodologies for study of phytochrome; a sensitive radioimmunoassay for this pigment; a rapid and efficient immunopurification capability; and newly developed cryoimmunocytochemical methods for observing its distribution in situ in green tissues. We are producing antibodies against phytochrome from several plant sources and have also begun the production of monoclonal antibodies against pea (Pisum sativum L.) phytochrome. These antibodies are essential for the application of the immunochemical methods used in this project. We will purify and quantitate phytochrome from green plant tissues and will initiate a characterization of the phytochrome thus obtained. We will also investigate phytochrome distribution in green plants, both at the intercellular and intracellular level, by immunocytochemical observation correlated with more routine biochemical observations. The results of this work will lead to the development of methodologies that may be used readily by others to expand our knowledge about phytochrome and its function in green plants and will provide answers to several critical questions concerning phytochrome and its function that could not be obtained in any other way.

22. HARVARD UNIVERSITY
Cambridge, Massachusetts 02138

UNRAVELING PHOTOSYSTEM II — $75,073

Lawrence Bogorad
Department of Cellular and Developmental Biology

The objective of this project is to understand Photosystem II--the part of the photosynthetic machinery that is involved in the evolution of oxygen, the extraction of an electron from water and its transfer to the beginning of the electron transport chain leading to the Photosystem I reaction center. Photosystem II-defective mutants of the blue-green algae Anacystis nidulans R2 are being generated and selected. These will be used as recipients of cloned DNA from normal A. nidulans or from other photosynthetic genomes. Genes on cloned DNA that compensate for the defects in the mutants will be identified and their products will be characterized as a way of identifying components of Photosystem II and studying their function.

23. HARVARD UNIVERSITY
Cambridge, Massachusetts 02138

EXPRESSION OF BACTERIAL GENES IN YEAST — $55,000 (FY 81 funds)
Helen Greer
Department of Biology

We have been studying the expression of bacterial genes in the yeast Saccharomyces cerevisiae in the hope that such knowledge will aid in the development of yeast strains that are capable of utilizing diverse energy sources, e.g., cellulose, for the production of ethanol by fermentation. The introduction of specific bacterial genes into yeast via yeast cloning vectors will tell us if bacterial promoters can be expressed under these conditions or if vectors which put cloned segments under a given yeast promoter will be necessary for future bacterial cloning in yeast.

Specifically, we have been investigating the expression in yeast of bacterial antibiotic resistance genes as well as structural genes for biosynthetic pathways from Gram-negative and Gram-positive bacteria. We have constructed several plasmids in which Tn5 (an Escherichia coli transposable element conferring kanamycin and neomycin resistance) has been transposed into different sites on E. coli - yeast hybrid integrating and episomal vectors. In addition, we have cloned the argG gene from the Gram-positive bacterium Streptomyces cattleya into an E. coli - yeast hybrid episomal vector. The Tn5 and argG plasmids are being used to transform yeast cells to determine if these bacterial genes can be naturally expressed in yeast or if certain mutations (either yeast or bacterial) will be required for expression.

24. HARVARD UNIVERSITY
Petersham, Massachusetts 01366

STRUCTURE AND FUNCTION OF FRANKIA VESICLES IN DINITROGEN FIXATION BY ACTINORHIZAL PLANTS — $53,027
John G. Torrey and John D. Tjepkema
Cabot Foundation, Harvard Forest

Symbiotic nitrogen fixation in the root nodules of woody dicotyledonous actinorhizal plants has been localized in terminal swellings of the filamentous bacterial symbiont, Frankia. Conclusive proof of the vesicles as sites of the dinitrogen-fixing enzyme nitrogenase has come from the demonstration that in cultures of Frankia grown in N-free synthetic medium vesicles differentiate during culture and their appearance and increase in number are accompanied by a proportionate increase in nitrogenase activity. The present proposal is to study vesicle structure in the root nodule and in the cultured organism and to define the nature of the vesicle envelope which protects the oxygen-labile enzyme within from destruction, fostering optimum activity. Using physiological and biochemical techniques, analyses will also be made of the factors influencing vesicle formation, shape and function. A comparison of vesicle structure and function will be made in the diverse strains of Frankia now available in pure culture. This knowledge will lead to methods of improving the effectiveness of the dinitrogen-fixing process in actinorhizal plants.

25. THE HELICON FOUNDATION
San Diego, California 92121

CONSTRUCTION AND ANALYSIS OF BACTERIAL STRAINS USEFUL IN CONVERSION OF CELLULOSE TO ETHANOL — $59,722
Richard W. Armentrout

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

26. UNIVERSITY OF IDAHO
Moscow, Idaho 83843

THE METABOLISM OF AROMATIC COMPOUNDS BY ACTINOMYCETES — $57,262
Don L. Crawford
Department of Bacteriology and Biochemistry

The goal of this project is to elucidate the pathways by which actinomycetes, particularly Streptomyces species, catabolize the aromatic plant polymer lignin and low molecular weight aromatic compounds structurally related to lignin. Such intermediates are released from lignin during its degradation by microorganisms. The approach utilizes mutagenesis to generate catabolically blocked mutants which accumulate lignin degradation intermediates in isolatable amounts, and gas chromatography-mass spectrometry to identify specific low molecular weight compounds. Polymeric intermediates are characterized by classical organic chemistry techniques. Both low molecular weight and polymeric lignin degradation intermediates are being isolated, and the pathways by which these intermediates are further degraded are being determined. The applications goal of this research is the development of biomass conversions which utilize Streptomyces to produce industrially useful phenolic chemicals and modified lignin polymers from renewable lignocellulosic plant residues. Once the pathway of lignin catabolism by Streptomyces is understood, specific lignin to chemical bioconversions will be studied.

27. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

FATTY AND AROMATIC ACID CATABOLIZING BACTERIA IN METHANOGENIC ECOSYSTEMS — $54,174

Marvin P. Bryant
Department of Dairy Science

In the complete anaerobic degradation of organic matter to CO_2 and CH_4, three metabolic groups of bacteria are mainly involved. Fermentative bacteria initially hydrolyze polymers and ferment the products mainly to fatty acids, CO_2 and H_2 but some aromatic compounds are also produced. A second group, we discovered, ferments the products of the first group to acetate, CO_2 and H_2. The terminal group includes methanogens which catabolize H_2-CO_2 and/or acetate to CH_4. The second metabolic group includes syntrophs which require methanogens to maintain a very low concentration of H_2 in the ecosystem in order to degrade short chain fatty acids (Syntrophomonas) to acetate and H_2 or acetate, propionate and H_2. A second species (Syntrophobacter) catabolizes propionate to acetate, CO_2 and H_2. The object of the work is to use anaerobic enrichment and roll-tube techniques to document and isolate (in co-culture with H_2-using Methanospirillum or Desulfovibrio) long chain fatty acid, e.g., stearate, degrading and aromatic degrading, e.g., benzoate, bacteria and to characterize their metabolism.

We have now isolated an apparently new species of gram-negative, polarly flagellated, short rod that degrades benzoate to acetate, CO_2, and, presumably, H_2, in co-cultures with CH_4 or sulfide serving as the final electron sink product. Studies are continuing on this and other bacteria degrading other aromatic compounds and stearate. These studies are adding to basic knowledge on biochemical ecology of methanogenic ecosystems.

28. UNIVERSITY OF ILLINOIS
Urbana, IL 61801

PHOTOSYNTHESIS IN INTACT PLANTS — $106,049

A. R. Crofts
Department of Physiology & Biophysics

In the intact plant, the photochemical reactions, and the reactions of electron transfer and proton tranport which they drive, are part of an integrated mechanism which responds to the physiological state of the plant as this is determined by environmental factors. Rates of electron transfer, and the control of cyclic and non-cyclic pathways, can be assayed directly by following the changes on illumination, of redox components of the chain, using spectrophotometry or indirectly using fluorescence techniques. The generation and utilization of the proton gradient can be assayed by following the 515 nm electrochromic change. Our research is aimed at developing instrumentation to facilitate such measurements. Four instruments have been completed (i-iv below), two are near completion (v-vi), and three more are at a design stage (vii-ix). The instruments are: 1) rapid kinetic fluorimeter with single flash for actinic and measuring beam; ii) dual-flash kinetic fluorimeter; iii) portable dual-flash fluorimeter; iv) computer-linked fluorescence induction fluorimeter; v) kinetic spectrophotometer with flash measuring beam; vi) kinetic spectophotometer for measuring pseudo-steady-state kinetics; vii) portable version of v); vii) very rapid kinetic photometer; and ix) portable version if iv). The portable instruments are designed for field use, and the remaining instruments for laboratory use, or as prototypes for field instruments.

The long term aim of the research is to understand how the physiological state of the intact system is determined by environmental factors, and to relate this to the decreased yields of photosynthesis found under adverse conditions.

29. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

MECHANISM OF PROTON PUMPING IN BACTERIORHODOPSIN — $51,826

Thomas G. Ebrey
Department of Physiology & Biophysics

The purple membrane of Halobacterium halobium probably represents the simplest biological solar energy conversion system. Light absorbed by bacteriorhodopsin, a small protein whose chromophore is retinal, directly leads to the transport of protons across the cell membrane. The resulting chemiosmotic potential can be used to make ATP. An additional feature of the purple membrane is its ability to pump protons over a wide variety of salt concentrations including in extreme saline environments. This proposal is to investigate the relationship between the transport of protons across the membrane and structure and conformation of bacteriorhodopsin. We have made an especially intriguing discovery, that the number of protons released by purple membrane sheets by light can be drastically altered by the proteolytic removal of a few amino acids from the C-terminal "tail" of bacteriorhodopsin. Thus we believe that we have a way of specifically controlling the release of some of the protons by altering a small part of the polypeptide chain. In addition the number of protons released has been shown to be a function of the salt concentration, which may in turn affect the protein conformation. We suspect this effect may be localized to the interaction of the C-terminal end of bacteriorhodopsin with the rest of polypeptide. This proposal centers around a set of experiments, based on these observations, to correlate the structure of the purple membrane with its energy transducing function.

30. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

DEVELOPMENT OF A GENETIC SYSTEM FOR BACTEROIDES SPECIES — $60,000

Jeffrey F. Gardner
Abigail A. Salyers
Department of Microbiology

Bacteroides is one of the major genera of the human colonic microflora and is involved in the contribution of dietary fiber to human nutrition. The objective of this project is to develop a genetic system in Bacteroides with which to study their polysaccharide degrading abilities and to determine the degree to which the Bacteroides genes involved in these processes are shared by different bacterial species in vivo. To do this a transformation and/or conjugation system is needed to move DNA into and out of Bacteroides strains for mapping and isolation of mutants. pEG1 is a chimeric plasmid constructed of a cryptic Bacteroides plasmid and the E. coli plasmid, pBR328. This plasmid is a good prospect for a cloning and shuttle vector between E. coli and Bacteroides and is being used as a transformation probe. The Bacteroides portion of pEG1 also allows it to be mobilized aerobically and anaerobically by incP conjugative plasmids between E. coli strains. Thus, it may be possible to introduce pEG1 into Bacteroides by conjugation. Random cloning of B. thetaiotaomicron DNA into E. coli plasmid and viral vectors has yielded one clone which complements an E. coli auxotroph. Maxicells will be used to determine if Bacteroides proteins are being produced by these clones. Several of the clones hybridize by Southern blot analysis to restriction fragments from B. thetaiotaomicron strains but not to those from other Bacteroides groups. Isolation of species-specific probes could make identification of Bacteroides species in vivo faster and cheaper than currently possible. To test this possibility experiments are being conducted to determine the sensitivity and reliability of using cloned DNA probes to directly and quantitatively determine in mixed samples the number of organisms that contain a particular gene or DNA sequence.

31. UNIVERSITY OF ILLINOIS
Urbana, IL 61801

BIOCHEMICAL AND BIOPHYSICAL STUDIES ON THE E. COLI AEROBIC RESPIRATORY CHAIN — $68,000

Robert B. Gennis
Departments of Chemistry and Biochemistry

Our research is directed towards elucidating the structure and function of the E. coli aerobic chain. We are particularly interested in the nature of the components of the electron transport chain, in how they interact with each other, and in the mechanism of energy transduction. The initial goal is to identify, isolate and characterize the E. coli cytochromes. Techniques we are using include preparative biochemistry, immunology, and genetics. Our emphasis has been on one of the two branches of the respiratory chain which contains cytochrome d as a terminal oxidase. This terminal oxidase has been purified to homogeneity and is presently being characterized. In addition to heme d, this complex also contains a b-type heme. Two subunits are indicated using SDS-polyacrylamide gels. This has been reconstituted in phospholipid vesicles and manifests ubiquinol-1 oxidase activity. A mutant in the cytochrome b/d complex has been isolated and characterized. Apparently, the entire branch of the respiratory chain containing cytochrome d is missing in this strain. The mutation has been mapped and the gene is presently being cloned. Immunological experiments indicate that only one component in the membranes from this strain contains heme. Presumably this component includes the other terminal oxidase, cytochrome O. These results strongly suggest a relatively simple structure for the respiratory chain. Future work will continue to stress the structural elucidation.

32. 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 — $70,000

John R. Laughnan
Department of Genetics and Development

This investigation involves studies of specific strains of maize at both the field and laboratory levels. The studies are based on the original finding that cytoplasmic reversions from S-type male sterility to the male-fertile condition are associated with disappearance of two mitochondrial-DNA (mtDNA) plasmids present in the mtDNA of S male-sterile plants. Reversions may be cytoplasmic or nuclear in origin. We are involved in characterization of the cytoplasmic revertants at both the genetic and mtDNA level, using restriction enzyme analysis and labelled plasmid probes. These probes are also being employed in attempts to determine the basis for nuclear reversion. We are investigating the degree of control over reversion events exercised by the nuclear genotype, and will also study the time course for disappearance of the plasmids in connection with the cytoplasmic reversion event. We are also investigating secondary transposition of the restorer element in strains of maize that have recently undergone nuclear reversion.

33. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

ACETOPHILIC METHANOGENIC CONSORTIA — $85,000
Ralph S. Wolfe
Department of Microbiology

The purpose of this study is to optimize the interspecies biocatalytic reactions in the fermentation of biomass to methane. To study a major limiting step in this system, the acetoclastic step, acetate adapted methanogens Methanosarcina barkeri and Methanosarcina mazei are being added as terminal biocatalysts in defined microbial consortia. These consortia are being constructed from pure cultures of a variety of heterotrophic bacteria. Comparative studies are being made on association of different species in consortia for optimal conversion of carbohydrate to methane. Immobilized consortia are being studied to determine their stability and efficiency. Methane digesters have a reputation of being slow and erratic in performance, facts that are related to the poorly-understood complex biochemistry and microbiology of the process. These studies are designed as an approach to optimizing critical biocatalytic interactions so that the reliability and efficiency of methane digesters may be improved.

34. THE INSTITUTE OF PAPER CHEMISTRY
Appleton, Wisconsin 54912

RAMAN MICROPROBE INVESTIGATION OF MOLECULAR STRUCTURE AND ORGANIZATION IN THE NATIVE STATE OF WOODY TISSUE — $44,300
Rajai H. Atalla
Division of Chemical Sciences

The objectives of this project are to establish, by Raman microprobe spectroscopy, the variability of molecular structure and organization in the cell walls of native woody tissue. The information to be developed is important for fundamental understanding of the structures in cell walls, as well as for analysis and design of industrial processes which use biomass as a primary resource. The specific objectives of the program are to determine the structures and molecular orientations of celluloses and lignins at different points within individual cell walls. The first species to be investigated will be Pinus taeda L (Loblolly Pine) which is of both academic and commercial interest. Samples of woody tissue will be sectioned both horizontally and longitudinally. Raman spectra will be recorded at different positions within sections of individual cell walls and within middle lamellae. The spectra will be recorded with incident laser polarization perpendicular and parallel to the planes of the cell walls. These spectra will reflect variations in chemical composition and in molecular orientation across the cell walls.

35. IOWA STATE UNIVERSITY
Ames, Iowa 50011

PROLINE METABOLISM IN PLANTS UNDER ENVIRONMENTAL STRESS — $60,778
Cecil R. Stewart
Department of Botany

The objective of this project is to understand the subcellular mechanism which causes proline to accumulate under salt and water stress. It is known that proline accumulates because synthesis is stimulated, oxidation is slowed and protein synthesis is impaired. The nature of the effect on proline oxidation will be studied by determining whether the effect is on the enzymatic machinery or transport in mitochondrial proline oxidation. Mitochondrial proline transport will be characterized and the effects of stress on it will be determined. Electron transport complexes will be isolated and the relationship between proline oxidation and these complexes will be determined.

Attempts will be made to get a cell free preparation containing the first enzyme in the proline biosynthetic pathway. This step appears to be affected by stress and, in order to determine the effect, we need to be able to measure the activity.

Experiments with excised leaves will be conducted to further characterize the salt effects on proline metabolism. Comparisons will be made between the effects of salt and those of desiccation. An understanding of the mechanism of proline accumulation will help tell us how plants adapt to environmental stresses. Perhaps this information can be used to increase the environmental adaptability of crop plants and other important biomass producing plants.

36. IOWA STATE UNIVERSITY
Ames, Iowa 50011

POST-ILLUMINATION CO_2 EVOLUTION AND PHOTORESPIRATORY METABOLITE LEVELS — $69,244 (FY 80 funds)
Cecil R. Stewart
Department of Botany

The objective of our current research is to determine why inhibitors of photorespiration in plants also inhibit photosynthesis. We are studying the effects of aminoacetonitrile (AAN), an inhibitor of glycine decarboxylation, on photosynthesis and photorespiratory metabolite levels in soybean leaves. We are testing the hypothesis that inhibiting photorespiration also inhibits photosynthesis because carbon is not recycled into the Calvin cycle. Levels of ribulose-1,5-bisphosphate (RuBP) are used to determine effects on Calvin cycle intermediates. AAN inhibits photorespiration as measured by the post-illumination burst and CO_2 evolution into CO_2-free air and causes glycine to accumulate. AAN inhibits photosynthesis under photorespiratory conditions, i.e., O_2 levels 10% and higher. However, the levels of RuBP are higher in conditions when photosynthesis is inhibited indicating that the inhibition of photosynthesis is not due to the lack of carbon in Calvin cycle intermediates. Rather these results suggest an inhibition of RuBP carboxylase by treatment with AAN under photorespiratory conditions. We are measuring levels of glyoxylate and glycolate and plan a series of experiments to determine if the carboxylase is inhibited and, if so, by what mechanism. Understanding how photosynthesis is inhibited by these compounds will increase our understanding of what limits photosynthesis under natural conditions and thus what limits biomass production. Photorespiration is energetically wasteful and we want to know why we cannot slow it down without also slowing photosynthesis.

37. C.F. KETTERING RESEARCH LABORATORY
Yellow Springs, Ohio 45387

THE BASIS FOR THE COMPETITIVENESS OF RHIZOBIUM JAPONICUM IN THE NODULATION OF SOYBEAN — $74,687

Wolfgang D. Bauer and William R. Evans
Enhancement of Plant Productivity Mission

The overall goal of these studies is to determine what characteristics might enable an inoculated strain of R. japonicum to compete effectively with indigenous rhizobia at infecting the host legume. With a knowledge of the factors that enable one strain to compete for the limited infection sites more efficiently than another strain it may be possible to select for strains that would have a high probability of effective nodulation in the field.

The methods developed in this laboratory permit the localization and timing of the initiation of infections in soybean within a time span of approximately one hour. The utilization of these newly developed methods should provide the means to establish whether a competitive advantage by one strain over another is due to superior numbers of bacteria in the infectible region of the root, to greater efficiency of infection, or to a more rapid rate of initiating infections.

Several important strains of R. japonicum will be compared with respect to the rates and relative efficiencies of initiating infections. The colonization of the host root, and particularly the narrow band of infectible root cells, will be studied by plate counts, immunological methods and scanning electron microscopy at various intervals after inoculation. R. japonicum is found to retain its viability and symbiotic infectivity after suspension in water at room temperature. Water suspension will be investigated as a novel, simple and inexpensive method for preserving rhizobia for laboratory and commercial inoculation.

38. MARTIN MARIETTA LABORATORIES
Baltimore, MD 21227

STUDIES OF PHOTOSYSTEM II USING ARTIFICIAL DONORS — $91,081

Richard Radmer and Bruno Velthuys
Biosciences Department

The objective of this project is to study the mechanism of photosynthetic water oxidation by the use of artificial donors. The approach utilizes specialized mass spectrometry and flash-kinetic spectrophotometry techniques developed in-house. Mass spectrometry studies will make use of competitive inhibitors of H_2O oxidation that have the ability to compete with and override H_2O oxidation without destroying the O_2 system (e.g. hydroxylamine and hydrazine). We will monitor the photooxidation products of these substrates along with evolved O_2 to determine the relative affinities of H_2O and the added substrate for the O_2-evolving site under various conditions. Similar experiments will also be done using substituted hydroxylamines and hydrazines. The goal of these experiments is to "map" the O_2-evolving site. Flash kinetic spectrophotometry will be used to study the photosynthetic oxidation of manganese ions. The oxidation of manganese is mediated by the rudimentary O_2 system, as a preparation for water oxidation. Characterization of this process will aid in our understanding of the chemistry of photosynthetic water oxidation.

39. UNIVERSITY OF MARYLAND
College Park, Maryland 20742

ENERGY-DEPENDENT CALCIUM TRANSPORT MECHANISMS IN PLANT MEMBRANES — $58,426

Heven Sze
Department of Botany

The objective of this project is to identify active calcium (Ca) transport systems in plants and understand the mechanisms that regulate Ca fluxes. Plant tissues will include tobacco tissue callus (Nicotiana tabacum) and oat roots (Avena sativa). The approach is outlined as follows: (1) Determine properties of ATP-dependent Ca transport in microsomal vesicles. 45-Ca transport will be measured by the filtration procedure. (2) Identify specific membrane types with active Ca transport. Non-mitochondrial membranes will be separated with continuous density gradients and membranes will be identified by enzyme or other markers. (3) Determine the types of Ca transport mechanisms by using specific inhibitors and activators. Two possible mechanisms are a H^+-dependent Ca transport mediated by a H^+-pumping ATPase and a direct Ca-pumping ATPase perhaps stimulated by calmodulin. (4) Determine whether phytohormones regulate directly or indirectly active Ca fluxes. (5) Determine whether and how Ca regulates Mg/KCl-ATPase activity and electrogenic H^+ pumps. (6) Identify and characterize Ca-pumping ATPase. Ca plays important roles in the regulation of various physiological and biochemical processes. These studies of active Ca transport systems are central to our understanding of the mechanism and regulation of energy-dependent solute transport and plant growth and development.

40. UNIVERSITY OF MASSACHUSETTS
Amherst, Massachusetts 01003

CONVERSION OF CELLULOSE TO ETHANOL BY MESOPHILIC BACTERIA — $75,000

Ercole Canale-Parola
Department of Microbiology

The objectives of this project are i) to obtain from natural environments, or by means of mutagenic techniques, strains of mesophilic bacteria capable of fermenting cellulose with maximum production of ethanol, and ii) to study the metabolic processes responsible for the anaerobic conversion of cellulose to ethanol by these bacteria. The procedures used for the isolation of ethanol producers are designed to select for diverse cellulolytic bacteria and involve culture media of varied composition, inocula from different natural environments, different types of cellulosic materials as growth substrates, and a variety of cultural conditions. The fermentation products and the phenotypic characteristics of the isolated strains are determined. Ethanol production from cellulose is studied under different cultural conditions, in pure cultures and in mixed cultures with Zymomonas mobilis. The regulatory effect of chemicals on cellulase activity and/or ethanol production, as well as the enzymatic steps involved in formation of ethanol, are investigated. Studies on the inducibility of the cellulase systems of the isolates by pentoses, hexoses, and disaccharides are carried out. The bacterial strains isolated in the course of this research and the information obtained on their metabolic processes will be useful in the development of efficient industrial processes for the conversion of cellulosic materials to ethanol.

41. MICHIGAN STATE UNIVERSITY
East Lansing, MI 48824-1312

CONTROLLED MEDIA FOR PLANT TISSUE CULTURE $50,000
Norman E. Good
Department of Botany and Plant Pathology

One objective of this research is to develop and test relatively stable substances which break down only slowly to yield a continuous supply of essential phytohormones which, of themselves, are biologically very labile. Thus, it is possible to provide plant tissue cultures with various steady-state low levels of auxins and cytokinins. Another objective is to develop hydrogen ion buffers suitable for use in plant tissue cultures. A third objective is to examine the effects of concentration gradients across pieces of cultured plant tissue - gradients of auxin, cytokinin, pH, sugar, electrical potential, etc.

42. UNIVERSITY OF MINNESOTA
St. Paul, Minnesota 55104

PHYSIOLOGY AND GENETICS OF METHANOTROPHIC BACTERIA $69,935
Richard S. Hanson
Gray Freshwater Biological Institute

Restriction fragments from pCH-SB1 which codes for the ability of Methylobacterium to oxidize methane to methanol (methane mono-oxygenase) in Methylobacterium organophilum strain R6-SB1 have been cloned into pBR322. Four unique libraries have been constructed. We intend to examine transcription of each fragment during growth on methane and heterotrophic substrates and identify proteins encoded by each fragment in E. coli minicells.

Chromosomal genes coding for enzymes of methanol metabolism and one-carbon assimilation will be cloned in E. coli using pRK290 as a vector. Inserts will be recognized by complementation of markers after mobilization of pRK290 hybrids into M. organophilum mutants lacking each enzyme.

Mutants lacking each activity will be examined for their ability to transform several substrates to products of commercial or environmental importance. The biotransformational activities and genetic homology of naturally occuring methylotrophs and laboratory isolates will be compared.

43. UNIVERSITY OF MINNESOTA
St. Paul, MN 55108

CORN STORAGE PROTEIN - A MOLECULAR GENETIC MODEL — $68,000
Joachim Messing
Department of Biochemistry

The objective of this proposal is to study the Zein multigene family in Zea mays. The approach uses the single-stranded DNA phage cloning system to study the primary structure of single cDNA clones made from the Zein mRNA population. The sequence of a number of representative cDNA clones will be compared to obtain an understanding of Zein protein structure. In addition, Zein related sequences will be obtained from genomic DNA directly and subjected to sequencing studies. The comparison of genomic sequences with cDNA sequences of the Zein multigene family will enable us to define the structure of the sequences flanking the Zein genes, to determine if RNA processing is likely to occur in Zein expression, and to compare the resulting features to genes found in animal systems. The analysis of the structure of the Zein genes and the Zein proteins are a prerequisite to design molecular changes for a molecular breeding program in corn, a major crop in our agricultural industry.

44. UNIVERSITY OF MISSOURI
Columbia, Missouri 65211

PHOTOSYNTHESIS AND CLONING IN CYANOBACTERIA - A SYSTEM FOR THE BIOCONVERSION OF SOLAR ENERGY — $55,246
Louis A. Sherman
Division of Biological Sciences

The aim of this proposal is to clone genes that code for photosynthetic functions, using the unicellular cyanobacterium Anacystis nidulans R2. This organism was chosen because it performs an aerobic photosynthesis nearly identical to that of plants, yet it possesses many important characteristics of microorganisms. In particular, this strain displays a high frequency of transformation, and contains two plasmids (33 and 5.3 MDa). We have also developed procedures for the isolation of temperature-sensitive, photosynthetic mutations. The presence of mutants, transformation, and cloning vectors would allow genes to be inserted into mutant cells and identified by phenotypic complementation. The appropriate genes can then be sub-cloned and transcribed and translated to allow a direct analysis of each gene product. The first stage in this procedure is to construct suitable cloning vectors. Since neither A. nidulans plasmid codes for a recognizable function, we have produced hybrid vectors that are capable of replication in both Escherichia coli and Anacystis nidulans. One such vector, pSG111, is composed of pBR328 and the 5.3 Mdal plasmid. This 8.2 Mdal plasmid can replicate in both organisms and has been used to produce a clone bank of Anacystis DNA. We are now constructing a derivative of this plasmid that contains the lambda cos site. This will enable us to clone large DNA fragments and will greatly speed our analysis. We have also isolated a number of important photosynthetic mutants, including those affecting cytochromes and chlorophyll-binding proteins. Using the gene bank in pSG111, we will soon begin complementation analysis in the mutants. Finally, we have also analyzed known clones for homology to Anacystis DNA. We are now in the process of cloning genes that code for the subunits in the H^+-translocating ATPase.

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

THE RESPIRATORY CHAIN OF ALKALOPHILIC BACTERIA — $68,835 (FY 81 funds)
Terry A. Krulwich
Department of Biochemistry

In view of the increased energy cost of life at extremely alkaline pH, the extraordinary qualitative and quantitative array of respiratory chain components of alkalophilic bacteria, and the normal growth yields and O_2 consumption rates of such organisms, it has been proposed that the obligately alkalophilic bacteria possess structural/functional properties of the respiratory chain such that particularly efficient energy conservation is facilitated. The respiratory chain components of Bacillus alcalophilus have been studied in comparison with its non-alkalophilic mutant derivative; a similar study of Bacillus firmus RAB and non-alkalophilic RABN is now partially completed. The alkalophiles contain high quantities of many distinct redox carriers as compared to their derivative and other non-alkalophiles. Determinations of H^+70 ratios are now in progress.

A system for study of the regulation of cytochrome expression, as a function of pH, has been developed. Failure of obligate alkalophiles to grow at pH 7.0 now appears to relate to the low membrane potentials produced by respiration at that pH, rather than a failure of pH homeostasis. Since alkalophilic cells are found to be viable at pH 7.0, incubations can be conducted for study of functional and regulatory aspects of respiration. These studies should provide further insights into fundamental aspects of energy transduction/conservation by membrane-associated processes.

46. NATIONAL INSTITUTE OF GENERAL MEDICAL SCIENCES
5333 Westbard Avenue
Bethesda, MD 20205

NUCLEIC ACID SEQUENCE DATA BANK — $40,000
Christine Carrico

The National Institute of General Medical Sciences is in the process of establishing a nucleic acid sequence data bank. It is anticipated that all available data on nucleic acid sequences will be collected and made available to the scientific community in the context of a sophisticated computer system. This computer system will provide both access to the sequence data and effective computer software for the analysis and manipulation of the data and will facilitate the exchange of data, ideas, and methods within the scientific community.

The work to be performed will be divided into two closely coordinated projects:

1. The first project will be for the collection, verification, and initial distribution of the nucleic acid sequence data.

2. The second project will provide for the establishment of the computer system and the implementation of the appropriate software for data base management, sequence analysis and manipulation, and user communication as described above.

(Multi-Agency supported)

47. UNIVERSITY OF NEBRASKA-LINCOLN
Lincoln, NE 68583-0718

BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERIZATION OF HIGHER PLANTS WITH REDUCED PHOTORESPIRATION — $48,051

Raymond Chollet
Department of Agricultural Biochemistry

This overall project is designed to further our understanding of mechanisms which may reduce photorespiration in C_3 plants. The major research thrust will involve a detailed investigation of photosynthetic and photorespiratory carbon metabolism in the few documented examples of naturally occurring higher plants with reduced photorespiration, including Moricandia arvensis and Flaveria linearis. The initial research objective is to evaluate critically the possibility that reduced photorespiration in the crucifer M. arvensis is due to a limited C_4-photosynthesis system similar to that previously proposed for the well-characterized C_3-C_4 intermediate Panicum milioides. The anatomical, physiological, and biochemical features of this crucifer which are strikingly similar to those of P. milioides are: (a) the presence of prominent leaf vascular bundle sheaths which contain numerous, centripetally-arranged mitochondria and starch-containing chloroplasts; (b) various intermediate photorespiratory CO_2 exchange characteristics; (c) a C_3-type PEP carboxylase exhibiting a maximal velocity which is 2- to 3-times that of the enzyme from C_3 plants; (d) aspartate and alanine aminotransferase activities which are 2- to 3-fold higher than in a representative C_3 species; and (e) C_3-type $\delta^{13}C$ values. However, the low or undetectable activities of the C_4 acid decarboxylating enzymes (NAD- and NADP-malic enzymes and PEP carboxykinase) and pyruvate, Pi dikinase in M. arvensis suggest that a limited C_4-like CO_2-concentrating mechanism is NOT responsible for reducing photorespiration in this crucifer. This view is further substantiated by the results from intact leaf $^{14}CO_2$ incorporation studies which indicate that after 6-s pulse photosynthesis in $^{14}CO_2$-air, only about 8% of the total ^{14}C fixed is in the C_4 acids malate and aspartate [see Holaday, Shieh, Lee, & Chollet, Biochim, Biophys. Acta 637 (1981) 334-341 and Holaday, Harrison, & Chollet, Plant Sci. Lett. (1982) in press].

48. UNIVERSITY OF NEBRASKA
Lincoln, Nebraska 68583-0722

VIRUSES OF EUKARYOTIC GREEN ALGAE — $53,000

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. Virus replication begins in the algae following isolation from the host and by 24 hours the entire population of algae lyse. The origin of the viruses is unknown. The identification and characterization of these viruses represents one of the first characterizations of eukaryotic algal viruses.

The objectives of this proposal will provide further information about these viruses. Using established methods, we intend to (i) characterize and compare the four viruses found to date, (ii) attempt to infect culture grown Chlorella, both for mass culture purposes and for developing a biological assay, (iii) determine the source of the viruses, (iv) characterize the receptor/attachment site and infection mechanism of these viruses, (v) determine if the viruses contain an enzyme(s) which degrades the algal cell wall, and (vi) begin studies on the life cycle of the viruses.

The research has several potentially important long range implications. Studies of these algal viruses will: (i) provide an opportunity to study a new type of virus-host relationship, (ii) determine the role these viruses play in symbiosis, (iii) demonstrate the usefulness of these dsDNA viruses as vectors for transferring genes into algae or higher plants, and (iv) determine if these viruses contain a new source of plant cell wall degrading enzymes.

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

GENE-ENZYME RELATIONSHIPS IN SOMATIC CELLS AND THEIR ORGANISMAL DERIVATIVES IN HIGHER PLANTS — $85,000
Roy A. Jensen
Center for Somatic-cell Genetics & Biochemistry

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

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

METHANE PRODUCING BACTERIA: IMMUNOLOGICAL CHARACTERIZATION — $45,000
Conway de Macario, E.,*Macario, A.J.L.,* & Wolin, M.J.°
*Laboratory Medicine Institute
°Environmental Health Institute

The objective of this project is to develop immunological methods and prepare specific antibodies for identification and classification of methane-producing (methanogenic) bacteria. The approach is to immunize rabbits with all methanogenic bacteria available in pure culture to prepare specific antisera. These antisera are titrated and their reactivity pattern with all known methanogens is established. Selected dilutions of each antiserum are used as calibrated probes for identification and rapid classification of new isolates. Utilizing immunological methods developed and the antibody probes, microorganisms isolated in our laboratory and in many others are being characterized. The data obtained are processed, analyzed and stored in a bank which is becoming an extremely valuable reference for immunological analyses of methanogenic bacteria. These studies are being applied to identification and enumeration of methanogenic bacteria in practically important methane producing systems, i.e. systems for production of natural gas from biomass, waste decomposition systems and natural ecosystems where methane production is an important component of the carbon cycle.

51. NORTH CAROLINA STATE UNIVERSITY
Raleigh, NC 27650

GENETIC BASIS OF LONG-CHAIN ALIPHATIC HYDROCARBON BIOSYNTHESIS IN BACTERIA — $36,049 (FY 81 funds)

Wesley E. Kloos
Department of Genetics

The objective of this research will be to gain preliminary information on the genetic basis of long-chain hydrocarbon biosynthesis in the aerobic bacteria Micrococcus and related Arthrobacter, and Pseudomonas maltophilia, and then estimate the feasibility of constructing bacterial strains with unusually high hydrocarbon composition. Genetic and biochemical studies of these aerobic, hydrocarbon-producing bacteria can also serve as models for approaching the regulation and construction of eukaryotic hydrocarbon biosynthetic systems. We will begin our search for the genetic basis of hydrocarbon biosynthesis by first looking for the presence of plasmids which may be carrying genes specifying enzymes for hydrocarbon biosynthetic pathways or in some way regulating hydrocarbon biosynthesis. Cleared lysates of various cultures will be subjected to agarose gel electrophoresis to determine plasmid presence. After screening, we will attempt to eliminate selected plasmids by the use of various curing agents and determine what, if any, effect their absence may have on hydrocarbon synthesis. We will also attempt to transfer selected plasmids, e.g., from "high" hydrocarbon-producing strains to "low" hydrocarbon-producing strains, or those not containing detectable levels of hydrocarbons, by using protoplast fusion and/or transformation, and observe any changes that might occur in the composition or quantity of hydrocarbons in the recipient. If time permits and we are successful in identifying plasmids specifying hydrocarbon biosynthesis, selected plasmids will be further characterized by restriction endonuclease analysis and electron microscopy.

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

THE ISOLATION AND CHARACTERIZATION OF β-GLUCOSIDASE GENE AND β-GLUCOSIDASE OF TRICHODERMA VIRIDE — $79,866

Darrel W. Stafford and Roger L. Lundblad
Department of Biology and Dental Research Center

The objective of this proposal is to genetically engineer an organism with the following properties: The organism should carry a multiple copy plasmid with all the necessary genes for cellulase production under the control of one strong promoter; the genes should carry information for the "leader" protein sequence which is necessary for the proteins to pass through the cell membrane into the environment; the genes themselves should have been mutated in vitro to eliminate end-product inhibition; and finally, the genes should be inserted into a yeast host so that the glucose produced from cellulose can be converted directly to ethanol.

A Trichoderma genomic bank of 30,000 clones has been constructed in E. coli using lambda or pACYC 184 vectors. The clones containing DNA encoding specifically cellobiohydrolase I, cellobiohydrolase II, or endoglucanase II are being selected by hybridization with synthetic DNA oligomers specific for these enzymes as deduced from published amino acid sequences. Portions of the cloned DNA will be sequenced by Maxam and Gilbert techniques to verify this identification. Amino acid sequencing of β-glucosidase is in progress. A synthetic oligomer specific for β-glucosidase will be synthesized and will be used to select genomic clones containing DNA encoding β-glucosidase.

A Trichoderma cDNA bank of 400,000 clones has been constructed in E. coli using lambda vectors. Differential screening with cDNA prepared with induced and uninduced mRNA has yielded a pool of clones specific for cellulose-induced Trichoderma. The clones containing each of the four enzymes of the cellulose complex will be identified using specific cloned genomic DNA as probes. Cellulase cDNA clones in an expression vector will be identified by the binding of antibodies specific for each of the cellulases.

53. THE OHIO STATE UNIVERSITY
Columbus, Ohio 43210

DEVELOPMENT OF GENETIC SYSTEMS FOR ANALYSIS OF THE OBLIGATE ANAEROBE METHANOBACTERIUM RUMINATIUM PS. — $62,804

John N. Reeve
James I. Frea
Department of Microbiology

Methane biogenesis is of enormous importance as a source of world energy and as a mechanism of waste disposal. This study is a genetically oriented investigation of a methanogenic species. The goals are to develop techniques to genetically manipulate methane producing species and to provide a detailed understanding of the genomic structure of the methanogen Methanobacterium ruminatium PS. Antibiotics are being screened to determine which inhibit the growth of methanogens and mutants are subsequently being isolated which are resistant to these antibiotics. Antibiotic-resistance-conferring mutations will be used to provide selectable traits in developing a transformation system. A number of procedures are being evaluated which convert methanogens to protoplasts to determine if protoplast regeneration can be obtained so that protoplast transformation systems can be developed. In vitro recombinant DNA techniques are being used to construct gene banks containing methanogen-derived DNA cloned in cosmid and phage vectors which are capable of replication in E. coli. These banks are being screened for the expression of methanogen genes in E. coli. Some auxotrophic mutations of E. coli are found to be complemented by methanogen-derived DNA sequences and these sequences, together with DNA sequences found to occur repetitively in the genome of methanogens, are being analyzed in detail. Restriction enzyme generated fragments of methanogen DNA, identified as being capable of complementing E. coli auxotophic mutations, will also be used as donor DNA to facilitate the development of a methanogen transformation system.

54. UNIVERSITY OF OKLAHOMA
Norman, Oklahoma 73019

DETERMINE IF INFLUENCE OF SINK DEMAND ON NITROGEN METABOLISM IN THE SOURCE LEAF CAN ACCOUNT FOR REGULATION OF PHOTOSYNTHETIC ACTIVITY — $50,000 (FY 81 funds)

John S. Fletcher, Leonard Beevers
Department of Botany and Microbiology

In vascular plants the photosynthetic rate of source leaves is subject to control by the metabolism of sink areas. It has been suggested that during reduced sink demand assimilates accumulate in the leaf and inhibit photosynthesis. Research directed primarily towards relating carbohydrate accumulation to a decline in photosynthesis has failed to show a clear relationship. No attention has been given to the possibility that nitrogenous compounds may regulate photosynthetic activity of the source.

Since the reduction and assimilation of NO_3^- is dependent upon photosynthesis for both reducing power and carbon skeletons, it is reasonable to speculate that both nitrogen metabolism and photosynthesis in the source leaf may be subject to sink control. Furthermore, disruption of normal nitrogen assimilation in the leaf may be instrumental in reducing the photosynthetic rate. Research is being conducted to establish the influence of fruit removal on nitrogen metabolism and photosynthesis of source leaves in green peppers. Subsequent experiments will be conducted with other species of plants reported to have varied degrees of sink control on photosynthesis as well as differences in the site of NO_3^- reduction, root versus leaf.

55. UNIVERSITY OF PENNSYLVANIA
Philadelphia, Pennsylvania 19104

FACTORS GOVERNING LIGHT DRIVEN ELECTRON AND PROTON TRANSLOCATION IN PROTEINS ACROSS MEMBRANES — $79,736
P. Leslie Dutton
Department of Biochemistry & Biophysics/G5

The experimental goal of this project is to understand more about the individual electron transfer steps that are involved in charge separation in the photochemical reaction center protein from Rhodopseudomonas sphaeroides and in the respiratory ubiquinol-cytochrome c oxidoreductase. Work is being done to organize these proteins in planar phospholipid bilayers (BLMs) between electrodes or in protein mono- and multilayer arrays deposited on planar substrates and electrode materials. From flash activation studies, current/voltage measurements, and from the effects of applied voltages across the oriented proteins it is hoped to obtain information on which reactions are electrogenic and what are the relative locations of the redox center in the profile of the protein. The results should increase our understanding of the function of these membrane redox proteins and make contributions to improving experimental information pertinent to progress in electron and proton transfer mechanisms.

56. PURDUE UNIVERSITY
West Lafayette, Indiana 47907

SEED PROTEIN GENES AND THE REGULATION OF THEIR EXPRESSION — $99,000 (10½ months)
Brian A. Larkins and Donald E. Foard
Department of Botany and Plant Pathology

The Bowman-Birk protease inhibitor (BBI) and the related family of isoinhibitors (PI I-IV) occur in soybean seeds and contain a high content of the sulfur amino acids. The isolation of genes for these isoinhibitors would provide information on their number and organization, and would provide probes for studying the regulation of their expression. Cloned copies of these genes might also eventually be used to raise the sulfur amino acid content of soybean seed protein. Messenger RNAs that direct their synthesis are identified and proteins synthesized in vitro are compared with the authentic proteins that accumulate in vivo by HPLC and amino acid sequence analysis of immunoprecipitates. The mRNAs are used to construct cDNA clones. Several of these clones have cDNA inserts of 400-500 nucleotides and probably contain the complete coding sequence of the mRNA. We are determining the nucleotide sequence of these clones to verify their authenticity and using them as probes to identify the corresponding genes in a library of soybean nuclear DNA.

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

DIFFERENTIAL GENE EXPRESSION IN C4 PLANT — $78,524

Anthony R. Cashmore
Department of Cell Biology

The small subunit of the chloroplast enzyme, ribulose-1,5-bisphosphate carboxylase, is encoded by nuclear DNA. When a cloned small subunit cDNA (pSS15) is hybridized to Eco R1 restricted pea DNA, five complementary fragments are observed. Two of these Eco R1 restriction fragments, of 3.3 and 8.0 kb, have been isolated by cloning, and are seen to correspond to a pair of tandemly duplicated, divergently orientated, small subunit genes which are separated by 10 kb of DNA. These genes have been sequenced and shown to contain two intervening sequences. The coding capacity of the first exon of these genes is almost exclusively devoted to encoding the 57 amino acids of the transit sequence for the small subunit precursor. Further studies will be concerned with characterizing the expression of these small subunit genes.

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

BIOMASS UTILIZATION: BASIC SECRETORY PHENOMENA AND THE CONSTRUCTION OF HYPERCELLULOLYTIC MUTANT OF TRICHODERMA REESEI — $119,894

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

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

59. SMITHSONIAN INSTITUTION
Washington, D. C. 20560

A PRIMARY LIGHT HARVESTING SYSTEM: PHYCOBILISOMES AND ASSOCIATED MEMBRANES — $50,000 (15 months)

Elisabeth Gantt
Radiation Biology Laboratory
Rockville, MD 20852

Absorption of light for photosynthesis is greatly enhanced in red and blue-green algae by the presence of phycobiliproteins which absorb energy in the wavelength region where chlorophyll has minimal absorption. The energy absorbed by the phycobiliproteins, which exist as supramolecular aggregates on the photosynthetic lamellae, is transferred with high efficiency to the photosynthetic reaction centers. The structure and composition of phycobilisomes are being studied in reference to their function in absorbing and transferring light energy. One major aspect being studied is the role of linker proteins which are assumed to be responsible for specific packing of phycobiliproteins assuring maximum energy transfer. Polypeptide analyses of phycobilisomes from numerous blue-green algae, phylogenetically related and nonrelated, suggest that a protein (ca. 31,000 m. wt.) involved in phycocyanin and allophycocyanin linking is present in all. In vitro reassociation of phycobilisomes is being used to establish the location and function of this protein. Another major aspect under investigation is identification and characterization of the component(s) responsible for anchoring the phycobilisome to the thylakoid membrane. A high molecular weight polypeptide component (ca. 95,000 m. wt.), present in many red and blue-green algae, is being characterized in Porphyridium cruentum. The 95,000 m. wt. protein separately isolated from phycobilisomes and from photosynthetic membranes (free of phycobilisomes), are being compared (immunologically, biochemically and spectroscopically). The results will add fundamental knowledge to our understanding of the organization of a very effective light harvesting system.

60. SOUTHERN ILLINOIS UNIVERSITY
Carbondale, Illinois 62901

REGULATION OF ALCOHOL FERMENTATION BY ESCHERICHIA COLI — $62,000

David P. Clark
Department of Microbiology

I intend to investigate how the production of alcohol by the bacterium Escherichia coli is regulated. E. coli produces ethanol when fermenting sugars under anaerobic conditions. The pathway for ethanol production is repressed by oxygen and other electron acceptors and is also inoperative when conditions are too acid. New advances in genetics, such as molecular cloning of the genes for enzymes involved in alcohol production, and the isolation of gene fusions will be used. Mutants which lack alcohol dehydrogenase and those which overproduce this key enzyme will be used as a starting point for the genetic analysis. The gene for alcohol dehydrogenase has been located on a large restriction fragment on a bacteriophage vector. Future work will involve subcloning of small fragments into high copy-number vectors and a detailed analysis of the regulatory elements associated with this gene. Further investigations, of a similar nature, will then be pursued for the second enzyme of the alcohol production pathway- acetaldehyde dehydrogenase. Understanding how alcohol production is regulated should allow the construction, by genetic engineering of more efficient producer organisms.

61. STANFORD UNIVERSITY
Stanford, California 94305

CLONING AND MAPPING OF EARLY SYMBIOTIC GENES OF RHIZOBIUM MELILOTI — $100,446

Sharon R. Long
Department of Biological Sciences

We are studying the symbiosis between the bacterium Rhizobium meliloti and its plant host, alfalfa (Medicago sativa). When these two organisms interact, they form root nodules and carry out enzymatic fixation of nitrogen to ammonia. The plant is thus able to grow without added nitrogen fertilizer. Our primary goal is to identify and locate the bacterial genes which control recognition and infection of the host plant. The genes needed for nodulation can be identified through mutants which cannot form nodules. We are producing such mutants by transposon mutagenesis of R. meliloti. We are taking two primary approaches: first, we are making mutants at random in R. meliloti and screening for nodulation-defective strains. Secondly, we are generating transposon mutations in DNA regions adjacent to known nodulation genes, to find out whether there is a cluster of many nod genes in these regions. When we have isolated mutants, we will further study the genes by obtaining them as cloned DNA sequences. One method for doing this is by directly cloning transposon-containing regions from DNA of mutant strains. Alternatively, we will clone nodulation genes by functional complementation of Nod^- mutants using a clone bank of wild-type R. meliloti DNA sequences in a broad host range cosmid vector. The mutant strains and cloned DNA sequences of the nodulation genes will be used to determine where the genes are located in the R. meliloti genome.

62. STANFORD UNIVERSITY
Stanford, California 94305

CARBON DIOXIDE AND THE STOMATAL CONTROL OF WATER BALANCE AND PHOTOSYNTHESIS IN HIGHER PLANTS — $66,000

Eduardo Zeiger
Department of Biological Sciences

The aim of this project is the characterization of the stomatal responses to carbon dioxide in studies with intact leaves, epidermal peels and isolated guard cell protoplasts. Species under investigation include Chlorophytum comosum, Commelina communis, Ginkgo biloba, Paphiopedilum harrisianum, P. insignes and Vicia faba. The role of guard cell chloroplasts in the stomatal responses to CO_2 is investigated in studies of chlorophyll a fluorescence transients. These transients reveal a CO_2 modulation of guard cell photophosphorylation and point to a mechanism coupling photosynthetic activity in the mesophyll with changes in stomatal conductance. The light-CO_2 interactions in stomatal responses are also analyzed in dual-beam experiments with monochromatic blue and red light provided by lasers. Other stomatal responses to CO_2 are characterized in studies of stomatal opening in darkness. Progress in these investigations should enhance our understanding of stomatal function, help us to evaluate the potential implications of increases in atmospheric CO_2 on the biosphere and provide means of improving agricultural practices in marginal lands.

63. UTAH STATE UNIVERSITY
Logan, UT 84322

BIOENERGETICS OF THE METHANOGENIC BACTERIA $67,000
Jack R. Lancaster, Jr.
Department of Chemistry and Biochemistry, UMC 03

The research in this laboratory is directed toward delineating the exact biochemical mechanism whereby energy is conserved by the methanogenic bacteria. Our present efforts are devoted to establishing a link between the various electron transfer components which have been described by this and other laboratories and ion gradients generated during methanogenesis and during ATPase activity. Our primary technique is low temperature electron paramagnetic resonance spectroscopy, which is undoubtedly the method of choice for studying electron transfer components in methanogens. We will also use a combination of other approaches, including redox titrations, enzymatic assays, membrane potential assay and manipulation, reconstitution techniques, protein purification, physical probes, and membrane sidedness reagents.

64. WASHINGTON STATE UNIVERSITY
Pullman, Washington 99164

REGULATION OF TERPENE METABOLISM $56,628
Rodney Croteau
Institute of Biological Chemistry, 6340

The volatile plant oils (essential oils) and plant derived resins are finding increasing use as replacements for petroleum based industrial feedstocks, and the potential for the large scale production and expanded use of these renewable resources is considerable. To realize this potential, fundamental knowledge of the biochemistry of essential oil and resin terpenes is needed, particularly as it applies to the regulation of terpene metabolism. This research project is intended to provide an understanding of such regulatory mechanisms at the enzyme level. This objective is being accomplished through the intensive investigation of two test plant systems (Salvia officinalis and Mentha piperita) which allow us to probe the control of both biosynthetic and catabolic processes involved in terpene accumulation. A major focus of the research is the possible hormonal mediation of developmental enzyme level changes, and the possible inductive processes mediated by the terpenes themselves. The new information gained will have important fundamental and practical consequences for the yield and composition of terpenoid oils and resins that can be made available for exploitation as renewable sources of chemical feedstocks.

65. WASHINGTON STATE UNIVERSITY
Pullman, Washington 99164

DNA TRANSFORMATION OF PLANT CELLS DEFECTIVE IN NITRATE REDUCTASE ENZYME ACTIVITY — $54,428

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

The objective of this project is to isolate and characterize the chl (chlorate resistant-nitrate reductase deficient) genes from Escherichia coli for use as selectable gene vectors for plant cell transformation. Initial data based on in vitro complementation tests between the E. coli chl mutant extracts and the tobacco cnx 68 mutant extracts suggested that the E. coli chl A gene may be the best choice for further work. This gene will be cloned, sub-cloned on a small fragment and characterized by restriction enzyme mapping. The nitrate reductase-deficient tobacco mutant cnx 68 protoplasts will be used as recipients of the E. coli chl A gene. Cells from this mutant are unable to grow on nitrate as the sole nitrogen source, but cells transformed with a functional chl A gene will be able to grow on nitrate and thus can be selected. This transformation system will provide a means for introducing new genes into plant cells.

66. WASHINGTON UNIVERSITY
St. Louis, Missouri 63130

CHARACTERIZATION OF, AND PLANT GENETIC ENGINEERING WITH, A GENE FOR SOYBEAN STORAGE PROTEIN — $60,875

Roger N. Beachy
Department of Biology, Box 1137

The purpose of this project is to isolate and to characterize by DNA sequencing a gene that encodes a subunit of the vicilin seed storage protein (the 7S storage protein) of soybean, Glycine max, and to genetically engineer this gene for expression in a different plant. Several genes have been isolated and DNA sequence analysis will be completed on one or more genes. The sequencing experiments verified that the entire gene, including the putative promoter, initiation and termination sequences, are present on the cloned gene. One gene for an α' subunit of the vicilin protein is currently being genetically engineered onto the tumor-inducing (Ti) plasmid of Agrobacterium tumefaciens such that its expression will be controlled by the promoter signals of the Ti-plasmid. The engineered gene (in collaboration with Dr. M. D. Chilton, this Department) will be transferred to tobacco cells. Subsequent experiments will ascertain if transcription and translation of the engineered gene has occurred. This research represents an attempt to determine if precise placement of the target gene within the Ti-plasmid will facilitate its expression following genetic engineering into the transformed tobacco cells.

67. WASHINGTON UNIVERSITY
St. Louis, MO 63130

DEVELOPMENT OF A TUMOR-INDUCING PLASMID VECTOR FOR PLANT GENETIC ENGINEERING — $68,000

Mary-Dell Chilton
Department of Biology

The Ti plasmids of Agrobacterium tumefaciens have the natural ability to bring about insertion of a sector of plasmid DNA, called T-DNA, into the chromosomes of a wide variety of host plants, causing the plant cancer called Crown Gall. Cells from the gall can be cultured free from the bacterium and stably maintain the foreign DNA introduced by the pathogen. In order to exploit this natural gene vector for the genetic engineering of the agriculturally important crop plants, several technical problems must be solved. The first is to "disarm" T-DNA by introducing one or more mutations into it, such that it does not cause the plant cells to become tumorous. This is essential in order to enable us to regenerate whole plants from the engineered plant cell lines. In the past year we have found that a single gene inactivation suffices to "disarm" T-DNA of our Ti plasmid vector, pTi T37. We have regenerated tobacco plants from plant cells containing "disarmed" T-DNA, and have shown that they contain the full length of T-DNA. Transmission of this foreign DNA to fl progeny of these fertile plants is under study this year. We have used this technique to introduce two eukaryotic genes (maize zein and yeast alcohol dehydrogenase) into whole tobacco plants. Similar work with soybean 11 S seed storage protein gene is underway. The yeast and maize genes do not function in tobacco. We are constructing chimeric forms of these genes, attaching the signals (promoters and terminators) from a T-DNA gene that does function in tobacco, in an effort to bring about expression of foreign genes engineered into this new plant host.

68. UNIVERSITY OF WASHINGTON
Seatlle, WA 98195

STUDIES ON THE CONTROL OF PLANT CELL ENLARGEMENT BY CELLULAR PARAMETERS — $53,000

Robert E. Cleland
Department of Botany

The goal of this research is to determine how plant cell enlargement is controlled at the cellular level. Cell enlargement is regulated by the balance between wall extensibility and the effective turgor pressure. When hormones such as auxin or environmental agents such as light alter the growth rate of plants, they must do so by changing one of these two parameters. We are concentrating our attention on the effective turgor pressure. A micro-pressure probe is being used to measure the turgor pressure directly. With it, the effect of auxin on the turgor pressure and the hydraulic conductivity of pea stem cells is being measured directly for the first time. An unexpected result is the finding that the actual turgor pressure is 1-2 bar lower than that calculated by the usual methods; it appears that this is due to the presence of considerable solutes in the cell wall solution. A second approach is to examine the relation between the growth rate and the import of solutes into soybean and pea stem cells. As cells enlarge, turgor pressure decreases due to dilution of the cell contents with water, unless new solutes are imported. We are finding a remarkable correlation between the amount of growth and the amount of solute import that occurs, suggesting that the principal factors controlling the growth rate may be the rate of osmoregulation. If so, research on the control of osmoregulation may lead to an increased ability to modulate the growth rate of plants, and thus their biomass production.

69. UNIVERSITY OF WASHINGTON
Seattle, Washington 98195

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

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

Xylose is a major structural component of hemicellulose. As such, it is abundantly available in waste streams produced from biomass, such as sulfite waste liquor from paper manufacture. The efficient utilization of this resource would be furthered by development of a xylose-fermenting yeast. The fission yeast Schizosaccharomyces pombe is an efficient fermenter of xylulose and we have shown that it transports xylose. In order to provide S. pombe with enzyme activity for the missing step, (xylose isomerase) we have cloned this gene from E. coli. From its DNA sequence, we can deduce the correct DNA site for fusions to a promoter sequence active in S. pombe. Yeast strains engineered with such xylose$^+$ expression plasmids will be tested for their ability to grow on xylose as carbon source and for their ability to produce ethanol when grown on xylose.

70. UNIVERSITY OF WASHINGTON
Seattle, Washington 98195

A STUDY OF THE GENETICS AND REGULATION OF METHANE OXIDATION — $72,499 (FY 81 funds)

Mary E. Lidstrom (formerly Mary L. O'Connor)
Department of Microbiology & Immunology SC-42

The objective of this project is to locate and identify the genes necessary for growth on C_1 compounds in methane utilizing bacteria. The approach utilizes mutants which are unable to grow on C_1 compounds. A plasmid cloning vector (pVK100) and its mobilizing plasmid (pRK2013) will be used to identify DNA fragments which complement the C_1 mutants. These fragments will then be physically mapped, using restriction enzymes, and genetically mapped, using transposon-generated mutants. The C_1 mutants will also be characterized physiologically concerning C_1 specific activities. The inducer of C_1 specific genes as well as the coordinate nature of their regulation will be determined using regulatory mutants and chemostat culture. The implications of this information are of primary importance to the optimization of any commercial process involving the use of methane utilizing bacteria as biological catalysts for chemical production.

71. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

DEVELOPMENT OF NITROGEN-FIXING MONOCOT-BACTERIA ASSOCIATIONS — $68,000
Winston J. Brill
Department of Bacteriology

The objective of this project is to understand interactions between nitrogen-fixing bacteria and the roots of corn. It may be possible to manipulate both the bacteria and plant to obtain a corn that will receive a significant proportion of its nitrogen from nitrogen fixation. The approach involves selection of corn lines that produce material with the capacity to support bacterial nitrogen fixation. Breeding of these corn lines has produced lines that may support up to 1% of the total plant nitrogen. The details of this association are being examined in hydroponically-grown plants. Both visible and electron microscopy are used to determine the root location of the nitrogen-fixing bacteria. Immunological approaches will be applied to identify the bacteria that are most efficient with respect to corn root binding and to rhizosphere nitrogen fixation.

72. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE — $39,000
Jerry L. Kermicle
Laboratory of Genetics

Alleles of the R gene in corn govern the presence, distribution and timing of anthocyanin pigmentation, plant part by plant part. We seek to understand the control of tissue specific expression in terms of the number, kind and arrangement of genetic components concerned. Some alleles are composed of more than one functionally independent genic element. Both tandem and displaced duplications have been identified. Individual elements may confer pigmentation only to the seed (Sc), only to the plant (P), or to both. A segregational analysis of (P):(Sc) allelism shows the determiners of tissue specific function to recombine with a frequency of 10^{-5} or less. To test whether R elements of different tissue-specific spectra share common components, (Sc) was mutagenized with the transposable element Dissociation, Ds. From combinations of the resulting variants with (P), gametes of typical (Sc) action were rescued with a frequency of 6×10^{-4}. Most were recombinant for flanking markers. The reciprocal crossover class included instances in which the effects of (Ds) were superimposed on (P). We infer that an R genic element is composed of a tissue specific segment of short genetic length and a longer segment that is common to alleles of different tissue specific activities. The function of tissue specific and tissue nonspecific components comprising a given genic element is cis-dependent. Spontaneous and induced mutations are being analyzed in parallel to those incited by Ds.

73. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

STARCH SYNTHESIS IN THE MAIZE ENDOSPERM AS AFFECTED BY STARCH-SYNTHESIZING MUTANTS — $48,956

Oliver E. Nelson
Department of Genetics

The objective of this research is to gain a more complete insight into the process of starch synthesis utilizing the various mutants of maize that disrupt synthesis as experimental probes. In spite of the fact that the starchy endosperms of cereal grains and starchy roots and tubers are vitally important in the nutrition of humans and domestic animals and the consideration that during their development these tissues constitute the principal sinks to which photosynthate is translocated, there are substantial voids in our knowledge of the process. To be specific, we don't understand how the process is initiated nor do we understand the relative importance of the various enzymes that catalyze the formation of starch from various substrates, nor what the rate-limiting step is in the process. The large number of maize mutants that affect endosperm starch synthesis are valuable tools since the net effect of the mutations is known, and by detecting the primary biochemical lesion in a mutant, we can understand the relative importance of that enzyme in biosynthesis.

74. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

PHYTOCHROME FROM GREEN PLANTS: ASSAY, PURIFICATION AND CHARACTERIZATION — $50,000

Peter H. Quail
Botany Department

This project is directed toward providing procedures for assaying, purifying and characterizing phytochrome from chlorophyllous tissue of both terrestrial and aquatic plants. The approach involves the use of polyethyleneimine (PEI) to remove chlorophyll from phytochrome-containing extracts, the spectral measurement of the phytochrome with a new, sensitive, microprocessor-based spectrophotometer designed by L.H. Pratt as part of a collaborative program and the use of a combination of ion exchange and affinity chromatography for purification. Phytochrome from green Avena is spectrally detectable in PEI-treated extracts at $\sim$ 0.5 - 1% the level of etiolated tissue. The peak position of Pr from the green tissue is almost 10 nm shorter (657 nm) than its etoliated-tissue counterpart and exhibits very rapid dark reversion. In addition, the green-tissue molecule displays immunological and chromatographic properties that differ from those of etiolated-tissue phytochrome and is not readily detectable in the $\sim$ 120 kdalton region of SDS gels. Mixed etiolated and green tissue extractions as well as further purification will be used to determine whether green tissue has a phytochrome molecule intrinsically different from that of etiolated tissue as the data might suggest or whether in vitro modification occurs in green-tissue homogenates. Axenic cultures of Mesotaenium are being used to obtain algal phytochrome. The significance of this program is that it will permit the assay and characterization of phytochrome from plants grown under natural conditions, a task previously precluded by interference from chlorophyll and the low levels of the chromoprotein in such tissue.

75. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

ONE CARBON METABOLISM IN ANAEROBIC BACTERIA: ORGANIC ACID AND METHANE PRODUCTION — $71,000

J. G. Zeikus
Department of Bacteriology

The project goal is to understand the pathways and regulation of carbon metabolism in acetic acid and methane producing bacteria. The approach involves comparison of growth parameters, intermediary metabolite formation and enzymatic activities during methanol, acetate, carbon monoxide and H_2/CO_2 metabolism by Methanobacterium thermoautotrophicum, Methanosarcina barkeri, Acetobacterium woodii and Butyribacterium methylotrophicum. M. thermoautotrophicum contains an active PEP carboxylase which functions in autotrophy. Both methanogens and acetogens contain high levels of vitamin B12 and CO dehydrogenase which function in acetyl CoA synthesis and probably in acetate cleavage to methane. Methanogens used GOGAT and alanine dehydrogenase to assimilate ammonia and to synthesize alanine, glutamate and aspartate. The anabolic and catabolic pathways of carbon transformation of M. barkeri are unified by common carbon metabolites. M. barkeri or B. methylotrophicum grow mixotrophically on methanol, CO_2 and acetate. Selected strains of B. methylotrophicum or M. barkeri grow on 100% carbon monoxide as the sole carbon and energy source. Notably, CO dehydrogenase activity was higher during growth of B. methylotrophicum on methanol than CO. The growth of B. methylotrophicum during steady-state continuous flow culture on methanol is less than 20 min.

76. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

MOLECULAR PLANT GENETICS — 2.0 SMY

Benjamin Burr and Frances A. Burr
Biology Department

Recent advances in molecular genetics suggest ways to aid traditional plant breeding in ameliorating higher plants for human use. Problems that need to be addressed include the isolation of agronomically important genes and the methodology for introducing foreign genes into desired crop species. Corn (Zea mays, L.) genes specifying storage proteins and enzymes involved in polysaccharide biosynthesis and degradation are being isolated by molecular cloning techniques. Genetic entities that are known to transpose within the corn genome have been described at the molecular level and are being isolated. The transposable elements may aid in the mobilization of genes for transfer and in their integration into a recipient genome. By studying where these elements integrate with respect to the structural gene for the enzyme sucrose synthetase, we have found that sequences several thousand nucleotides from the 5' end of the gene are important for regulation. It is anticipated that the cloned transposable element sequences will be useful as probes to isolate other genes for which we have transposable element mutations.

77. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

MECHANISMS OF ENERGY CONVERSION IN PHOTOSYNTHESIS — 3.8 SMY

Geoffrey Hind
Biology Department

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

78. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

THE REGENERATION OF PLANTS FROM CROWN GALL TUMORS — 1.0 SMY

Daniela Sciaky
Biology Department

The genetic engineering of plants using the T-DNA of Agrobacterium tumefaciens requires that the engineered plant cell be regenerated into a fertile plant. Two separate occurrences are being studied with respect to understanding how crown gall callus can organize into shoots. One event is the spontaneous formation of shoots from Nicotiana otophora cells transformed by pTiB6806. At present, we are determining whether the T-DNA in these shoots has undergone some modification when compared to the T-DNA of undifferentiated transformed cells. The second event is the localization of a 2.7 kb insert in the T-DNA of pTiA66 leading to the requirement for auxin to induce tumors in some plants. When these tumors are placed in tissue culture they sometimes form shoots and lose their auxin requirement. Sequences homologous to the 2.7 kb insert are present in other regions of the Ti plasmid of A66 and in the A66 chromosome. The primary structure of the junction between the insert and T-DNA is being determined in order to understand how the insert causes this shooting morphology.

79. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

THE PHYSIOLOGY AND BIOCHEMISTRY OF CYANOBACTERIA — 2.0 SMY
Harold W. Siegelman
Biology Department

The cyanobacterium Microcystis aeruginosa is a noxious bloom former in freshwaters which may sometimes be toxic to animals and potentially harmful to man. A worldwide collection of toxic and non-toxic isolates was obtained. The molecular structures and composition of the photosynthetic energy collection systems of M. aeruginosa are being made. These systems include phycobilisomes, chlorophyll, carotenoids, and reaction centers which are being characterized in specific isolates. The influence of light intensity and temperature on the energy collection systems is being examined with turbidostat cultures. The phycobilisomes are characterized by gel filtration, hydrophobic interactions chromatography, electron microscopy, and biliprotein stoichiometry. The toxins of several isolates of M. aeruginosa are under examination. They are composed of one to over 20 toxic pentapeptides, and their compositions, sequences, and structures are being determined by amino acid analysis, mass spectroscopy, and automated amino acid sequence methods. The M. aeruginosa toxins are reputedly hepatotoxins, but detailed pathological studies indicate that the toxins initially cause rapid blood clotting. The lipid and carbohydrate composition of several isolates of M. aeruginosa are being examined by chromatographic and mass spectral methods as potential sources of useful intermediates for chemical feedstocks and fuels.

80. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

PLANT CELL GENETICS — 0.5 SMY
Harold H. Smith
Biology Department

The objective of this project is to gain an understanding of genetic controls in plant development through the use of cell and tissue cultures and the analysis of genetic tumors. The genetic basis of spontaneous tumor formation in certain plants is being studied by isolating particular chromosomes that, when transferred to another distantly related species, (intersectional hybrids in Nicotiana), cause tumors to develop. These chromosomally-defined, tumor prone plants are being grown in tissue culture to determine their differences from normal plants in growth factor requirements, such as for indole-acetic acid; and with regard to differences in quantity and isozyme characteristics of superoxide dismutase, an enzyme which has been shown to be characteristically deficient in mammalian tumors. Experiments are also underway to determine base sequence homologies between Ti-plasmid DNA, from Agrobacterium tumefaciens, and that of the genetically-conditioned tumorous Nicotiana hybrids.

81. LAWRENCE BERKELEY LABORATORY
Berkeley, CA 94720

RESONANCE STUDIES IN PHOTOSYNTHESIS — 0.3 SMY

Alan Bearden
Donner Laboratory

Our studies provide information about the membrane locations and structures of photochemical and electron-transport components in green-plant (chloroplast) photosynthesis. Using time-dependent electron paramagnetic resonance spectroscopy at liquid-helium range temperatures, together with membrane orientation techniques we are able to sense the "sideness" of protein constituents that display paramagnetic resonance signals. These components include the photochemical participants of Photosystems I and II, cytochromes, plastocyanin, the Rieske iron-sulfur protein, and various quinone complexes. The use of added, non-reactive, paramagnetic "relaxer" components such as rare-earth compounds has been highly useful in this regard. In addition, we have completed studies of the role of nuclear tunneling in the electron-transport chain of the photosynthetic bacterium, Chromatium. These studies have afforded an important test of the Hopfield-Jortner theory of such processes. Theoretical work has also been completed which complements and extends the previous studies. The general applicability of these latter studies to many systems in chemical and solid-state physics has been an unexpected product of these biophysical investigations.

82. LAWRENCE BERKELEY LABORATORY
Berkeley, CA 94720

PLANT BIOCHEMISTRY: REGULATION OF PHOTOSYNTHETIC METABOLISM AND SECONDARY BIOSYNTHESIS — 5.0 SMY

James A. Bassham
Chemical Biodynamics Division

The objectives of this project are to determine photosynthetic and biosynthetic paths in green plants and the mechanisms of regulation in these paths. Both metabolic regulation (feedback inhibition, energy charge, etc.) and regulation of gene expression are studied in plant tissue, including whole plants and organs, cells and organelles isolated from leaves, and plant tissue culture. Subtask I (Regulation of Metabolism and Gene Expression) focuses on key enzymes and processes of metabolism in photosynthetic cells, and is directed to obtaining a better understanding of control of primary carbon and nitrogen metabolism in these cells, including specialized cells found in certain plants (C4 plants). Plant cell tissue culture is used for investigation of gene expression during differentiation and plant regeneration. Subtask II (Secondary Plant Metabolism: Plant Pigments) includes similar goals focussed on secondary products (alkaloids, terpenes) and also investigation of structures and mechanism of action of non-chlorophyllous plant pigments.

83. LAWRENCE BERKELEY LABORATORY
Berkeley, CA 94720

MAPPING PHOTOSYNTHETIC GENES IN PROCARYOTIC AND EUCARYOTIC CELLS — 0.8 SMY

John E. Hearst
Chemical Biodynamics Division

The central theme of the work is to interface molecular genetics with physical chemistry in the study of photosynthesis. Specifically, this proposal outlines experiments aimed at the isolation of genes from Rhodopseudomonas capsulata coding for polypeptides in the photosynthetic apparatus (PSA). Once identified, the genes coding for the reaction center (RC) and light harvesting (LH) polypeptides will be sequenced. The protein sequences inferred from the DNA sequence will be a first step in the understanding of the molecular details whereby these polypeptides mediate the conversion of light into the chemical potentials that drive cellular metabolism. The gene organization of the PSA sequences will be basic to models explaining the induction and regulation of the PSA components.

The effort will include mapping the genes coding for components of the photosynthetic apparatus of a eukaryotic organism in the nuclear or chloroplast DNA. The genes will be isolated and sequenced to study elements of regulation of expression. These studies should provide not only a better understanding of how cells coordinately regulate information in distant parts of the cell, but also in collaboration with others in our laboratory to provide insight as well as materials for the study of the processes of photosynthesis.

84. LAWRENCE BERKELEY LABORATORY
Berkeley, CA 94720

ENERGY CONVERSION IN PHOTOSYNTHESIS — 2.5 SMY

Kenneth Sauer, Melvin P. Klein
Chemical Biodynamics Division

The physical chemistry of the conversion of photon energy to chemical potential in the photosynthetic light reactions is being studied using spectroscopic techniques. We are exploring (a) the molecular structure and organization of photosynthetic membranes, (b) the dynamics of photon absorption, excitation transfer among the pigment molecules and trapping of the excitation in photosynthetic reaction centers, (c) the kinetics and energetics of charge separation in the reaction centers, and (d) the mechanism of water oxidation leading to the formation of O_2 in higher plants and cyanobacteria. We use optical absorption, circular dichroism, linear dichroism and fluorescence polarization to characterize the important pigment proteins and to measure the intermolecular interactions which are essential for efficient photon utilization. Through fluorescence lifetime measurements in the sub-nanosecond region and transient EPR studies in the microsecond time regime we are able to follow and interpret the initial steps of charge transfer in the reaction centers. Studies using X-ray absorption and EPR provide insights into the role of manganese and other essential components in the water oxidation process. In each instance we are focussing on determining the basis physical and chemical principles involved in the conversion of solar energy by photosynthetic organisms.

85. LAWRENCE BERKELEY LABORATORY
Berkeley, CA 94720

PHOTOCHEMICAL CONVERSION OF SOLAR ENERGY — 2.0 SMY
Lester Packer, Rolf J. Mehlhorn, Alexandre T. Quintanilha
Membrane Bioenergetics Group, Energy & Environment Division

This program seeks to characterize the molecular events associated with the photochemical conversion mechanisms in bacteriorhodopsin: this light- and temperature-stable protein, which operates via a photocycle to develop a proton current, contains retinal as a chromophore and is the only protein found in the purple membrane isolated from halobacteria.
Approach: Bacteriorhodopsin is investigated in the natural membrane and in reconstituted artificial membranes. Chemical modification with a wide variety of group-specific reagents is used to identify those amino acids which are essential for the photocycling activity and the simultaneous proton pumping through the molecule. These studies are aided by the investigation of isotope effects, and the use of laser flash photolysis measurements and ESR spin probe and labeling techniques which correlate individual steps in the photocycle with electrochemical and structural events within and across the protein. Analogous studies are being undertaken on a recently discovered retinal-lacking protein (mutant R_1mW), which can be reconstituted into a similar light activated proton pump by the addition of retinal and retinal analogues. Anticipated benefits of this program include understanding of the molecular basis of the photochemical energy conversion by retinal proteins and their use for development of photovoltaic cells.

86. MICHIGAN STATE UNIVERSITY
East Lansing, MI 48824

ANALYSIS OF FACTORS INFLUENCING THE EFFICIENCY OF PHOTOSYNTHESIS — 3.3 SMY
Charles J. Arntzen
MSU-DOE Plant Research Laboratory

In photosynthesis, the trapping of solar energy and conversion of this energy to chemical form is mediated by pigments and enzymes localized in the internal membranes of the chloroplast. The objectives of this project are to characterize the structural organization of these membranes to identify features which regulate, and potentially limit, the quantum efficiency of photosynthesis. A major approach is to isolate and study light-harvesting pigment-proteins. Procedures have been developed to use non-ionic detergents to isolate a native photosystem I particle which can subsequently be depleted of four polypeptides and their associated antenna chlorophyll. We are now attempting to purify this light-harvesting complex in a form which retains the long wavelength absorbing forms of chlorophyll a. In parallel experiments, the light-harvesting complex which serves photosystem II is being characterized. The reconstitution of these isolated components into various model membranes is being attempted to learn how microenvironments around the complex influence its self-association and/or specific interaction with the photosystem II reaction center complex. A second major approach is to use developmental or mutant systems to characterize the synthesis and turnover of membrane components. A series of cytoplasmically-encoded photosynthetic mutants of tobacco are now being analyzed which are blocked in photosystem II activity. We are comparing protein composition of these membranes to isolated photosystem II complexes to identify, and to learn the function and organization of polypeptides catalyzing the photosystem II reactions. The long range goal of these lines of research will be to determine when and how the light reactions of photosynthesis can be modified via genetic or cultural techniques to increase the productivity of solar energy conversion via photosynthesis.

87. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

DIFFERENTIAL GENE EXPRESSION IN RHIZOBIUM — 2.0 SMY

Barry K. Chelm
MSU/DOE Plant Research Laboratory

The interaction of bacteria of the genus Rhizobium with a legume host to establish a symbiotic, nitrogen-fixing relationship requires a series of developmental steps in both the bacterium and the host plant. The objective of this project is to identify the underlying molecular mechanisms by which the expression of the bacterial genome is regulated during this process. We have focused primarily on the Rhizobium japonicum/ soybean system. Specific genes whose expression is regulated during nodule development have been isolated by hybridization screening of an R. japonicum DNA fragment library carried in an E. coli phage lambda vector. The hybridization probes for this screen were radioactively labelled RNA isolated from differentiating bacterial cells. The genes thus far isolated are being further characterized for their expression and structure while other genes with differing expression patterns are isolated. The isolated genes will be genetically manipulated and reintroduced into rhizobia using broad host range plasmids in order to establish the importance of these genes for the process of nodulation. High resolution analysis will also be carried out on the transcriptional promoter/regulatory regions in order to determine the molecular mechanisms by which they are regulated. This analysis has been started on the R. japonicum nitrogenase operon (nif HDK). These further understandings of the processes regulating legume nodulation and nitrogen fixation should enlighten the viewpoint from which the symbiotic relationship might be further optimized.

88. MICHIGAN STATE UNIVERSITY
East Lansing, MI 48824

A STUDY OF THE MECHANISM AND REGULATION OF CELLULOSE BIOSYNTHESIS IN PLANTS AND BACTERIA — 0.5 SMY

Deborah P. Delmer
MSU-DOE Plant Research Laboratory

The objective of this project is to understand, at the biochemical level, the mechanism and regulation of the pathway for cellulose biosynthesis. For comparative purposes, we are studying the process both in higher plants (cotton fibers--Gossypium hirsutum and cultured cells of soybean--Glycine max) and in Acetobacter xylinum, a bacterium which secretes cellulose as an extracellular pellicle, Results of our previous studies show that UDP-glucose serves as a precursor to cellulose both in plants and A. xylinum. We are currently analyzing the structure of additional negatively-charged sugar derivatives which appear to be precursors to cellulose beyond the level of UDP-glucose. Attempts at synthesis of cellulose using preparations derived from permeabilized or broken cells are traditionally hampered by the very low levels of enzyme activity obtained. Our studies show that the existence of a transmembrane electrical potential in intact cells is crucial, both in plants and in A. xylinum for maintaining active cellulose synthesis. Since this potential is lost upon cellular disruption, our results can offer an explanation for the low activities observed in cell-free preparations. We continue to study the mechanism of regulation of this process by electrical potentials. We have also recently found that the enzyme system in A. xylinum is activated by guanosine triphosphate in combination with a loosely-bound protein factor; we continue to explore this phenomenon and also continue to seek ways to stabilize the enzyme system in cell-free preparations. Understanding of the mechanism of cellulose biosynthesis is of primary importance because it could lead to an ability to design effective environmental, chemical, or genetic manipulations which could lead to altered regulation of the rate of cellulose deposition in plants. A specific example could be in effective design of herbicides which effect this process specifically in plants.

89. MICHIGAN STATE UNIVERSITY
East Lansing, MI 48824

REGULATION OF PROTEIN FORMATION IN PLANTS: SIGNIFICANCE IN GROWTH REGULATION — 0.3 SMY

Philip Filner
MSU-DOE Plant Research Laboratory

The XD line of cultured tobacco cells have the potential for dividing with a doubling time of 2 days when grown on nitrate, ammonium of mixed amino acids as a N source. However when grown on urea the doubling time is 4 days because of low levels of urease. The cells of the culture are capable of increasing their urease level after 40 generations of growth on urea; the high urease variants overgrow the slower growing cells. The phenomenon of adaptation is slower than known mechanisms of enzyme de-inhibition, activation or induction but faster than what may be expected for conventional mutations assuming a frequency of 10^{-7}. Such a selection process may require 100 generations to become evident. The dynamics of the phenomenon described above suggests selection for gene amplification as in bacteria, yeast and animal cells. A major objective is to determine whether such a mechanism can explain the appearance of high urease cells. One objective is to ascertain whether the phenomenon is unique to urease or whether it is more general. Another objective is to determine whether the trait is expressed in differentiated tissues generated from the cells.

90. MICHIGAN STATE UNIVERSITY
East Lansing, MI 48824

RESISTANCE OF PLANTS TO ENVIRONMENTAL STRESS — 2.8 SMY

Andrew D. Hanson
MSU-DOE Plant Research Laboratory

This project investigates the metabolic components of adaptation to, and injury from, environmental stresses, and has the long-range objective of developing novel approaches to breeding plants for stress-adaptation. Accordingly, research is conducted in collaboration with genetic programs and plant-breeding programs of the Crop and Soil Sciences Department, MSU. Overall aims are: (1) To characterize metabolic changes elicited by environmental stress, particularly water-stress; (2) To investigate the biochemical and genetic basis for the regulatory effects of stress on metabolism; (3) To seek both natural and induced variation in pathways of stress metabolism, in order to evaluate the adaptive value of such pathways by physiological-genetic experiments and to develop stress-resistant germplasm. Work is in progress on three topics: the accumulation of glycine betaine in cereals and other crops subjected to water stress and to salinity; the accumulation in barley of the toxic indole alkaloid gramine in response to high-temperature stress; the control of gene expression in the aleurone layer of barley seeds by environmental stresses.

91. MICHIGAN STATE UNIVERSITY
East Lansing, MI 48824

ACTION AND SYNTHESIS OF PLANT HORMONES — 3.0 SMY
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 senescence and stress in plants. Ethylene promotes aging and the appearance of stress symtoms, cytokinins retards them. Ethylene also promotes growth in aquatic plants.

a) Regulation of stress etylene synthesis: Plants under stress produce ethylene. Stress ethylene seems to have a dual function. It mediates some of the stress symptoms in some instances and is involved in eliciting the resistance reaction in other cases. Ethylene synthesis is very rapidly induced through enhancement of the activity of 1-aminocyclopropane-1-carboxylate (ACC) synthase, an enzyme involved in ethylene biosynthesis. Research will mainly concentrate on the mechanism by which ACC synthase is regulated. We shall examine whether ACC synthase is newly synthesized in response to stress or whether it is activated.

b) Regulation of aging in plants by ethylene and cytokinins: Catabolic activities, e.g., membrane breakdown, leading to irreversible deterioration of the cell are promoted by ethylene and retarded by cytokinins. It will be investigated how these two hormones affect membrane turnover and what membrane components are involved. Investigations will also focus on the mechanism by which ethylene synthesis is induced by ethylene at the onset of senescence (positive feedback).

c) Regulation of growth in aquatic plants by ethylene: The mechanism(s) by which growth is enhanced in deep-water rice under flooded conditions, another stress response involving ethylene, will be investigated (funded in part by the National Science Foundation).

92. MICHIGAN STATE UNIVERSITY
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PLANT CELL WALL PROTEIN — 2.5 SMY
Derek T.A. Lamport
MSU-DOE Plant Research Laboratory

Extracellular matrix is the evolutionary sine qua non of eukaryotes. Morphogenesis is the endless variation on a relatively few extracellular themes in the form of macromolecules consisting mainly of glycoproteins such as collagen, extensin, elastin, fibronectin, etc., which, with few exceptions, also contain hydroxyproline. This hydroxyproline glycoprotein "formula" provides a universal glue for sticking eukaryotic cells together . . . a formula surprisingly similar in both plants and animals, with profound implications for the origin of multicellular from unicellular organisms, and the evolution of morphogenetic machines capable of recognizing "self" and "nonself". Thus our approach to cell wall glycoproteins is a fundamental study of the extra-cellular matrix of photosynthetic eukaryotes ranging from the most primitive single-celled photosynthetic protists (Chlamydomonas) through more advanced protists (Volvox), to advanced angiosperms such as Acer and Solanum. Current objectives include determination of arabinogalactan protein amino acid sequences, assessment of the role hydroxyproline enhancement plays in disease resistance, and the role of isodityrosine in crosslinking the extensin network.

93. MICHIGAN STATE UNIVERSITY
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REGULATION OF FLOWERING — 2.5 SMY

Anton Lang
MSU-DOE Plant Research Laboratory

Whereas evidence for hormone-like (translocatable) promoters of flower formation ("florigen", "floral stimulus") has been available for almost 45 years, unequivocal evidence for analogous inhibitors of flowering ("antiflorigen") has been obtained only recently, mainly by this task. This discovery markedly affects our ideas about the regulation of flowering. Failure of plants to flower under certain environmental conditions has been attributed mainly, if not exclusively to the lack of promoters of flowering; now we find that it may also be caused by the presence of potent inhibitors of flowering. The inhibitors share important physiological properties with the promoters: they are not specific in a taxonomic nor a physiological sense since they appear to be the same in different species and genera, and in different environmental (photoperiodic) response types. Current work is directed primarily at the isolation and identification of antiflorigen, using extraction and diffusion procedures combined with bioassays.

Flower formation is of great fundamental interest as well as potential practical interest - fundamental because it involves a profound alteration of the growth pattern of the plant, namely cessation of vegetative growth and "metamorphosis" of leaves and stems into seemingly new organs, the flowers; practical because flowering is the first stage of reproductive development in seed plants and thus the premise for fruit and seed production, but is on the other hand antagonistic to continued production of vegetation biomass. It can thus be considered as a problem of energy partitioning in the plant.

94. MICHIGAN STATE UNIVERSITY
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INTERACTION OF NUCLEAR AND ORGANELLE GENOMES — 2.5 SMY

Lee McIntosh
MSU-DOE Plant Research Laboratory

The purpose of this research program is to construct a model of nuclear:organelle genome interaction necessary for development of photosynthetic competence in higher plants. The initial analysis of the genes involved in these processes is being carried out by cloning, identification, and localization of both chloroplast and nuclear genes for Zea mays and several other crop species. These gene "libraries" are the basis for the study of the regulation of photosynthetic development. Transcriptional regulation is being investigated by DNA sequence analysis of differentially regulated chloroplast genes. An example of this is a class of genes expressed only in the light as compared with those which are transcribed constitutively.

Experiments leading to characterization of a class of nuclear "regulatory" genes which control chloroplast development are also an integral part in determining the mechanism of assembly of photosynthetically active plastids. While we now have complete banks of chloroplast genes from maize and several other higher plants, the identification of a class of postulated nuclear genes which act in chloroplast biogenesis is a potentially more complex problem. Thus, cytoplasmic control of chloroplast gene expression is being studied by the use of nuclear mutants which lack specific chloroplast gene products. These mutants will allow the identification of elements coded for by the nucleus and which influence chloroplast development.

95. MICHIGAN STATE UNIVERSITY
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SENSORY TRANSDUCTION IN PLANTS — 2.8 SMY

Ken Poff
MSU-DOE Plant Research Laboratory

We seek to understand the mechanisms of acquisition of environmental information in several types of organisms as a basis for understanding sensory transduction in plants. Non-visual light and temperature perception are both under investigation. We will study the "blue-light" photoreceptor pigment system(s) which control(s) numerous light responses in flowering plants by using specific inhibitors and mutants as probes blocking specific initial steps in the transduction sequence. We will also study light sensing in the cellular slime mold Dictyostelium in which we have identified several photoreceptor pigments and their subcellular localization. Isolation and analysis of photoreactor pigment mutants which will permit dissection of the components of the photoreceptor transduction sequence are in progress. Thermotaxis by Dictyostelium is being used as a unique 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.

96. MICHIGAN STATE UNIVERSITY
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GENETIC IMPROVEMENT OF PHOTOSYNTHETIC PRODUCTIVITY — 0.5 SMY

Chris Somerville
MSU-DOE Plant Research Laboratory

The long term objective of this task is to develop genetic modification systems for the improvement of specific physiological processes in plants. The primary initial emphasis of the task is focused upon the chloroplast enzyme RuBP carboxylase/oxygenase (RubisCO). Because of the dual function of this enzyme, the composition of the gaseous environment is a strong determinant of net photosynthesis in C_3 plants. O_2-inhibition of photosynthesis is directly attributable to RuBP oxygenase activity. This task is attempting to determine if the RuBP oxygenase activity serves some unknown indispensable function, and if not, whether the activities can be genetically altered to eliminate this RuBP oxygenase activity, using in vivo genetic selection procedures and/or recombinant DNA techniques. Successful elimination of this futile RuBP oxygenase cycle, in which previously fixed CO_2 is released to the atmosphere, will lead to increased net photosynthesis, ultimately resulting in significant increases in crop yields.

97. MICHIGAN STATE UNIVERSITY
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DEVELOPMENTAL BIOLOGY OF NITROGEN-FIXING ALGAE — 3.8 SMY

C. Peter Wolk
MSU-DOE Plant Research Laboratory

Certain cyanobacteria (blue-green algae) trap solar energy via photosynthesis and utilize the chemical reducing power to assimilate and fix atmospheric nitrogen gas (N_2) to produce ammonia which then is utilized as the nitrogen source for cellular growth. The objective of this project is to understand the cellular differentiation processes which are required to allow N_2 fixation to proceed. The assimilation of N_2 and the incorporation of this reduced nitrogen into organic molecules occurs in specialized cells called heterocysts. These cells have been isolated and biochemically characterized. The following projects are currently being pursued in an effort to further understand the development of the specialized cells. Using recombinant-DNA technology we are constructing a DNA clone which should complement a characterized mutation in Anabaena, and which should replicate in both Anabaena and E. coli. We are also constructing a physical-genetic map of the Anabaena genome using the cosmid cloning technique. Lastly, we are exploring various means for transferring genetic information into Anabaena via the selection of infective, transforming viruses. The significance of these lines of research will be in the development of genetic tools for the manipulation of cyanobacteria to produce photoautotrophic, nitrogen fixing cells which could be of use in developing commercial techniques for biological solar energy conversion.

98. MICHIGAN STATE UNIVERSITY
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SYNTHESIS, METABOLISM, AND ROLE OF ABSCISIC ACID — 3.0 SMY

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

The objective of this project is to determine how the synthesis of abscisic acid (ABA) is enhanced by water stress, and how ABA regulates the loss of water by closing stomata. In order to determine which parameter of the cell water status causes the increase in ABA, thin sections of bean (Phaseolus vulgaris) leaves are treated with different osmotica, viz. those that penetrate rapidly and therefore increase the osmotic potential and those which do not penetrate and cause plasmolysis, and thus zero turgor. At the biochemical level an attempt will be made to develop an in vitro system that will incorporate mevalonic acid into ABA. Since the bulk ABA in a leaf often does not correlate with stomatal closure, studies on compartmentation of ABA and its metabolites will be carried out by isolation of chloroplasts and vacuoles from protoplasts. For measuring ABA in the epidermis and in isolated guard cells, a very sensitive technique for measuring ABA is required; this is being developed with a capillary column and electron capture detector. Following rehydration of stressed leaves ABA is rapidly degraded; however, this degradation occurs more slowly in the light than in dark. The possibility that this difference is due to ethylene will be investigated. The information obtained in this study will be useful for growing plants under conditions where water supply is limiting.

99. OAK RIDGE NATIONAL LABORATORY
Oak Ridge, TN 37830

ENERGY AND NUTRIENT UTILIZATION EFFICIENCY IN INTENSIVE FOREST BIOMASS PRODUCTION: A STUDY OF SITE-SEEDLING INTERACTIONS — 1.5 SMY

S. B. McLaughlin
Environmental Sciences Division

This study addresses plant physiological and soil-plant nutrient processes which are important considerations in the utilization of marginal land for intensive silvicultural production of energy. Specifically it examines interactions between processes by which plants acquire and utilize resources (the rate and seasonal kinetics of net assimilation; allocation of energy between various metabolic pools during the seasonal growth, storage, and mobilization cycle; partitioning of growth between roots and shoots; and water and nutrient relations) and the biological and physical processes by which nutrients are transformed and cycled within the soil-plant system. These processes are being studied in the field on four tree species: yellow poplar (Liriodendron tulipifera), loblolly pine (Pinus taeda), sweet gum (Liquidambar styriciflua), and black locust (Robinia pseudoacacia) under variable rates of nutrient and moisture supply. In addition to examining basic differences between species' physiological strategies for resource allocation, comparisons of the mechanisms of growth enhancement by the mycorrhizal symbionts Pisolithus tinctorus (loblolly and sweet gum) and Glomerus fasciculatus (poplar) are being made by contrasting process-level responses between inoculated and non-inoculated seedlings. 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.

100. SOLAR ENERGY RESEARCH INSTITUTE
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BASIC PHOTOBIOLOGY RESEARCH — 1.2 SMY

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

This work encompasses three areas of electron-transport studies associated with photobiological H_2 metabolism. Task 1 will determine the degree of integration among H_2 metabolism, N_2 fixation, photosynthesis, and the multiplicity of energy-conversion mechanisms present in photosynthetic bacteria, particularly Rhodopseudomonas and Rhodospirillum species. A broad spectrum of mutants is being generated with defective redox components operative in autotrophic or heterotrophic, anaerobic or aerobic, and light or dark growth modes. These defects are being characterized by pleiotrophic losses of other growth modes, spectral and enzymatic properties, and protein separation and quantitation techniques. The data will be used to amplify H_2 metabolism in organisms utilizing the less energy intensive, ammonia-insensitive hydrogenase reaction rather than the nitrogenase pathway. Task 2 has led to the first reported isolation and purification of hydrogenase from a eukaryotic source (Chlamydomonas reinhardtii). The physical, chemical, and catalytic properties of this purified enzyme will be characterized, and the results will be compared with those from procaryotic hydrogenases. Special emphasis will be placed on the physical changes and chemical alteration of the algal enzyme induced by oxygen inactivation. Task 3 will reconstitute electron-transport pathways associated with the primary charge separation of photosynthesis using isolated and artificial redox components. Monolayers of bacterial reaction-center complexes have been carefully characterized on water/air interfaces and then transferred to solid substrates as monolayer and multilayer structures. Multilayer structures with reaction centers sandwiched between donor and acceptor molecules will be constructed and light-driven electron transport demonstrated. The ultimate goal is to show how such a system may be coupled to energy-dependent reactions.

INDEX OF INVESTIGATORS

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