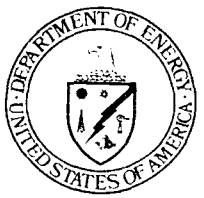
DOE/ER-0110

Annual Report and Summaries of FY 1981 Activities

October 1981



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INTRODUCTION

This report summarizes the activities of the Biological Energy Research (BER) program of the Office of Basic Energy Sciences during Fiscal Year 1981. The BER program was established in 1979. Initially, a number of on-going biological sciences projects were transferred from DOE's Office of Health and Environmental Research into this new energy sciences program. FY 1980 and 1981 have been years of program growth and definition. Roughly two-thirds of projects underway at the end of FY 1981 were initiated since 1979.

The BER Program

The future implementation of innovative biotechnologies to produce fuels and chemicals depends upon the development of a sound understanding of the biological principles involved. Thus the Biological Energy Research sub-program goal is to generate the fundamental data and conceptual understanding in the botanical and microbiological sciences requisite to formulating strategies for enhanced biomass productivity, for novel and improved fermentations and for biosystems capable of saving energy. The strategy of the Biological Energy Research program is to support studies that are not only scientifically meritorious but likely to contribute to the longer term development of entirely new or greatly improved biotechnologies relating to energy. The program is aimed at building the base of understanding of the physiology, biochemistry and genetics of plants and microorganisms required for the future exploitation of the techniques of genetic manipulation, cell culture and others.

The program focuses on understanding the basis of biomass productivity in green plants and its limits, how plants adapt to suboptimal conditions of growth as will be encountered in the utilization of marginal lands and waters, and the mechanisms of microbial conversion of various biomass forms. Of special interest are the anaerobic microorganisms, which are able to carry out fermentations with high efficiency. Other microorganisms of interest include the thermophilic species which have optimal growth and conversion rates at high temperatures. An integral part of the program is the development of information on genetics which may ultimately be used to tailor new or improved microorganisms and plants which will facilitate the production of fuels or petroleum conserving chemicals or will lead to the use of biotechnologies to conserve energy.

While the program is yet a young one, technical achievements are beginning to emerge from various projects. Several examples are to be seen from longer tenured projects including the discovery of thermophilic bacteria which can ferment a broad range of sugars to ethanol, a better understanding of the role of plant growth substances in response to environmental stress factors, development of chemical analytical procedures to probe the structure of plant cell wall polysaccharides and the critical re-examination of the advantages of the technique of saccharification of cellulose and hemicelluloses using hydrogen fluoride.

Program Activities

A total of 62 formal research proposals plus many more informal proposals were considered during FY 81. Of these 25 new projects were initiated. Each of the formal proposals was subjected to peer review by independent reviewers and in most cases, the scrutiny of a panel of active research scientists. Those projects which are currently supported also are subjected periodically to peer review in order to qualify for renewed funding.

By the end of FY 1981 each of the "on-site" or program project type activities supported by BER will have been peer reviewed by site visit teams within the previous 18 months. This is one facet of the project assurance effort to maintain an overall high quality program.

In initiating new projects there were a number in which collaborative efforts were a significant aspect of the work proposed. For example, the contracts covering studies on the genetics of anaerobic microorganisms (Ohio State University, Purdue University, University of Illinois) each has one co-investigator who is a microbiologist with expertise in the microbiology of anaerobic organisms and a co-investigator who is experienced in microbial genetics. This illustrates an increasingly common situation where more than one kind of expertise is required to make inroads into a problem.

In FY 1981 continuing progress was made in providing additional balance in the scope of the BER program. Projects were initiated in areas dealing with environmental stress mechanisms in plants, genetics of anaerobic microorganisms, anaerobic digestion and methanogenesis, plant growth mechanisms, and molecular genetics of plants and microorganisms. The program depends on unsolicited proposal submissions exclusively. The fact that proposals are being submitted in areas covered by the BER program scope is indicative that knowledge of the program is being disseminated within the research community. It should be recognized that there is an enormous amount of research necessary for exploitation of biological systems in the energy context.

During FY 1981 the BER program contributed to the support of two particularly important scientific meetings:

1. "Trends in the Biology of Fermentations for Fuels and Chemicals", Dec. 2-7, 1980, Brookhaven National Laboratory.[a]

2. "Genetic Engineering of Microorganisms for Chemicals", University of Illinois, May 26-29, 1981.[b]

[a] A. Hollaender, et al, editors, Plenum Press, 1981.
[b] A. Hollaender, R. DeMoss, S. Kaplan, J. Konisky, D. Savage, R. Wolf, Editors (in press).

At the end of FY 1981 the BER program had 85 research projects plus several other contracts covering meetings and other activities. The distribution of the funding is illustrated below:

BREAKDOWN BY TYPE OF INSTITUTION OR ACTIVITY
WHERE BER FUNDS WERE SPENT IN FY 81

	No. of Projects at end of FY 81	FY 81 Funding	% of Total Funds
University Contracts	56	$3,804,209	53
University on-site facilities (principally Michigan State Univ.)	14	1,450,000	20
National Laboratories (Brookhaven National Laboratory, Lawrence Berkeley Lab and SERI)	12	1,718,347	24
Other research institutions (federal, state, industrial, non-profit)	7	222,513	3
Conferences, Symposia	3	42,500	<1
TOTAL	92	$7,237,569	

During FY 81 the BER program had the advantage of the able assistance of Dr. Palmer Rogers of the Department of Microbiology of the University of Minnesota who came for six months on an appointment under the Intergovernmental Personnel Act (IPA) of 1970. Dr. Rogers' assistance in initiating an informal liaison group among federal agencies concerned with the support of microbiological research was a significant contribution to the program. The objective of such a group is to provide a continuing vehicle for interchanging information among agencies on topics of common interest such as program content, trends in research, problems of research support and others. A similar interagency group focussing on the plant sciences has been in existence for several years.

In addition to his many duties, Dr. Rogers collaborated to produce a paper which places in perspective the importance of fundamental biological research contributions to future developments in biomass technologies (Rabson, R., Rogers, P., "The Role of Fundamental Biological Research in Developing Future Biomass Technologies", Biomass, in press).

The BER effort is still in the early stages of development. In areas as dynamic as those covered in the plant and microbiological sciences, change is a part of the operation of the program. As new ideas develop and different problems emerge, the program also will evolve. It is necessary to anticipate needs for knowledge for the future and to do this most effectively reliance is placed on inputs from the research community on a continuing basis.

The growing involvement of industry in the development of biotechnology gives priority to the question of government-industry-university roles in research in these areas. It seems to be merely a matter of time before the technical barriers are overcome to using advanced genetic techniques and cell culture methodology on a broad commercial scale. At least that appears to be the rationale for the large and increasing commercial investment in these areas. Despite this influx of new industrial funds, the amount of research necessary to exploit these methods optimally is still extremely large. The limiting factor ultimately, in all likelihood, will not be the availability of techniques for genetic and cell manipulation; rather, the deterring factors will more likely relate to a lack of understanding of the biology of the system which is targeted for improvement. Thus the Biological Energy Research program which aims at understanding fundamental genetic, biochemical and physiological mechanisms will strongly complement the new reserach initiatives in biological topics undertaken by industry as well as the more applied, energy oriented federal programs. Undoubtedly, there is a need for more and better dialogue between the major parties on these questions, in addition to the occasional interaction at meetings, to assure most rapid progress with minimal duplication of effort.

Finally, it would be a gross oversight if mention were not made of the many persons in the research community who have contributed their time, ideas and other assistance to the BER program. These persons have reviewed proposals by mail and served as review panel members. They have made suggestions about research needs at workshops and elsewhere. They have reviewed on-going projects as site visitors. They have instilled enthusiasm to the BER research program. To these many people, both in the U.S. and abroad, grateful thanks are offered in appreciation of their essential efforts on behalf of this research program.

In the following pages research project summaries are provided for each of the efforts supported in FY 81. Projects are funded normally on an annual basis with provision for longer term funding by virtue of multiple year commitments. In most cases projects are given peer review every third year.

The data provided for the individual project contracts indicates the funding (direct and indirect costs) for one year unless otherwise noted. With respect to national laboratory and other "on-site" funding the figures indicated for the projects frequently include either all or part of the salaries of the principal investigator(s) and accordingly the levels appear larger than most other BER projects. 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 — $99,445 (FY 80 funds)

Robert M. Pearlstein
Organic & Polymer Chemistry Section

Chlorophyll (Chl) in vivo and in isolated lipoprotein complexes has spectroscopic and photochemical properties that distinguish it from monomeric chlorophyll in a solution. Present ideas regarding the origin of these distinct properties tend to emphasize Chl-Chl interactions, rather than the interactions between Chl and the protein to which all in vivo Chl is now known to be bound. One reason is that, in any native Chl-protein complex so far isolated, there have been at least two Chl molecules, some of the interactions between which cannot be easily separated from those between Chl and protein. We reported the first 1:1 pigment-protein complex -- formed by interaction of the water-soluble Chl derivative, chlorophyllin, with the apoprotein of myogolobin (Mb) -- that undergoes photoconversion to a long-wavelength (690 nm) absorbing form resembling in vivo Chl. We subsequently prepared complexes of apoMb with chlorophyllide, a water-insoluble derivative structurally closer to Chl itself. These include high-molecular-weight complexes with biomimetic circular dichroism spectra. Current work focuses on preparing a greater variety of heme apoprotein-Chl derivative complexes. Spectroscopically biomimetic complexes will be further studied by attempts to grow single crystals for x-ray diffraction analysis, and to oxidize or photo-oxidize the bound pigment in order to characterize the resulting cation radical.

2. BRANDEIS UNIVERSITY
Waltham, Massachusetts 02154

EFFECT OF LIGHT ON RESPIRATION AND DEVELOPMENT OF PHOTOSYNTHETIC CELLS — $41,465

Martin Gibbs
Institute for Photobiology

1. Chloroplast Respiration. The breakdown of starch within the chloroplast demands a constant supply of ATP(phosphate cycle) and NAD(P)(hydrogen cycle). We have documented in a spinach chloroplast particle fortified with ferredoxin, glyceraldehyde-3-P and NADP that NADPH produced by the oxidation of glyceraldehyde-3-P to glycerate-3-P can transfer its electrons to ferredoxin which, in turn, is used to reduce O_2 to H_2O_2, nitrate to ammonia and protons to H_2 (if algal hydrogenase is present). If NAD is substituted for NADP, then NADH-ascorbate peroxidase generates NAD in the presence of H_2O_2 and ascorbic acid. These experiments can explain the long-standing questions of (a) O_2 consumption by chloroplasts, (b) reduction of nitrate and nitrite to ammonia by darkened chloroplasts and by roots, (c) growth of algae in the dark with nitrate as the sole source of nitrogen, and (d) the evolution of H_2 in the dark by algae adapted to a H_2-metabolism.
2. Hydrogen Metabolism in Intact Algae. The addition of glucose or acetate to H_2-adapted Chlorella or Chlamydomonas results in an increase in H_2 consumption. There is evidence that ethanol is derived from acetate and possibly mannitol from glucose. Also glycerol is formed which is in all probability produced from starch. Ethanol dehydrogenase linked to NAD and NADP and a NADH-dependent fructose-6-P reductase were detected in the algal extracts.

3. UNIVERSITY OF CALIFORNIA
Santa Cruz, California 95064

RESPIRATION OF ROOTS: RESPONSE TO LOW O_2 STRESS — $46,266

Harry Beevers
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.

4. UNIVERSITY OF CALIFORNIA
Los Angeles, California 90024

ENERGY CAPTURE AND USE IN PLANTS AND BACTERIA — $71,775

Paul D. Boyer
Department of Chemistry
Molecular Biology Institute

Our project concerns how plants use sunlight to produce adenosine triphosphate (ATP). ATP serves as the "currency of the cell" for processes such as syntheses, replication, growth and ion transport. A hypothesis we have developed is that energy from light is used to change the binding of reactants at catalytic sites on the ATP synthase of the chloroplast thylakoid membranes.

We are measuring the fate and properties of ^{32}P-, ^{3}H- and/or ^{18}O-labeled ATP that is transitorily tightly bound to the catalytic site. Measurements are made during steady state or when energization is suddenly removed. Fate of the γ-^{32}P and ^{3}H-ADP moieties may yield rate constants for release of ATP, ADP and/or P_i. Increases in sensitivity and precision of ^{18}O measurements may allow direct measurement of the rate of interconversion of the bound ATP to ADP and P_i. Related studies with the purified CF_1-ATPase are on the nature and extent of ATP modulation of oxygen exchanges accompanying ATP cleavage.

How sunlight energy is used by plants is inadequately understood. We hope to establish whether or not the binding change mechanism for ATP synthesis is a key part of the overall photosynthesis process.

5. UNIVERSITY OF CALIFORNIA
Davis, California 95616

FLUORESCENCE PHOTOBLEACHING MEASUREMENTS OF PLANT MEMBRANE VISCOSITY: EFFECTS OF ENVIRONMENTAL STRESS — $40,200

R. W. Breidenbach, D. W. Rains
Plant Growth Laboratory

Temperature and salinity are two of the major limits on production of many crops, and various lines of evidence suggest that the plasma membrane plays a central role in both chilling injury and injury by salinity.

Our experiments will examine the viscosity of plant cell membranes by means of fluorescence photobleaching recovery measurements of lateral diffusion rates of membrane proteins and lipid analogs. The membrane viscosities for chilling-sensitive and chilling-resistant plants will be determined at various temperatures, to see whether the viscosity in chilling sensitive plants increases abruptly at the critical temperature for chilling injury. Membrane viscosities will also be compared for salt-tolerant and salt-intolerant lines of alfalfa and barley, at high and low salt concentrations. The use of photobleaching recovery of labeled proteins to measure viscosity is particularly appropriate since many proposed cellular mechanisms are directly dependent on lateral motion of membrane proteins.

6. UNIVERSITY OF CALIFORNIA
La Jolla, California 92093

HYDROGENASES, THEIR CLONING, PREPARATION, CHARACTERIZATION AND MODIFICATION FOR USE IN PHOTOBIOREACTORS — $75,000

R. G. Bartsch, M. D. Kamen, N. O. Kaplan
Department of Chemistry A-002

In our attempts to utilize hydrogenase as a component of a biophotolytic fuel gas generator (Benneman, Berenson, Kaplan and Kamen, Proc. Natl. Acad. Sci. USA 70, 2317 (1973)) we were impressed with the paucity of information about the structure, the detailed reaction mechanism(s) and the relative stabilities of the enzymes. Our comparative studies of the properties and occurrence of hydrogenase activities in forty different species and strains of microorganisms complement those of others in demonstrating that at least three classes of hydrogenase enzymes exist: those with a single Fe_4S_4 cluster, e.g., Chromatium vinosum type hydrogenase; those with three Fe_4S_4 clusters, eg. Desulfovibrio vulgaris or Clostridium pasteurianum-type hydrogenases; those with flavin plus Fe_4S_4 and Fe_2S_2 clusters, e.g., Alcaligenes eutrophus-type hydrogenase; and there may be other types not as yet recognized. In addition widely different rates of inactivation of the various enzymes have been recognized.

We have prepared crystalline Desulfovibrio vulgaris hydrogenase and propose to begin x-ray crystallographic studies of the protein in the expectation that the three-dimensional structure of this hydrogenase will permit understanding of the mechanism by which a low potential electron carrier reduces the reaction site, presumably an Fe_4S_4 cluster, and how a proton, in turn, is reduced. Aided by detailed structural and mechanism information about the hydrogenases we expect to be able to select from among the available wild-type enzymes or to genetically modify one of the hydrogenases to make an optimal component of a functional hydrogen-generating photolytic system.

7. UNIVERSITY OF CALIFORNIA
Irvine, California

BIOENERGETICS OF SALT TOLERANCE — $135,000

Janos K. Lanyi, Department of Physiology & Biophysics, UC-Irvine
Lester Packer, UC-Berkeley, Dept. of Physiology & Anatomy

The goal of this research is to aid the development of highly salt-resistant crops by identifying and describing those evolutionary adaptive features which enable salt tolerant (halophilic) organisms to overcome the deleterious effects of salt. The cytotoxic effect of salt in halotolerant cells is prevented by coupling powerful sodium ion extrusion systems to the transmembrane gradient of protons. In a special case, the halobacteria, two mechanisms of sodium extrusion are presently known: an indirect but more rapid one, based on the light-driven proton pump bacteriorhodopsin and a proton/sodium antiporter, and a newly discovered light driven pump that will directly transport sodium across membranes. In the different membrane systems which have been examined the exit of sodium is accompanied by either potassium ion uptake, the synthesis of organic osmoregulatory substances, or both. Halobacterial membranes, and halotolerant plant root membrane vesicles will be investigated for rates and specificity of sodium transport. We are describing the sodium transport mechanisms involved, and are now attempting to isolate and characterize at the molecular level the transport proteins. The general plan is to use the methods and concepts arising from work with bacteria in parallel studies of salt transport in halophytic plant cell membranes.

8. UNIVERSITY OF CALIFORNIA
Los Angeles, California 90024

METHANOGENESIS FROM ACETATE, A KEY INTERMEDIATE IN NATURE — $62,486

Robert A. Mah
School of Public Health
Center for the Health Sciences

The objectives of this proposal are 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. 1. Isolation. Inocula were obtained from eight geographical locations ranging from the La Brea Tar Pits, Los Angeles, California to sediment from the Salton Sea, Salton City, CA. Over 15 different samples were inoculated into acetate enrichments or directly diluted into acetate roll-tube agar. In most cases, enrichment cultures were incubated at 25°C, 37°C, and 55°C. When active methane formation occurred, roll tubes were inoculated. Several isolations of acetate-using organisms are currently in progress. Evidence indicates different morphological and physiological characteristics for several of these new cultures. 2. H_2 Experiments. H_2 utilization by Methanosarcina strain 227 differs from Methanococcus mazei strain S-6. In strain 227, very little acetate was converted to methane when H_2 was present; however, more methanol was converted to methane (and less oxidized to CO_2) when H_2 was present. Strain S-6 does not use H_2/CO_2 preferentially; methanol and acetate were metabolized before H_2/CO_2 and the rate of methanogenesis was faster on methanol or acetate. 3. Mutant Isolation. Bromoethanesulfonic (BES) acid resistant mutants of Methanosarcina occurred at high frequency(1 in 10^4) and were isolated after one single exposure to BES. Resistance was a heritable character and was not due to nonbiological inactivation of BES. Inhibition by BES in sensitive strains was reversed by addition of coenzyme M in Methanosarcina but not in Methanospirillum.

9. 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 are employing specific selective pressures designed to alter the structural gene for yeast alcohol dehydrogenase. The mutants produced by this technique, selected by resistance to allyl alcohol, show alterations in turnover number, and in the kinetic constants for substrate and for cofactor. A number of the amino acid substitutions involved in these mutants have been localized, and their positions in the molecule are consistent with the effects that these substitutions have on the kinetics of the enzyme. No mutants have so far been produced with a higher turnover number for the production of ethanol than the wild type, but we have only explored in detail a small number of the available mutants. We expect over time to build up a "functional map" of the molecule which will aid in our understanding of its function and also suggest where directed mutational changes might best be applied.

The biochemical mechanisms of regulation of yeast alcohol dehydrogenase are also under intensive investigation. The two cytoplasmic isozymes are regulated by slightly different mechanisms, and we are currently investigating the role in this regulation of several cytoplasmic-mitochondrial shuttles. The ability to turn the two isozymes on or off will also increase our control over the fermentation process.

10. UNIVERSITY OF COLORADO
Boulder, Colorado 80309

STUDIES OF PLANT CELL WALLS AND OF PLANT-MICROBE INTERACTIONS — $245,000
Peter Albersheim, Alan G. Darvill,
Michael McNeil, Barbara S. Valent
Department of Chemistry

This laboratory is studying the structure and function of plant cell wall polysaccharides. Recent research has shown that fragments of wall polymers are biologically active and appear to be involved in regulating many physiological processes, such as defense against microorganisms and insects, stimulation and inhibition of growth, stimulation of organogenesis, and perhaps even the control of when plants flower. We have recently proposed that these biologically active fragments are hormones and we believe the total number of such wall fragment hormones could be greater than 100. The finding of these biologically active wall fragments has brought together what were three separate projects of this laboratory: studying the structure and function of cell wall polysaccharides, studying host-pathogen interactions, and developing methods for the isolation and structural analysis of complex carbohydrates. Indeed our newly developed methods for purifying and analyzing the structure of complex carbohydrates have been invaluable in determining that hormones are covalently bound within the structure of the cell wall polysaccharides. It was only after we developed sensitive methods of structural analysis that we realized that the polysaccharides of plant cell walls are immensely complex in their structure. This led us to ask what functions, other than providing support for the plant, could account for such complexity. And thus we found the regulatory role of some of the wall fragments. In the future, we will continue to refine biological assays for the wall fragment hormones, to purify the biologically active wall fragments, and to determine their structures. These studies require a wide variety of expertise. We have that expertise because of the diversity of the training of the members of our large research group and because we have available sensitive and sophisticated analytical instruments.

11. CORNELL UNIVERSITY
Ithaca, NY 14853

STUDIES OF PHOTOSYNTHETIC ENERGY CONVERSION — $65,000

Roderick K. Clayton
Section of Plant Biology

The pigmented membranes of plants and photosynthetic bacteria contain "antenna" pigment-protein complexes (chlorophylls and carotenoid pigments bound to specific proteins) that absorb light energy and deliver this energy to photochemical reaction centers, also specific pigment-protein complexes. The reaction centers then mediate a photochemical separation of charge as in a solar cell, with electrons transferred from chlorophyll to acceptor molecules such as quinones or iron-sulfur compounds. This charge separation initiates the coordinated transport of electrons and protons across the membrane, through specific carriers: quinones, Fe-S compounds and cytochromes. The resulting electrochemical gradient is the source of energy for ATP formation. Using combinations of biochemical and optical techniques we study the composition, structure and function of the antenna and reaction center complexes, in isolation and in their natural setting. We have elucidated many details of the photochemistry and the subsequent electron and proton transfer. This work has led others to construct artificial reaction centers patterned after the natural ones, capable of acting as efficient photovoltaic cells. Through measurements with polarized light we have defined the orientations of pigment molecules within the complexes and in relation to the membrane. We are studying the nature of the binding of chlorophyll to protein by exploiting the reversible dissociation of chlorophyll from an antenna complex. For this work we use photosynthetic bacteria, which offer experimental advantages over green plants; especially the ease with which reaction centers and antenna complexes can be isolated and studied.

12. CORNELL UNIVERSITY
Ithaca, NY 14853

EFFECTS OF FREEZING AND COLD ACCLIMATION ON THE PLASMA MEMBRANE OF ISOLATED CEREAL PROTOPLASTS — $73,496

Peter L. Steponkus
Department of Agronomy

One form of freeze-thaw injury to isolated protoplasts is the result of volumetric contractions which decrease the resilience of the plasma membrane. As the extent of expansion is limited by an absolute tolerable surface area increment (TSAI) value, sufficiently large surface areal contractions are irreversible. This lesion quantitatively accounts for the sensitivity of non-acclimated protoplasts to freezing injury. Tolerance of the plasma membrane to surface areal contractions exhibits genotypic diversity and is increased three-fold during cold acclimation. Proposed experiments address the characterization and quantification of the components of the stress-strain relationship of the plasma membrane in order to determine the molecular mechanism for contraction and expansion and alterations due to cold acclimation and genotypic diversity. Analyses of the stress-strain relationship of the plasma membrane by micropipette aspiration indicate that membrane material is deleted from or incorporated into the membrane during contraction and expansion. Several parameters which influence the TSAI value have been measured including γ_r, the resting tension; γ_c, the critical lysing tension; k_A, the area elastic modulus; and $\delta A(\gamma,t)$ a parameter which describes the extent of exchange between the membrane and an extrinsic reservoir.

13. CORNELL UNIVERSTIY
Ithaca, NY 14853

MICROBIAL ECOLOGY OF THERMOPHILIC ANAEROBIC DIGESTION — $61,485
Stephen H. Zinder
Department of Microbiology

This study will endeavor to provide an integrated understanding of the ecology of microbial populations in a thermophilic (60°C) laboratory-scale digestor. Shredded municipal solid waste (organic reaction - primarily cellulose) will be the substrate. Standard digestor parameters will be assayed routinely. Important microbial populations, including methanogens, cellulolytic and fermentative bacteria will be enumerated and isolated using anaerobic habitat and niche simulating media and techniques. Volatile fatty acid-oxidizing bacteria will be enumerated on lawns of Methanobacterium thermoautotrophicum. Numbers and morphotypes of cells will be enumerated using phase microscopy and by staining cells with acridine orange and then observing them using epifluorescence microscopy. Cells which autofluoresce blue-green when excited by light of the proper wavelengths will be enumerated by epifluorescence microscopy and their numbers and morphology will be compared with cultural counts of methanogens. Transmission electron microscopy will be used to study ultrastructure of microbial populations, and will be used along with scanning electron microscopy to study microbial attachment to cellulose. The metabolic activities and physiological properties of various digestor populations will be studied by incubation of sludge samples in serum bottles with ^{14}C-radiotracers. The relative contributions of acetate breakdown and carbon dioxide reduction to methanogenesis will be assessed, as well as the fate of other precursors. An attempt will be made to correlate the activities of microorganisms measured in situ with those of numerically dominant microorganisms. New thermophilic anaerobes may be isolated, which would increase our knowledge of microbial diversity, and which could be industrially useful.

14. FLORIDA STATE UNIVERSITY
Tallahassee, Florida 32306

GUARD CELL BIOCHEMISTRY: RESPONSE TO ENVIRONMENTAL STIMULI CAUSING CHANGES IN GAS EXCHANGE — $74,310
William H. Outlaw, Jr.
Department of Biological Sciences

Virtually all gas exchange between plants and the environment occurs through stomata on leaf surfaces. Thus, CO_2 entry into the leaf is the first regulatory step in photosynthesis. The apertures of these stomata are variable. They are optimized for meeting the opposing priorities of minimizing water loss while maximizing CO_2 uptake by integrating environmental signals favorable for photosynthesis (light) with substrate availability (internal $[CO_2]$) and water status/history of the plant. Even though the biochemical events in guard cells during opening are known in outline, almost nothing is known about the processing of the signals and the initiation of stomatal opening (or closing). The most attractive hypothesis is that each of the signals affect an ion pump located on the cell plasma membrane. I propose to investigate this hypothesis by: (a) measurement of abscissic acid (ABA) in guard cells, (b) characterization of guard cell plasma membrane ATPase, (c) in vivo and in vitro effects of ABA, light, $[CO_2]$ and fusicoccin (FC) on K^+-stimulated guard cell ATPase, (d) mechanism of water stress "memory" in preventing stomatal opening. Part of this research will depend upon the development of two new technologies: (a) improved methods for the isolation of pure guard cell protoplasts; and (b) adaptations of microfluorometric techniques to the measurement of Sub pmole quantities of NAD(P)H.

15. UNIVERSITY OF FLORIDA
Gainesville, FL 32610

INVESTIGATION OF THE TRANSPOSITION OF MITOCHONDRIAL DNA AND ITS RELATIONSHIP TO FERTILITY IN ZEA MAYS — $60,258

Rusty J. Mans
Department of Biochemistry & Molecular Biology

Segments of the mitochondrial genome of maize undergo reorganization or transposition upon the reversion of cytoplasmically male sterile (cms) plants to fertility. Sequences present in high molecular weight mitochondrial DNA (mtDNA) of normal, fertile plants are detected as a pair of double-stranded, plasmid-like DNAs in S-type cms plants that fail to exsert pollen. At frequencies exceeding 20%, the S-type cms plants revert to fertility i.e. exsert pollen grains. Concomitantly, the plasmid-like DNAs are lost as discrete entities and are detected as segments of the high molecular weight mtDNA of the cytoplasically revertant, fertile plants. We want to define this transpositional event and to show that reorganization of the mtDNA sequences results in altered genomic expression. We will utilize restriction endonuclease fragment mapping and limited deoxynucleotide sequence analysis to define the reorganization of the DNA. We will seek changes in the size and templating activity of in vitro transcripts of appropriate segments of the mitochondrial genome from sterile and revertant, fertile lines as a measure of altered expression. We will resort to DNA cloning technology to propagate chemically significant amounts of segments of the maize genome and as a mechanismm of enrichment for specific sequences.

The physiologic manifestation of the plasmid-like, mitochondrial DNAs can be depressed (i.e. sterile plants rendered fertile) by a genetically mapped nuclear gene. Nuclear mutants exhibiting new "restorer to fertility" genes have been characterized in maize. The molecular components, biochemical functions and mode of interaction of the nuclear gene products regulating expression of the maize mitochondrial genome are unknown. Definition of the transposition event observed in the mitochondria should provide a model approach as well as a biochemical assay for the nuclear/mitochondrial interaction of the nuclear restorer gene.

16. UNIVERSITY OF GEORGIA
Coastal Plain Station
Tifton, Georgia 31793

DEVELOPMENT OF INNOVATIVE TECHNIQUES THAT MAY BE USED AS MODELS FOR THE INCREASE OF BIOMASS PRODUCTION WITH GRASSES AND OTHER SPECIES — $36,000

Glenn W. Burton
Wayne W. Hanna
Agronomy Department

We are developing unique plant breeding methods that we believe will reduce the time required per unit of yield increase and are using pearl millet as the test organism. To maximize biomass production, we are making and evaluating Pennisetum species hybrids in order to transfer genes for pest resistance, drought tolerance, perennial growth habit, and apomixis from wild species to cultivated pearl millet. We are developing special techniques of creating cytoplasmic-male-sterile mutants to permit commercial production of F_1 hybrid seed in pearl millet. We believe these techniques can be used in other species and can ultimately increase their biomass yields. We are developing and evaluating sorghum inbreds with brown midrib genes that reduce lignin 30% to produce sorghum hybrids that can maximize metabolizable energy. We are also studying the genetics, linkage, and agronomic potential of a number of radiation induced mutants. This year we released Tifway II, a radiation induced mutant of Tifway turf bermuda-grass that is more frost tolerant and more resistant to nematodes and weed invasion.

17. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

MICROBIAL DESULFURIZATION AND DENITROGENATION OF FOSSIL FUELS — $80,613
William R. Finnerty
Department of Microbiology

This research is developing basic data-bases regarding the microbial desulfurization and denitrogenation of fossil fuels. The approach concerns the construction of microorganisms that serve as highly specific biocatalysts in the oxidation of fossil fuels. Microorganisms have been selected which catalyze the oxidation of dibenzothiophene (DBT) and carbazole to characteristic water-soluble S- and N-containing end products. The microorganisms are pregrown in a selective medium which induces the enzyme systems catalyzing these specific oxidations and used as a non-growing, strictly biocatalytic reagent. The organic sulfur-oxidizing microorganism has been optimized to yield 100% conversion of dibenzothiophene to water-soluble products in 5 hrs. The organic-nitrogen oxidizing microorganism has been optimized to yield 80% conversion of carbazole to water-soluble products in 8 hrs. These microorganisms contain plasmid-encoded genes specifying the oxidation of dibenzothiophene and carbazole. The respective DBT plasmid and carbazole plasmids have been cured from the parent strain resulting in the loss of this specific metabolic capability. These microorganisms have been tested with high-sulfur petroleum and demonstrated to functionally effect desulfurization at high efficiency (90%). The biological process is expected to provide an efficient technology for the desulfurization and denitrogenation of high-sulfur petroleum.

18. UNIVERSITY OF GEORGIA
Athens, GA 30602

ENVIRONMENTAL STRESS MEDIATED CHANGES IN TRANSCRIPTIONAL AND TRANSLATIONAL REGULATION OF PROTEIN SYNTHESIS IN CROP PLANTS — $72,000
Joe L. Key
Department of Botany

This project is directed to an analysis of the influence of physical stresses (heat shock, water, salt, anaerobiosis, etc.) on the patterns of mRNA and protein synthesis and of the mechanisms through which the stresses induce the observed responses. When tissues of soybean seedlings are shifted from a growth temperature of 28°C to 40°C, there is a rapid shift from polyribosomes to monoribosomes followed by accumulation of a low level of polyribosomes. Synthesis of most 28° proteins is greatly decreased in association with the appearance of 8 or so new bands of proteins ("heat shock" proteins) as viewed by SDS gel analysis; one or two additional bands present in 28°C tissue are increased several fold at 40°C. The response is initiated at 32°-35°C and is maximized at 40°-42.5°C. This new pattern of protein synthesis persists, with some relative changes in different bands, for several hr. Return of the tissue to 28°C after 4 hr at 40°-42.5°C, results in a normal pattern of protein synthesis within 4 hr.

This new pattern of protein synthesis results from the induction at the elevated temperature of a new set of mRNAs, based both on in vitro translation-2D gel analysis and northern blot analyses using labeled cDNA clones as probes. The new mRNAs are detectable within 2 min of initiation of "heat shock" and accumulate for 1 to 2 hr; the "heat shock" mRNAs rapidly disappear following transfer of tissue back to 28°C.

While the different stresses studied to date share some common properties, the others do not produce a "heat shock" pattern of proteins. cDNA clone-northern blot analyses indicate, however, that several of the stresses do induce the accumulation of some mRNAs which are also induced by heat shock.

Current transcription and translation studies are directed to an analysis of the mechanism(s) operative in the "heat shock" response.

19. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

THE MICROBIOLOGY AND PHYSIOLOGY OF ANAEROBIC FERMENTATIONS OF CELLULOSE — $190,000

Harry D. Peck, Jr., Lars G. Ljungdahl
Department of Biochemistry

The microbiology and physiology of individual microbial types involved in the conversion of cellulose to ethanol, acetate, methane and hydrogen are being investigated. Emphasis is placed on the physiology of the newly discovered bacterium, Thermoanaerobacter ethanolicus, which produces ethanol from a large variety of sugars by means of a yeast-type fermentation at 80°C. Attempts will be continued to modify the organism for greater substrate and ethanol tolerance. The regulatory properties of its alcohol dehydrogenase will be studied with regard to ethanol formation. New thermophilic microorganisms capable for fermenting cellulose to useful products will be isolated and studied from samples obtained from the Icelandic Thermal Springs. Existing programs on the enzymology and bioenergetics of the sulfate reducing bacteria, methanogenic bacteria and homoacetate fermentors will be continued with emphasis on the role of H_2 and hydrogenase in interspecies H_2 transfer and the nature of their electron transfer proteins. We have observed that cellulose is most rapidly fermented to products when cellulytic microorganisms are grown in association with a second bacterium capable of fermenting cellobiose. These associations will be investigated using thermophilic bacteria with regard to the parameters responsible for successful association and the factors effecting product formation.

20. UNIVERSITY OF GEORGIA
Athens, Georgia 30602

PHYTOCHROME PROPERTIES AND FUNCTION IN PHOTOSYNTHETICALLY COMPETENT PLANTS — $55,211

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 energy is converted to chemical energy via photosynthesis. At present, however, virtually nothing is known about the properties of phytochrome in green plants. The overall goal of this research, therefore, is to characterize phytochrome in photosynthetically competent tissues. We shall rely heavily upon methods that we have recently developed for this purpose. These methods include a radioimmunoassay for phytochrome, an immunopurification procedure for phytochrome, and immunocytochemical procedures for the visualization of phytochrome in situ. Specific goals are: 1) production of antibodies against phytochrome from several plant sources; 2) quantitation of phytochrome in green plant extracts; 3) purification of phytochrome from green plants; 4) characterization of phytochrome isolated from green plants; and 5) determination of the localization of phytochrome in situ in green plants. This research will provide answers to several questions concerning phytochrome and its function that could not be answered in any other way.
(Collaborative with Project #59, University of Wisconsin)

21. HARVARD UNIVERSITY
Cambridge, Massachusetts 02138

EXPRESSION OF BACTERIAL GENES IN YEAST — $55,000
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.

22. UNIVERSITY OF IDAHO
Moscow, Idaho 83843

THE METABOLISM OF AROMATIC COMPOUNDS BY ACTINOMYCETES — $48,334
Donald L. Crawford
Department of Bacteriology & Biochemistry

The actinomycetes are filamentous bacteria, some of which are known to degrade the complex aromatic polymer lignin. Many of these actinomycetes also degrade simple hydroxyl- and methoxyl-substituted benzoic acids and phenylpropanoid compounds structurally related to lignin. Lignin is a major structural component of vascular plants and the second most abundant organic compound on earth next to cellulose. In the current research, we are studying the possibility of utilizing this renewable resource as a source of useful chemicals, particularly as a source of low molecular phenolic compounds. Our principal objective is to utilize lignin decomposing actinomycetes of the genus Streptomyces to biologically convert lignin to selected low molecular weight phenols. In current research we are elucidating the pathways of degradation of low molecular weight aromatic lignin fragments released as catabolic intermediates from lignin as it is decomposed by selected Streptomyces. By characterizing the pathways, we will be able to identify potentially valuable intermedicate phenolic compounds. Once they are identified, we will produce groups of genetic mutants blocked in the further metabolism of these compounds. Such mutants will then be grown on lignin. The mutants will decompose the lignin and the desired low molecular phenolic chemicals will accumulate, hopefully, in yields sufficient to make the bioconversions economic on an industrial scale.

23. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

FATTY AND AROMATIC ACID CATABOLIZING BACTERIA IN METHANOGENIC ECOSYSTEMS — $58,719
Marvin P. Bryant
315 Animal Sciences Laboratory

In the complete anaerobic bacterial 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 only recently discovered, ferment the products of the first group to acetate, CO_2 and H_2. The terminal group includes diverse methanogens which catabolize acetate and/or H_2-CO_2 to produce methane. The second metabolic group includes obligate syntrophs which require the methanogens to maintain a very low concentration of H_2 in the ecosystem in order to degrade fatty acids and possibly aromatic compounds. The object of the work is to use anaerobic enrichment and roll tube culture techniques to document and isolate (in co-culture with H_2-using methanogens or other H_2-using bacteria) the propionate and long-chain fatty acid degrading bacteria and characterize their metabolism. Similar techniques will be used to attempt to document the bacteria involved in benzoic acid degradation and, possibly other aromatic compounds. These studies will add fundamental knowledge to the area of biological methane formation.

24. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

PHOTOSYNTHESIS IN INTACT PLANTS — $101,951
A. R. Crofts
Department of Physiology and 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 technqiues. 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. Three instruments are under development, and nearing completion: i) a kinetic spectrophotometer with flash measuring beam; ii) a kinetic fluorimeter with flash measuring beam; iii) a portable flash kinetic fluorimeter for use in the field. The first two instruments are for laboratory use, and will be set up for use either with suspensions of photosynthetic systems in cuvetes, or with intact leaves.

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.

25. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

DEVELOPMENT OF A GENETIC SYSTEM FOR BACTEROIDES SPECIES — $42,000

Jeffrey F. Gardner
Abigail A. Salyers
Department of Microbiology

Bacteroides is one of the major genera in the microflora of the human colon. A number of colonic Bacteroides species degrade plant polysaccharides (mainly hemicelluloses) and mucins. Not only do these Bacteroides species probably play an important role in the microecology of the human colon, but they may also be useful for microbial conversion of biomass into energy. At present, there is no genetic system for Bacteroides, although some conjugative plasmids have been reported. We propose to develop systems for transformation and/or conjugation of DNA into Bacteroides, using as DNA probes vectors constructed for use in Escherichia coli (e.g. pBR328) or vectors which we will construct by inserting genes of known function (from E. coli) into cryptic plasmids from Bacteroides. These latter plasmids will also be used as cloning vectors for cloning genes in Bacteroides which are not likely to be expressed in E. coli. A transformation or conjugation system will enable us to use the technique of transposon mutagenesis and, in addition, to construct Hfr strains of Bacteroides (for genetic mapping) by introducing conjugative plasmids which can insert into the chromosome.

26. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

STUDIES ON THE ESCHERICHIA COLI AEROBIC RESPIRATORY CHAIN — $64,000

Robert B. Gennis
Chemistry & Biochemistry Department

Our goal is to elucidate the structure and function of the E. coli aerobic respiratory chain. This will provide us with a system with which to approach fundamental questions concerning the bioenergetics of this organism as well as to answer questions concerning how complex membrane-bound enzyme networks are constructed. The major advantage with E. coli as a system is the ability to utilize genetics in conjunction with biochemical techniques. The initial phase of this work is directed primarily at the structural analysis of the E. coli cytochromes. The number and kind of cytochromes present is still not known. Our approach is to use immunological techniques to identify all the different heme-protein complexes in the membrane. This will give us good quantitative assays. We have also obtained a mutant which lacks about half of the cytochromes, apparently corresponding to the complete absence of one of the two parallel electron transport chains. Such a simplified organism will greatly assist our analysis. Preparative biochemical methods are also being utilized. Soon, we hope to be able to isolate defined components of the respiratory chain and begin to look at the specific functions.

27. 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 — $68,450

John R. Laughnan
Department of Genetics and Development

Spontaneous reversions of S cytoplasmic male-sterile (cms-S) maize that are either cytoplasmic or nuclear, may arise through transposition of a fertility element, F. Recent studies that identify the mitochondrion as the site for genetic determination of cms-S support this view; they also indicate that cms-S plants carry two unique mtDNA plasmids. Hybridization studies show that cytoplasmic reversions to fertility are associated with insertion of plasmid sequences into the main mitochondrial genome. We will study numbers of independently occurring cytoplasmic reversions, using restriction enzyme and hybridization techniques to further characterize the insertion event. We also plan to determine whether nuclear reversions involve transposition and insertion of mtDNA plasmid sequences. These studies will use as a probe an already cloned region of the S-2 mtDNA plasmid that, in nick-translated and ^{32}P-labelled form will be used to hybridize with isolated nuclear DNA of revertants, and in ^{125}I-labelled form will be used in in situ studies. The spectacular influence of nuclear genotype over both kinds of reversion events, and the time course for disappearance of the mtDNA plasmids associated with cytoplasmic reversion will be studied at the plasmid level. Secondary transposition within the nucleus, of F element arising by primary reversion events, will also be studied.

28. UNIVERSITY OF ILLINOIS
Urbana, Illinois 61801

ACETOPHILIC METHANOGENIC CONSORTIA — $85,000

Ralph S. Wolfe
Department of Microbiology

Evidence indicates that acetophilic methanogens are responsible for about 70% of the methane produced in anaerobic digestors. We have assembled a collection of acetophilic methanogens and are studying their nutrition, metabolism, and mass culture: Methanosarcina mazei has been obtained from R.A. Mah, a modified medium for its cultivation has been developed, and the organism is now being cultivated at the 5-liter stage. We propose to scale up mass culture of this organism. We have obtained the "fat rod" from Hewser and Zehnder; its nutrition (and cultivation) is being reevaluated. We have Methanosarcina barkeri under culture on acetate. From Lake Kivu we have a number of enrichments on acetate. We have developed several new isolation techniques, and these are being used and evaluated in our study of acetophilic methanogens. Our goal is to define those acetophilic methanogens that would be most suitable to use in the construction of acetophilic consortia.

29. IOWA STATE UNIVERSITY
Ames, IOWA 50011

POST-ILLUMINATION CO_2 EVOLUTION AND PHOTORESPIRATION METABOLITE LEVELS — $69,244 (FY 80 funds)

Cecil R. Stewart
Department of Botany

The purpose of this project is to determine whether or not the post-illumination CO_2 burst (PIB) can be a valid, rapid and reliable measure of photorespiration. Further, we want to determine if this measurement would be useful for systematic screening of compounds that could limit photorespiration.

A small assimilation chamber adapted to the use of leaf discs and which suitable both for accurate measurements of gas exchange and very rapid sampling for further metabolite analysis has been built. This chamber, coupled to an infrared gas analyzer with a small sample cell and sufficient sensitivity, allows us to measure PIB that is independent of the flow rate in a range where boundary layer resistance is not the major limiting factor of photosynthesis.

In order to validate the PIB as a measure of photorespiration after correction for non-steady state conditions, it is compared to the rate of disappearance of glycine and glycolate. A colorimetric method which does not involve the separation of glycine from other amino acids is being used and allows us to perform rapid, routine measurements of the glycine pool. These rates will be compared under different O_2 and CO_2 concentrations in various C_3 plants.

The effects of compounds which inhibit specific steps in the photorespiratory pathway on the PIB, apparent photosynthesis, and photorespiratory metabolite levels will be determined to assess their potential for limiting photorespiration.

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

THE BASIS FOR THE COMPETITIVENESS OF RHIZOBIUM JAPONICUM IN THE NODULATION OF SOYBEAN — $24,484

Wolfgang D. Bauer
William R. Evans

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.

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. Presently it is not known how to select for rhizobia that are superior in any of these respects or what characteristics are of critical importance to the root surface competitiveness of inoculum strains. The purpose of this research proposal is to determine what these characteristics may be.

31. MARTIN MARIETTA LABORATORIES
Baltimore, Maryland 21227

PHOTOCHEMISTRY AND ENZYMOLOGY OF PHOTOSYNTHESIS — $80,000
R. Radmer, J. Golbeck, and B. Velthuys
Department of Biosciences

This project, which is devoted to studies of basic photosynthetic processes, consists of three subprojects. 1) Mass spectrometer studies use specially designed systems to rapidly monitor gas exchange occurring in response to actinic light. These studies concern a) photosystem II donor reactions, and b) light-induced gas exchange in algae. 2) Biochemical studies focus on the role of copper and iron in photosynthesis. Recent studies concern a) the role and mechanism of polyphenol oxidase, a copper-containing chloroplast enzyme, and b) iron-sulfur proteins operating on the reducing side of photosystem I. 3) Spectrophotometric studies, in conjunction with polarographic measurements, concern the electron-transport mechanisms of C_4 plants. Current studies are devoted to a) the cyclic operation of photosystem I, and b) photosystem II assays using artificial donors such as tetraphenylboron.

32. UNIVERSITY OF MASSACHUSETTS
Amherst, Massachusetts 01003

CONVERSION OF CELLULOSE TO ETHANOL BY MESOPHILIC BACTERIA — $75,000
E. Canale-Parola
Department of Microbiology

The project has two major objectives. One of these objectives is to obtain from natural environments, or by means of mutagenic techniques, strains of mesophilic bacteria capable of fermenting cellulose with maximum production of ethanol. The other is to achieve an understanding of the metabolic processes responsible for the anaerobic conversion of cellulose to ethanol by bacteria. The proposed research project involves: 1) the selective isolation from natural environments of different types of mesophilic bacterial strains capable of fermenting cellulose with formation of ethanol; 2) the study of the metabolic pathways used by these bacteria to produce ethanol from cellulose; 3) the investigation of physiological mechanisms that regulate ethanol production by the bacterial isolates; 4) attempts to isolate mutant strains that produce ethanol as the major or only non-gaseous product of cellulose fermentation; and 5) the study of cultural conditions leading to high ethanol yields from cellulose. An important practical application of the proposed work is the possible use of the cellulolytic bacterial isolates and mutant strains in the production of ethanol from paper, wood and other cellulosic materials present in urban and agricultural waste.

33. UNIVERSITY OF MINNESOTA
St. Paul, Minnesota 55108

CORN STORAGE PROTEIN - A MOLECULAR GENETIC MODEL — $84,462
Joachim Messing
Department of Biochemistry

Alterations in the genetic program of a plant require a deep understanding of the genetics of the plant cell on the molecular level. As the molecular genetics of eukaryotic cells has been successfully approached by studying multigene families like globin, egg chorion, or immunoglobulins, the storage protein of corn, Zein, may serve as a model system for plants. The synthesis of the Zein protein family is directly coordinated by a developmental program in the endosperm. The heterogeneity of the protein and mRNA in the endosperm has been studied. Mutants which are affected in the overall synthesis are available. A number of different inbred lines exist with a broad range of phenotypes.

In the current proposal we want to use a new molecular cloning system to define sequences of unique and conserved character by DNA sequencing. As an initial step we want to focus on a sub-family defined as a group of mRNA's hybridizing to one arbitrary chosen genomic clone. This approach should lead to a) the isolation of cDNA clones which can be discriminated against the others of the same family by their evolutionary affinity; b) dissection of cloned cDNA's in small overlapping restriction fragments and their storage in a single-stranded DNA phage cloning system; c) the diagnosis of unique sequences and the one gene, one mRNA, one gene-product relationship; d) the determination of the nucleotide sequences of the cDNA subfamily and e) the analysis of their developmental program.

34. UNIVERSITY OF MISSOURI
Columbia, Missouri 65211

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

The object of this project is to produce a system in the cyanobacteria capable of cloning individual genes important in the bioconversion of solar energy. The main goal of this work will be to produce strains that are highly efficient in the bioconversion of solar energy. The approach taken will be to clone genes involved in photosynthesis. This technique has three requirements: transformation (the ability to exchange extracellular DNA), photosynthetic mutants, and cloning vectors. We already possess the first two items and the primary goal of this work will be to construct the appropriate vectors. This will be done using recombinant DNA techniques to form a circular DNA capable of replicating in both cyanobacteria and bacteria and conferring antibiotic resistance. DNA fragments can be added to this vector and transformed into mutant cells. The growth of these cells is evidence that the correct gene has been cloned. The cloned DNA will then be grown in bacteria and used to construct newer, possibly more efficient strains. Once the system has been developed, it may be possible to clone other genes that are involved in energy production into these organisms.

35. UNIVERSITY OF NEBRASKA
Lincoln, Nebraska 68583

BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERIZATION OF HIGHER PLANTS WITH REDUCED PHOTORESPIRATION — $50,000

Raymond Chollet
Department of Agricultural Biochemistry

This project is designed to further our understanding of mechanisms which may reduce photorespiration in C_3 plants by exploiting two different, but complementary, experimental systems. Since the majority of higher plants, including all gymnosperms and most dicots and major agronomic crops, are C_3 species, control of the apparently wasteful process of photorespiration and the associated O_2 inhibition of photosynthesis represents an enormous potential for increasing net photosynthesis and thus biological productivity and phytomass production. One facet of this project will involve a detailed investigation of photosynthetic and photorespiratory carbon metabolism in the crucifer Moricandia arvensis, involving biochemical, physiological and ultrastructural studies with intact leaf tissue and isolated leaf cells, protoplasts and organelles. Since several independent reports, including preliminary experiments in this laboratory, indicate that this species represents one of the few documented examples of a naturally occurring higher plant with reduced photorespiration, it is of considerable importance to determine the biochemical mechanism(s) involved. Such detailed knowledge of mechanisms which have evolved to reduce photorespiration may provide viable approaches to the eventual practical solution of this problem. A second phase of the research will involve a detailed comparative study of the physicochemical and enzymic properties of ribulosebisphosphate carboxylase/oxygenase purified from various diploid and tetraploid cultivars of perennial ryegrass which reportedly differ with respect to photorespiration and from representative C_3 plants. Such a comparative biochemical approach will undoubtedly yield definitive insight into the question of whether increased ploidy (i.e., nuclear gene dosage) has qualitatively altered the properties of this important bifunctional enzyme in favor of more efficient carboxylation and thus net photosynthesis and dry matter production.

36. CITY UNIVERSITY OF NEW YORK
1 Gustave L. Levy Place
New York, New York 10029

THE RESPIRATORY CHAIN OF ALKALOPHILIC BACTERIA — $68,835 (2 years)

Terry Ann Krulwich
Department of Biochemistry
Mount Sinai School of Medicine

Several observations have led to the consideration of the possibility that the respiratory chain of the alkalophiles is especially adapted to achieve unusually efficient energy transduction. Moreover, mutational loss of the alkalophilic property, due to loss of a NA^+/H^+ antiporter, may result in the concomitant loss of the alkalophilic capacity for energy transduction. A characterization of the cytochrome components of the respiratory chain of wild type Bacillus alcalophilus has been conducted in collaboration with other investigators who have expertise in the relevant techniques. A comparative study would now be conducted on the non-alkalophilic mutants, and a parallel set of strains of a second alkalophilic species would be examined. A functional study of the respiratory chain would be undertaken. The regulatory effects, indicated by the reduced cytochrome levels in the non-alkalophilic strains, would be studied using a newly isolated mutant. Finally, a chromophoric membrane component that is observed in spectra of wild type, but not non-alkalophilic, membranes would be studied. The underlying hypothesis for this proposal is that alkalophilic bacteria may offer a system for clarification of some of the mechanistic bases for efficient bioenergy conversion.

37. NEW YORK STATE DEPT. OF HEALTH
Albany, New York 12201

METHANE PRODUCING BACTERIA: IMMUNOLOGICAL CHARACTERIZATION — $45,029
Everly Conway de Macario
Division of Laboratories and Research

The goal is to produce specific antibodies to methanogenic bacteria for: a) immunological classification; b) characterization of new isolates; c) identification of methanogens in microbial communities including pure cultures isolated from the communities; and d) identification and characterization of phenotypic variants of these strains.

The approach will be to prepare antibodies, in several animal species, against a variety of strains of pure cultures. Specificity techniques including cross absorption will be used to delineate the specificity of antibody reactions. Immunofluorescent and agglutination procedures will be used to determine antigen-antibody interactions.

The results of these studies can be applied to analysis of 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.

38. STATE UNIVERSITY OF NEW YORK
Binghamton, New York 13901

GENE-ENZYME RELATIONSHIPS IN SOMATIC CELLS AND THEIR ORGANISMAL DERIVATIVES IN HIGHER PLANTS — $81,000
Roy A. Jensen
Department of Biology, Science III

The feasibility of managing cultured plant cells in the haploid state allows the exploitation of microbiological approaches with these eucaryotic cells (which are amenable to manipulations leading to regeneration of an entire plant organism). Our prime objective is to establish an ideal experimental system of biochemical genetics that is rigorously defined by particular gene-enzyme relationships in cultured totipotent cells of Nicotiana sylvestris, a true diploid species of tobacco. The enzymological basis of the ability to synthesize aromatic amino acids, i.e., tyrosine, phenylalanine and tryptophan, is being characterized in culture cells and in organismal tissues. Particular emphasis is placed upon 3-deoxy-D-arabinoheptulosonate 7-phosphate (DAHP) synthase, shikimate dehydrogenase, chorismate mutase, arogenate dehydratase and arogenate dehydrogenase. An appreciation of the total pathway and its regulation will be established through the isolation of regulatory mutants and structural gene mutants. In order to assure the study of these mutations within an organismal context, our approach will be that of screening for temperature-sensitive mutations. A beginning will be made to study the interfacing link between primary and secondary metabolism of aromatic compounds through the characterization of phenylalanine ammonia lyase and its regulation in both wild-type and mutant cell lines.

39. NORTH CAROLINA STATE UNIVERSITY
Raleigh, North Carolina 27650

GENETIC BASIS OF LONG-CHAIN ALIPHATIC HYDROCARBON BIOSYNTHESIS IN BACTERIA — $36,049

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.

40. UNIVERSITY OF NORTH CAROLINA
Chapel Hill, NC 27514

ISOLATION AND CHARACTERIZATION OF β-GLUCOSIDASE GENE AND β-GLUCOSIDASE OF TRICHODERMA VIRIDE — $83,007

Darrel W. Stafford
Roger L. Lunblad
Department of Zoology & Dental Research

As fossil fuels are exhausted, energy from biomass will undoubtedly become an important source of energy. There is, for example, more than 10^9 tons of waste cellulose per year in the U.S. We have begun studies which will lead to the production of an organism genetically engineered to live on cellulose and produce ethanol. Our goal is 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.

We have selected the cellulases from Trichoderma reesei for our initial studies because they have been studied more thoroughly than cellulase from any other organism. DNA from a high cellulase-producing strain has been isolated and a plasmid clone bank containing inserts of restriction endonuclease-cleaved T. reesei DNA has been obtained. Intact RNA has been isolated from cultures induced to produce cellulase and from uninduced cultures to be used in screening the clones for cellulase genes. Future work will involve the isolation of the cellulase proteins, identification of the amino acid sites responsible for the end-product inhibition, and modification of the genes to eliminate this inhibition.

41. THE OHIO STATE UNIVERSITY RESEARCH FOUNDATION
Columbus, Ohio 43210

DEVELOPMENT OF GENETIC SYSTEMS FOR ANALYSIS OF THE OBLIGATGE ANAEROBE METHANOBACTERIUM RUMINATIUM PS — $64,460

John N. Reeve
James I. Frea

Auxotrophic and drug resistant mutant strains of Methanobacterium ruminatium will be isolated. Deoxyribonucleic acid from the drug resistant strains will be used as a donor to develop a genetic exchange system. If successful, this study will provide the first genetic exchange system available for a member of the unique methanogenic group of organisms. Recombinant DNA techniques will be used to introduce and evaluate the expression of methanogen derived DNA in Escherichia coli.

42. 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

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.

43. UNIVERSITY OF PENNSYLVANIA
Philadelphia, Pennsylvania 19104

FACTORS GOVERNING LIGHT DRIVEN ELECTRON AND PROTON TRANSLOCATION IN PROTEINS ACROSS MEMBRANES — $79,000

P. Leslie Dutton
Department of Biochemistry & Biophysics

Reaction center proteins (RC) are natural biological photochemical devices that convert light energy into that useful to cellular function: this includes (a) separation of charge within the protein, directed across a membrane, generating an electric potential and (b) generation of a redox potential difference which drives electron transfer through an associated protein.

The early part of this project has been directed toward obtaining a different view of electron transfer processes: that is, a direct measurement of electron transfer. As such we have gone to some lengths to compare electron transfer patterns and electrogenic measurements obtained over the past ten years from optical studies, with those from direct measurements using voltage and current clamp techniques. We have obtained complete agreement between these disparate methods.

At the biological preparative and manipulative level we are developing two structural approaches: (a) incorporation of RCs in planar bilayers of phospholipid and (b) formation of monolayers of RCs. Further we are developing an electrochemical series of RCs modified by systematic replacement of the natural quinone with quinones of different E values (for the Q/Q reaction). Wide and systematic kinetic variations have been obtained with the different quinones.

With (a) the direct method of electron transfer and (b) study of the RCs in vectorially ordered systems and (c) systematic electrochemical modification it is hoped to obtain practical information important to understanding the factors that govern electron transfer rates in devices coupled to the generation of electric potentials.

44. PURDUE UNIVERSITY
West Lafayette, Indiana 47907

DEVELOPMENT OF GENETIC SYSTEMS IN SPECIES OF CLOSTRIDIUM — $65,000

Jean E. Brenchley
Leonard E. Mortenson
Department of Biological Sciences

Bacteria belonging to the genus Clostridium represent a group of anaerobic bacteria with diverse fermentation pathways that are extremely important in agriculture and industry. Although some studies of the physiology of clostridia have been done, little is known about the genetics of these organisms. Because genetic studies provide a valuable approach to understanding complex physiological processes, we plan to develop procedures that will allow genetic experiments with clostridia. We will construct strains needed to test for genetic transfer by transduction, transformation, and conjugation. After at least one procedure for genetic transfer has been established, we plan to construct a vehicle carrying a transposable element for future use in mutagenesis and mutant characterization. The utility of the techniques will be illustrated by isolating and characterizing mutants of C. pasteurianum that are unable to fix nitrogen. The analysis of these mutants will provide important information about nitrogen fixation in an anaerobic microorganism and, simultaneously, serve as a model system for the application of the genetic procedures.

45. PURDUE UNIVERSITY
West Lafayette, Indiana 47907

SEED PROTEIN GENES AND THE REGULATION OF THEIR EXPRESSION — $15,000 (3 1/2 months)

Don Foard
Brian Larkins
Department of Botany and Plant Pathology

Our recent studies on the Bowman-Birk and related protease inhibitors of soybean have focused on the identification and isolation of the messenger RNAs (mRNAs) directing their synthesis. The yield of polysomes and mRNAs has been improved by using carefully frozen young ovules. The mRNA has been fractionated by centrifugation on both non-denaturing and denaturing (DMSO-formamide) sucrose gradients. In vitro protein synthesis directed by selected mRNA fractions has been performed in a wheat germ system. Fractions containing mRNA for the Bowman-Birk inhibitor have been identified by means of a specific immune serum. The antigen-antibody complex was precipitated by using Cowan I strain Staphylococcus aureus cells. Through this approach we have recovered a radioactive polypeptide showing the same mobility on SDS polyacrylamide gels as the native Bowman-Birk inhibitor. With insufficient reducing agent this protein forms a dimer which is also observed in non-reduced and alkylated samples of the native protein. Experiments are in progress to further characterize this protein by means of N-terminal sequence analysis and cyanogen bromide cleavage. We are also starting to screen cDNA clones of soybean midmaturation stage mRNAs by means of coupled mRNA selection and immunoprecipitation in order to isolate a specific probe for the corresponding genome sequences.

Experiments assaying the expression of maize zein genes in Xenopus laevis oocytes have also been done. Conditions have been established for injecting optimal concentrations of cloned genes into oocyte germinal vesicles. Under these conditions the oocytes remain viable for several days. Experiments in which high specific activity amino acids have been injected into the oocyte have shown only very low incorporation into alcohol soluble proteins, however. The low efficiency of expression of the heterologous genes may result from an inefficient promotor (regulatory) sequence, or improper orientation of the gene in the cloning vehicle. Future experiments will focus on this problem by using smaller subclones of the genomic sequence containing variable amounts of the 5' flanking region of the gene, as well as "spliced" heterologous promotor sequences.

46. THE ROCKEFELLER UNIVERSITY
1230 York Avenue
New York, New York 10021

DIFFERENTIAL GENE EXPRESSION IN C4 PLANTS — $75,261

Anthony R. Cashmore
Department of Cell Biology

Major products of leaf nuclear gene expression are the small subunit of ribulose-1,5-bisphosphate carboxylase and the chlorophyll a/b binding polypeptides. These polypeptides are made on cytoplasmic ribosomes but they are found within chloroplasts where they perform primary roles in the photosynthetic process. We are interested in examining the factors involved in the differential expression of the genes encoding these polypeptides in maize. Cloned DNA sequences complementary to mRNA encoding the precursor to the major chlorophyll a/b binding polypeptide have been isolated. These cloned DNAs will be used as hybridization probes to isolate the corresponding nuclear genes. Structural and hybridization studies will be performed in an attempt to understand the factors involved in regulating the expression of these genes.

47. RUTGERS MEDICAL SCHOOL
College of Medicine & Dentistry of New Jersey
Piscataway, New Jersey 08854

SECRETORY MECHANISMS OF MICROORGANISMS: CELLULASE SECRETION BY TRICHODERMA — $75,000

Bijan K. Ghosh, Dept. of Physiology & Biophysics
Bland S. Montenecourt, Douglas Eveleigh,
Cook College, Rutgers University,
Department of Biochemistry & Microbiology

Cellulosic biomass can be developed as a renewable resource to supply 10% of the U.S. energy needs. This abundant material is naturally recycled through the action of microbial enzymes, cellulases. As cellulose is an insoluble material, these enzymes must be secreted through the cell membrane and the cell wall in order to reach, and to hydrolyze the substrate in the external environment. The economy of an industrial conversion process will depend in part on the cost of enzyme production. Increased synthesis and secretion of the cellulases per unit of cell mass will reduce the cost of the cellulase enzymes. Considerable headway has been made in developing high cellulase yielding strains of the fungus, Trichoderma reesei. However, high synthetic capacity does not necessarily result in a greater ability to transport the proteins through the cell membrane since synthesis and secretion are under independent controls. Knowledge of the physiological events regulating enzyme secretion in fungi is lacking. The aim of this investigation is to understand the mechanism of cellulase secretion in Trichoderma reesei. The specific goals are to identify the sequence of events and the subcellular location leading from initial synthesis in the ribosome-mRNA complex until release of the cellulases from the external cell surface. Knowledge of the critical steps in enzyme secretion will allow modification either by simple physiological means or genetic engineering resulting in enhanced secretion. The results of this investigation will allow for the development of more efficient microbial strains for cellulase production as well as application to other enzymes of industrial importance.

48. SMITHSONIAN INSTITUTION
12441 Parklawn Drive
Rockville, MD 20852

A PRIMARY LIGHT HARVESTING SYSTEM: PHYCOBILISOMES AND ASSOCIATED MEMBRANES — $40,000

Elisabeth Gantt
Radiation Biology Laboratory

Phycobilisomes and their association with the photosynthetic membranes are being studied in reference to their function in absorbing and transferring light energy used in photosynthesis. In red and blue-green algae (cyanobacteria) they act as an energy sink funneling the energy absorbed by phycobiliproteins through allophycocyanin to chlorophyll with an efficiency of up to 95%. Structurally, phycobilisomes have the same fundamental phycobiliprotein arrangement. Allophycocyanin is in the center near the photosynthetic membrane. Stacked rods composed of phycocyanin, or phycocyanin-phycoerythrin radiate peripherally from the allophycocyanin. In addition to phycobiliproteins, certain uncolored polypeptides appear to be required for phycobilisome stability. The objectives under study are:

1) The structure and mechanisms of energy transfer occurring to and within the core of the phycobilisomes.

2) The specific binding of phycobiliproteins within phycobilisomes.

3) The identification and isolation of anchor component(s) between the photosynthetic membranes and phycobilisomes.

Two long wavelength absorbing and emitting allophycocyanin forms (APC B and I) have been purified. Energy transfer in these forms is believed to occur by a delocalized exciton mechanism which provides for more efficient transfer to chlorophyll.

By direct association of specific phycobiliproteins into functionally active complexes, the role of binding proteins are being explored in cyanobacteria and red algae. Our recent success in the reassociation of phycobilisomes from allophycocyanin and phycoerythrin-phycocyanin will be utilized for further studies in characterizing the components involved in the core structure and in the attachment of the peripheral phycobiliprotein rods.

49. SRI INTERNATIONAL
Menlo Park, California 94025

REGULATORY MECHANISMS IN THIELAVIA TERRESTRIS — $49,999 (FY 80 funds)
Daniel Tusé
Medical Sciences Department

SRI International has isolated a strain of the thermophilic fungus Thielavia terrestris. This organism produces a heat stable cellulase that can degrade both amorphous and crystalline cellulose to glucose; hence it may be of interest for converting cellulosics into fuels. We are studying the regulatory mechanisms used by this fungus to sense and attack insoluble extracellular polymers. We are presently investigating the culture parameters leading to the production and release of cellulolytic enzymes. One of the primary questions asked in this research is: Is the physical contact between the fungal cells and an insoluble, extracellular polymer such as cellulose, a necessary phenomenon for the induction of lytic enzymes? We have approached the problem by growing the organism in two-chamber fermenters, where cell mass and an insoluble substrate are separated by a semipermeable membrane to prevent cell-polymer contact. Our results indicate that no contact between high MW, crystalline cellulose and the cells is necessary for cellulase induction. This leads us to believe that a diffusable agent may be responsible for initiating the induction process. We are presently attempting to identify the nature of the inducer by studying the production of the various enzyme fractions (crystalline, and amorphous cellulose degrading activity, and beta glucosidase) by culturing the cells in the presence of regulatory compounds such as cyclic AMP, cyclic GMP, various enzyme inhibitors, and membrane depolarizing agents. We are also attempting to locate the genetic information involved in the process of cellulose degradation. Although the genes involved are probably chromosomal, there exists the possibility that extrachromosomal elements may be involved in the regulatory process. These genes may exist as plasmids, or be part of intracellular organelles such as mitochondria. The information generated by these studies will help elucidate the mechanisms involved in eukaryotic enzyme induction, the behavior of cells towards polymers, and will allow us to better establish a genetic basis with which to improve cellulolytic organisms for the eventual efficient conversion of biomass into usable energy.

50. STANFORD UNIVERSITY
Stanford, California 94305

CARBON DIOXIDE AND THE STOMATAL CONTROL OF WATER BALANCE AND PHOTOSYNTHESIS IN HIGHER PLANTS — $65,637
Eduardo Zeiger
Department of Biological Sciences

Stomatal regulation of gas exchange of plant leaves imposes a primary control on water balance and photosynthetic efficiency of higher plants and affects the water loss of land covered by vegetation.

CO_2 is a major stomatal regulator yet its mode of action in stomatal function remains unknown. We propose to investigate the role of CO_2 in the stomatal responses in intact leaves, epidermal peels and isolated guard cell protoplasts in order to analyze the responses to CO_2 at the cellular, tissue and organ levels. Three alternative hypotheses for the mode of action of the stomatal regulator will be tested in experiments utilizing high resolution techniques such as null balance gas exchange and measurements of ion fluxes in isolated guard cells and guard cell protoplasts. Biological variability and physiological manipulation are incorporated in the experimental design to separate the direct responses of the guard cells from epidermis/mesophyll interactions and the effect of CO_2 uptake by photosynthesis. Stomata from CAM plants in which CO_2-induced stomatal closure in the light and opening in darkness occur under normal physiological conditions will be studied in order to integrate the sensing of environmental clues with the cellular responses modulating ion transport in the guard cells.

With the higher atmospheric levels of CO_2 and the increasing pressure on limited agricultural land resulting from industrialization and rapid population growth, scientific knowledge provides a unique expandable resource to preserve and improve human welfare. It is hoped that the elucidation of the basic mechanisms controlling the stomatal responses to CO_2 and its effects on water balance and photosynthetic efficiency of plants will contribute to our basic knowledge of plant physiology and help in improving available agricultural practices as well as methods for environmental control.

51. UNIVERSITY OF UTAH
Logan, Utah 84322

BIOENERGETICS OF THE METHANOGENIC BACTERIA — $75,000
Jack R. Lancaster, Jr.
Department of Chemistry and Biochemistry

I propose to study the mechanism whereby the methanogenic bacteria conserve in a biochemically useful manner the energy released by methane production from H_2 reduction of CO_2 or from acetate metabolism. The goals of this project are to identify the electron-transfer components in energy-coupled methanogenesis, describe the nature of the interactions of these components with substrate and with each other, and define the mechanism(s) whereby energy is conserved by this process. Since recent evidence suggests the involvement of transmembrane ion gradients in this process, the research will at least initially focus on the roles of the soluble and membrane-bound electron transfer components which I have recently observed by low temperature electron paramagnetic resonance spectroscopy.

52. WASHINGTON UNIVERSITY
St. Louis, Missouri 63130

CHARACTERIZATION OF, AND PLANT GENETIC ENGINEERING WITH, A GENE FOR SOYBEAN STORAGE PROTEIN — $53,655
Roger N. Beachy
Department of Biology

The objectives of this project are (1) to isolate and study the organization of the gene encoding the 11S seed storage protein from soybean (Glycine max L. Merr.), and (2) to insert this gene into the tumor inducing (T_i) plasmid of Agrobacterium tumefaciens and to look for stable transfer and expression of the gene after tumor induction in tobacco stems. Research in our laboratory has been directed toward the study of the (in vivo and in vitro) synthesis of the major seed storage of soybean. We have learned: 1) when during seed development these proteins accumulate in the seed; 2) when the messenger RNAs are most abundant; 3) the extent of relatedness between some of the protein subunits and to some degree; 4) the steps in protein-processing that occur during protein accumulation. We will use cloned cDNAs (previously identified) to isolate the gene for the 11S storage protein from a genomic library of soybean DNA fragments cloned in λ-phage. The structural gene will be characterized with respect to intervening sequences and sequences of DNA upstream from the coding sequences that might be expected to contain the gene promoter. In collaboration with Dr. M.D. Chilton (this university), the characterized gene will be inserted into the Ti-plasmid of Agrobacterium tumefaciens. Tumor tissues induced by the engineered plasmid will be examined for stable incorporation of the soybean gene(s), for transcription of the genes, and for production of the 11S seed storage protein. These studies will represent a rigorous attempt to use the A. tumefaciens plasmid to transfer plant DNA from one plant species to another. Concurrently, we will be thoroughly characterizing the gene for the 11S seed storage protein, a biologically and nutritionally important protein.

53. WASHINGTON UNIVERSITY
St. Louis, Missouri 63130

DEVELOPMENT OF A TUMOR-INDUCING (TI) PLASMID VECTOR FOR PLANT GENETIC ENGINEERING — $85,116
Mary-Dell Chilton
Biology Department

Agrobacterium tumefaciens and its tumor-inducing (Ti) plasmids constitute a promising vector system for the introduction of desirable genes into higher plant cells. This bacterium incites galls (malignant tumors) on plants by a mechanism whose details are not known, but whose result is that a specific part of the Ti plasmid is covalently joined to plant nuclear DNA. This foreign DNA (called T-DNA) is stably maintained and expressed in the transformed plant cell. There are two barriers to be overcome if we are to exploit the Ti plasmid as a vector for plant modification. The first is that T-DNA must be "disarmed"--genetically modified in such a way that its insertion into the plant genome does not cause tumors. We propose to introduce mutations into T-DNA fragments by in vitro mutagenesis of recombinant DNA plasmids carrying small parts of T-DNA. These will be returned to Agrobacterium and caused to reinsert into the Ti plasmid to test their effect on tumor induction. The second obstacle to genetic engineering is that we must devise means of inserting the desired DNA fragment into T-DNA at desired locations. We propose to approach this problem by inserting the desirable DNA into recombinant plasmids containing T-DNA fragments; these will then be return to Agrobacterium and caused to reinsert into the Ti plasmid. The target gene will be soybean seed storage protein gene, and the host plant will be tobacco. This model genetic engineering experiment will test rigorously the feasibility of using Ti plasmids as vectors.

54. UNIVERSITY OF WASHINGTON
Seattle, Washington 98195

STUDIES ON THE CONTROL OF PLANT CELL ENLARGEMENT BY CELLULAR PARAMETERS — $52,100
Robert E. Cleland
Botany Department

Plant cell enlargement is controlled at the cellular level by four parameters; the wall extensibility (WEx), the hydraulic conductivity, the osmotic potential gradient ($\Delta\Psi_s$) and the wall yield stress (Y). Whenever the growth rate changes, it must be caused by a change in one or more of these parameters. We are studying the role of each of these parameters in the induction of rapid cell elongation by auxin and fusicoccin (FC) in oat coleoptiles.

Auxin causes WEx to increase, primarily by inducing cells to excrete protons which lead to acid-induced wall loosening, and secondarily by increasing the ability of the walls to respond to protons. Auxin has no effect on Y, and does not increase $\Delta\Psi_s$, although auxin-induced water uptake leads to a greater rate of solute accumulation. If the cuticle is removed, coleoptile cells take up solutes from the solution until some maximum internal concentration is achieved; auxin decreases this maximum concentration. FC causes very rapid elongation at first, due to proton-induced increases in WEx, but then the growth rate falls precipitously when the walls lose their capacity to respond to protons.

The endogenous enlargement of corn root cells, the light-induced expansion of bean leaves and the auxin- and FC-induced growth of soybean hypocotyl sections are all initiated by excretion of protons, leading to increases in WEx. In each case $\Delta\Psi_s$ decreases, indicating that changes in turgor are not promoting cell elongation.

55. UNIVERSITY OF WASHINGTON
Seattle, Washington 98195

FERMENTATION TO ETHANOL OF XYLOSE PRESENT IN BIOMASS BYPRODUCT SOLUTIONS — $91,877

Benjamin D. Hall
Clement Furlong
Mary O'Connor
Department of Genetics and Microbiology

Objective: To develop a technically feasible xylose fermentation by yeast.

Description and Approach:
The yeast Schizosaccharomyces pombe can ferment xylulose efficiently. Studies are currently in progress to determine whether:

1. S. pombe can take up xylose either when grown in glucose medium or in a non-repressing medium (succinate).
2. S. pombe possesses a xylose isomerase enzyme activity and, if so, how the level of this enzyme is regulated by the carbon source used for growth.
3. Whether under any conditions, S. pombe can ferment xylose to some extent.

We assume that the answer to Question 3 will be "No" under all conditions. Accordingly, experiments are in progress to clone from Rhodotorula glutinis the gene necessary for xylose fermentation which S. pombe lacks.

Given a xylose-fermenting S. pombe strain, we shall carry out growth studies on various carbon sources, measuring in each instance the nature and yield of various fermentation products. Further genetic manipulation, either by mutant selection or DNA engineering will be carried out as needed to develop an organism which efficiently converts xylose in spent sulfite liquor into ethanol.

Final Product Expected: A strain of yeast which can be grown simply and cheaply and which will ferment xylose to ethanol even when glucose is present in the medium.

56. UNIVERSITY OF WASHINGTON
Seattle, Washington 98195

A STUDY OF THE GENETICS AND REGULATION OF METHANE OXIDATION — $72,499 (18 months)

Mary L. O'Connor
Department of Microbiology & Immunology, SC-42

Objective: The purpose of this project is to locate and identify the genes necessary for growth on methane in the facultative methane-oxidizer, Methylobacterium ethanolicum.

Description and Approach: Mutants unable to grow on methane but capable of growth on methanol have been isolated. A plasmid cloning vector (pRK290) and its mobilizing plasmid (pRK2013) will be used to identify DNA fragments which complement the methane-negative mutants. These fragments will then be physically mapped, using restriction enzymes, and genetically mapped, using transposon-generated mutations.

The methane-negative mutants will also be characterized physiologically concerning methane-specific activities. The inducer of methane-specific genes as well as the coordinate nature of their regulation will be determined using regulatory mutants and chemostat culture.

Final Product: This information is of primary importance to the optimization of any commercial process involving the use of methane oxidizers as biological catalysts for chemical production.

57. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

DEVELOPMENT OF NITROGEN-FIXING MONOCOT-BACTERIA ASSOCIATIONS — $72,000
Winston J. Brill
Department of Bacteriology

The purpose of this project is to determine the mechanisms by which certain nitrogen-fixing bacteria are able to utilize monocot-produced photosynthate as a source of energy. The energy source(s) are capable of supporting nitrogen fixation by the bacteria. The long-term application of such studies is to develop an association between nitrogen-fixing bacteria and cereal plants. However, as background information, we need to understand rhizosphere ecology, microbial physiology, and plant physiology as they relate to the system. The main tool that we use for these studies is a collection of corn genotypes, some of which support bacterial nitrogen fixation and some that do not.

58. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE — $35,600
Jerry L. Kermicle
Laboratory of Genetics

Previous studies of the R locus have centered on a class of component which governs the presence, distribution and timing of anthocyanin pigmentation, plant part by plant part. A variety of such determiners of gene expression occur naturally in cultivated races of maize. The determiner components serve as particularly favorable landmarks in the chromosome for working out the structure of allelic complexes. Recently, the emphasis of our work has shifted toward study of R genes as functional units. Are there components in addition to the determiners that are essential for R action? Spontaneous and induced recessive mutations of given alleles are being used to study this question. Our first positive evidence derives from four instances in which mutation was caused by insertion of the controlling element Dissociation (Ds) into R. In each, the site of Ds insertion mapped not at the determiner site but slightly distal. The inhibiting effect of Ds on R expression in these instances evidently is caused by Ds interrupting the relationship between the determiner component and others essential to R function. We are characterizing variants induced by the chemical mutagen ethyl methanesulfonate to determine more precisely the number and location of the components involved.

59. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

PHYTOCHROME FROM GREEN PLANTS: ASSAY, PURIFICATION AND CHARACTERIZATION — $58,000

Peter H. Quail
Botany Department

Phytochrome regulates key, productivity-determining facets of growth and development throughout the life-cycle of green plants. Elucidation of the mechanism by which this regulation occurs requires a full understanding of the molecular properties of the photoreceptor. Yet our current knowledge of these properties is restricted to data from etiolated seedlings despite evidence of potential qualitative differences between green and etiolated tissue phytochrome. Direct investigation of green-tissue phytochrome has been hampered by the lack of a convenient method for eliminating chlorophyll which interferes with the spectral assay, and by the relatively low levels of the chromoprotein in light-grown plants. The proposed research is directed (a) at providing procedures for overcoming these difficulties, and (b) at purifying and characterizing phytochrome from green tissue.

Preliminary experiments show that polyethyleneimine (PEI) precipitates chlorophyll from crude extracts leaving phytochrome in the supernatant. Further refinement of this observation will provide a simple, rapid and widely implementable procedure for the direct spectrophotometric assay of phytochrome in crude extracts of green tissue. Phytochrome will be purified from green tissue using immunoaffinity and Blue-sepharose chromatography in a proteolysis inhibiting buffer. The chromoprotein will be purified from a green alga for the first time as well as from the normally used terrestrial species. The molecules from these sources will be characterized and cross-compared using a series of standard analytical procedures. This information will permit delineation of any potential differences between phytochrome from green and etiolated tissue and terrestrial and aquatic species.

The significance of the proposed program is twofold: (a) it will provide new, readily implementable methodology for the assay and purification of phytochrome from green tissue; and (b) it will approach a number of currently unresolved issues in phytochrome research directly relevant to plants grown under natural conditions.
(Collaborative with Project #20, University of Georgia)

60. UNIVERSITY OF WISCONSIN
Madison, Wisconsin 53706

ONE CARBON METABOLISM IN ANAEROBIC BACTERIA: ORGANIC ACID AND METHANE PRODUCTION — $70,000

J. G. Zeikus
Department of Bacteriology

The metabolism of methanol, acetate and CO was compared in Methanosarcina barkeri, a methanogen, and Butyribacterium methylotrophicum. A defined mineral medium was designed for growth of B. methylotrophicum on H_2/CO_2 or methanol/acetate. A mutant, derepressed for formate dehydrogenase, was selected from the neotype Marburg strain of B. methylotrophicum that grew on CO as the sole energy source for growth. The mutant, called the CO strain, also grew via simultaneous consumption of CO and methanol. However, the organism consumed CO in preference to hydrogen when grown on syn gas (i.e. H_2/CO). The CO strain contained a very active CO dehydrogenase >3 µmol CO oxidized/ min/mg protein when grown on methanol or CO as energy source. During growth on CO/methanol butyrate was not a significant end product. A detailed analysis of the thermodynamics of growth on glucose, CO and methanol revealed that B. methylotrophicum possessed greater metabolic efficiency than aerobic methylotrophs or heterotrophs. M. barkeri was grown on acetate or acetate and methanol as the sole carbon and energy sources for growth. A significant intramolecular redox mechanism was discovered when M. barkeri was grown on acetate alone. Greater than 15% of the C-1 of acetate was transformed to CH_4, whereas 15% of the C-2 of acetate was converted to CO_2. The addition of methanol did not catabolite repress acetate metabolism but did increase the amount of C-2 acetate converted to CO_2 and the amount of acetate transformed to end products. A strain of M. barkeri was selected that grew on 100% CO as the sole energy source. However, during mixotrophic growth on H_2/CO or methanol/CO, significant CO consumption was not detected until the end of growth. Both M. barkeri and B. methylotrophicum were grown on methanol in continuous culture.

61. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

MOLECULAR PLANT GENETICS — $185,000
Benjamin Burr
Frances A. Burr
Biology Department

Recent advances in molecular genetics suggest new ways to change the genetic constitution of plants to make them more efficient energy converters and to adapt them to new requirements of food, fiber, and fuel production. The techniques of isolating genes from plants and transferring them to other species are only just being developed. Problems to be overcome include the physical identification and purification of genes to be transferred as well as learning how to introduce foreign genes, conferring beneficial properties, into target crop species. Corn genes involved in starch synthesis and degradation and genes encoding storage proteins are being isolated and their structural features which bear on gene expression are being described. Genetic elements known to transpose within the corn genome are also being investigated. These transposable elements may aid in the mobilization of genes for transfer and in their integration into a recipient genome. Emphasis is on the Ac-Ds controlling element system. We wish to elucidate the structure of these elements, determine how frequently they or their components occur in the maize genome, and learn their mechanism of transposition.

62. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

REGULATION OF PLANT REPRODUCTION, GROWTH AND RESPIRATION BY LIGHT AND NITROGEN METABOLISM — $75,000
William S. Hillman
Biology Department

Higher plants fix solar energy through photosynthesis, a process widely investigated in isolated cell components. However, in whole plants, the ultimate products and yields are greatly influenced by aspects of physiology such as reproduction, respiration and nitrogen metabolism that still demand study at the multicellular, organismic level. Excellent experimental systems are provided by the Lemnaceae (duckweeds), rapidly growing aquatic plants, since their growth rate, flowering, respiration and nitrogen metabolism can be profoundly controlled by low energies of light. As in other plants, this low-energy control is exerted not through photosynthesis but through the plant photomorphogenic pigment, phytochrome. Portions of this work also concern photoperiodism--the control of reproduction by the daily length of illumination--which is in itself an important model for the time-dependent actions of many other environmental factors. The overall purpose of this task is thus to advance our understanding of how surprisingly small changes in external factors--a few minutes of dim light, for example--can make profound differences in the utilization of the same basic energy or nutrient supplies by intact higher plants. (Dr. Hillman died on 9th February 1981)

63. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

MECHANISMS OF ENERGY CONVERSION IN PHOTOSYNTHESIS — $255,000
Geoffrey Hind
Biology Department

Chemiosmotic potential is the energetic basis for life processes. In photosynthetic membranes, solar energy is converted into chemiosmotic potential by ion pumping linked to electron transport; in the purple membrane of Halobacterium, it is directly generated across the pigmented membrane without external electron flow. Biomimetic solar energy conversion devices might be developed to drive ion pumping processes for special applications, but they must be independent of solution-phase reductants and oxidants. The purple membrane of Halobacterium and cyclic electron transport pathway of photosynthetic membranes may satisfy this criterion.

In C-3 photosynthesis, cyclic electron transport around photosystem I provides some of the energy for CO_2 fixation, whereas in bundle sheath chloroplasts of C-4 plants, virtually all electron flow is cyclic; the same applies wherever high ratios of ATP to reductant are required, as for example, in the nitrogen-fixing heterocysts of cyanobacteria. Chloroplasts of stomatal guard cells may perform only cyclic electron transport, though this is questioned. The role and pathway of cyclic electron flow in the above photosynthetic membranes will be elucidated by membrane resolution and reconstitution combined with conventional and flash spectroscopy. Cyclic generation of chemiosmotic potential across reconstituted and synthetic membranes will be attempted using these photosynthetic systems and the purple membrane of Halobacterium.

64. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

PHOTOSYNTHETIC MEMBRANES — $110,000
John M. Olson
Biology Department

When the structure and function of photosynthesis membranes are understood in sufficient detail, it may prove possible to construct solar energy converters based on biological principles and able to transform sunlight into chemical or electrical energy with an efficiency comparable to that of silicon cells. In photosynthetic membranes, light absorbed by antenna chlorophyll is converted to excitation energy which is transferred to photochemical reaction centers. The primary photochemical reaction is an electron transfer from an excited chlorophyll dimer to an acceptor molecule, either a quinone or an iron-sulfur center. This primary reaction drives photosynthetic electron transfer in a system of cytochromes, quinones, iron-sulfur proteins, and other components.) The efficiency of energy trapping depends on the spatial organization of antenna chlorophyll in relation to reaction centers. Structural relationships between bacterio-chlorophyll a (Bchl a)-proteins and reaction centers are being studied in unit-membrane vesicles from green bacteria, because the structure of the Bchl a-protein is known to a resolution of 0.28 nm and can in principal be identified and localized within the unit membrane. Bchl a-proteins have already been isolated and characterized, and currently lipids are being investigated. Eventually reaction centers will also be isolated and studied. When the interactions between these membrane components are elucidated, the resulting structural picture of the membrane should explain its function in the primary steps of energy conversion.

65. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

GENETIC ENGINEERING IN PLANTS — $155,000
Daniela Sciaky
Biology Department

Crown gall is a naturally occurring genetic engineering system where bacteria transfer and express their genes in plants. The components of crown gall responsible for transfer and integration of bacterial DNA in plants will be isolated and used to construct a plant cloning vector using the now conventional molecular cloning techniques. This will require: 1) the construction of a cloning vehicle capable of replication in the bacterial host (Agrobacterium tumefaciens) and integration into the plant DNA; and 2) the characterization of the bacterial DNA responsible for insertion and expression in the plant host so that insertion of foreign genes into the cloning vector will not inactivate the system or not allow the foreign genes to be expressed.

In the course of construction of this vector or transfer plasmid, the boundaries of the T-DNA (the bacterial DNA found in plants) on the plasmid responsible for tumorigenesis (the Ti plasid) will be delineated. Other genes involved in the final expression of T-DNA will be mapped and their ability to trans complement the T-DNA will be determined.

Spliced plant RNA, derived from the nucleus, is now know (phaseolin gene of French bean, leghemoglobin gene of soybean). If expression of nuclear plant genes requires splicing (as is the case for some mammalian genes), then foreign DNA inserts into T-DNA will have to preserve the correct splicing pattern. Therefore, plant RNA homologous to the T-DNA will be characterized by restriction digests and sequencing to determine whether and where these messages are spliced.

66. BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

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

The overall objective of this project is to gain an understanding of genetic controls in plant development through the use of cell and tissue culture and the analysis of genetic tumors. Various enzymatic techniques provide protoplasts, that is, wall-less cells, for fusion and parasexual hybridization, making possible expanded studies of somatic cell genetics. Interspecific hybridization has been accomplished in this way and the methods of fusing somatic protoplasts are being extended to intergeneric hybridization and even to interkingdom fusion. These new methods for combining more widely divergent genomes than previously possible may find use in the production of entirely new kinds of living organisms. Haploid cell lines are established by anther culture and these cultures are selected for resistance to analogs and antimetabolites, then analyzed to determine the causes for the resistance. These new methods are expected to find use in the production, more rapidly than previously possible, of plants resistant to various pollutants, thus permitting biological productivity to be maintained.

The genetic basis of spontaneous tumor formation in plants is studied by isolating particular chromosomes that, when introduced into another species, cause tumors to develop. These chromosomally-defined, tumor-prone plants are then grown in tissue culture to determine their differences from normal plants in physiological or biochemical growth-factor requirements. The experiments demonstrate the importance of a genetic component in causing tumors.

67. LAWRENCE BERKELEY LABORATORY
Berkeley, California 94720

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

Application of time-dependent electron paramagnetic resonance (EPR) spectroscopy to determination of structure-function relations of photochemical steps in green-plant photosynthesis. By using newly-developed methods of EPR data acquisition, with special attention to making measurements of spin relaxation for paramagnetic oxidation-reduction components, we plan to sort out the role of various electron donors and acceptors in these photochemical reaction centers. Our methods make use of saturation-recovery and direct detection resonance techniques and the use of oriented membrane samples prepared by drying under controlled atmospheres. So far our studies have shown the magnetic orientations of several iron-sulfur acceptor components and have, due to the enhanced signal-to-noise ratio, shown several new paramagnetic components whose specific roles are being further investigated. Careful application of these new techniques has also shown that many of the before-assumed components may lie in new positions in the electron-transport chain on the acceptor side of Photosystem I. In addition, these techniques show promise in studies of the oxygen-evolving complex of Photosystem II in vivo. The extension of these studies to iron-sulfur components in the electron-transport chain of oxidative phosphorylation is also planned. This research has as its goal the elucidation of reaction pathways for the photochemical steps in photosynthesis and associated bioenergetic processes. Investigations as to the role of electron-tunneling processes in biological electron transport are crucial in many bioenergetic pathways (photosynthesis, oxidative phosphorylation, and the creation of biomass); we are examining experimentally tunneling processes in a particularly sensitive photosynthetic bacterial system where charge-transfer spectra measured at cryogenic temperatures may give a definitive answer. This is important in general terms and to a test of the Hopfield theory of such processes specifically.

68. LAWRENCE BERKELEY LABORATORY
Berkeley, California 94720

PLANT BIOCHEMISTRY $380,000
James A. Bassham
Henry Rapoport

The objectives of this proposal are to determine photosynthetic and biosynthetic pathways of carbon and nitrogen metabolism in plants and the mechanisms of regulation in these paths, and to employ this knowledge to improve productivity, product quality, and other characteristics of plants through techniques of plant cell tissue culture, recombinant DNA, cloning and plant propagation. Investigations include elucidation of metabolic pathways in green plants and location of sites of metabolic regulation.

Subtask I (Regulation of Metabolism and Gene Expression) includes determination of the mechanisms of such regulation; isolation of differentiated plant cells such as leaf cells and use of these cells as test systems for studies of regulation; plant cell tissue cultures as (1) a means of studying plant cell differentation, (2) for plant improvement through cell selection, plant regeneration and cloning, and (3) as donor and acceptor cells for genetic information in the form of DNA to be transferred from one species to another through techniques of recombinant DNA.

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-chlorophillous plant pigments.

69. LAWRENCE BERKELEY LABORATORY
Berkeley, California 94720

PHOTOCHEMICAL CONVERSION OF SOLAR ENERGY — $100,000
Lester Packer

This program seeks to characterize the "Photochemical Conversion of Solar Energy" by bacteriorhodopsin, a light- and temperature-stable retinal protein derived from purple membranes of halobacteria. Unlike photosynthesis which depends on redox reaction, bacteriorhodopsin operates via a photocycle to develop a protein current directly.

Approach: Bacteriorhodopsin both in the natural membrane and in reconstituted artificial membranes is being investigated. Chemical modification with site specific reagents is used to identify amino acids essential for photocycle activity and for proton conduction through the molecule. These studies are being aided by the investigation of isotope effects, Laser flash photolysis measurements and ESR spin probe techniques that measure alterations in electrical potential at and across the membrane surfaces during the process of energy conversion. A newly discovered light energy converter, halorhodopsin, which is a retinal protein that may act by a direct mechanism for developing a sodium current in halobacteria, is also now being characterized. These studies are being aided by the recent discovery of other bacterial mutants.

Anticipated benefits of this program include understanding of the molecular basis of the light energy conversion by retinal proteins and their use for development of photovoltaic cells and model systems for desalination.

70. LAWRENCE BERKELEY LABORATORY
Berkeley, California 94720

ENERGY CONVERSION IN PHOTOSYNTHESIS — $250,000
Kenneth Sauer
Melvin P. Klein

Spectroscopic studies currently provide the best approach to investigating the physics and chemistry of the conversion of photon energy to chemical potential in photosynthetic membranes. Important aspects of the problem include (a) the structure of the photosynthetic membranes at the molecular level, (b) the absorption of photons, formation and nature of electronic excited states and transfer of excitation to the reaction centers, (c) charge separation in the reaction centers, and (d) water oxidation and the formation of O_2 in higher plant photosynthesis. The spectroscopic methods include optical absorption, fluorescence, circular and linear dichroism, EPR and X-ray absorption. Fast kinetic studies of electronically-activated components are carried out using fluorescence lifetime, depolarization relaxation and electron spin polarization measurements. Our findings have led to the formulation of the Pebble Mosaic Model of membrane organization, which predicts a mechanism for excitation transfer among defined chlorophyll proteins. Our EPR studies serve as the basis for the Radical-Pair Mechanism of charge separation in the photosynthetic reaction centers. Our studies of water oxidation have revealed the role of manganese in storing oxidizing equivalents prior to O_2 release. In summary, this research spans the events that connect the initial absorption of solar photons to the eventual production of stable, high-energy chemical products in photosynthetic organisms.

71. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

ANALYSIS OF PHOTOSYNTHETIC MEMBRANES — $209,000
Charles J. Arntzen
MSU/DOE Plant Research Laboratory

Research is conducted to learn the nature of factors which influence, and potentially limit, the quantum efficiency of photosynthesis. Primary emphasis is directed at understanding the interactions among functional components of chloroplast internal membranes (thylakoids) and the structural organization of these components. Procedures for isolation of photosystems I and II, as well as the light-harvesting pigment-proteins associated with each of these complexes, have been developed. The various components are being utilized to reconstitute functional complexes into artificial membranes. Covalent modification of pigment proteins by protein phosphorylation and the role of protein processing in membrane assembly is being analyzed. These studies will provide an understanding of control mechanisms which regulate photosynthetic light reactions.

72. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

ENZYMATIC MECHANISMS AND REGULATION OF PLANT CELL WALL BIOSYNTHESIS — $92,000
Deborah P. Delmer
MSU/DOE Plant Research Laboratory

One of the goals of the Plant Research Laboratory is to conduct research on processes that determine the conversion of solar into chemical energy (biomass) in plants. This task specifically addresses this goal as it seeks, as its major objective, to elucidate the pathway of conversion of reduced carbon into cellulose, the world's most abundant organic compound. For these studies, we use primarily the developing cotton fiber as an experimental system because this cell produces a nearly pure cellulosic cell wall. The principal investigator has also begun comparative studies using Acetobacter xylinum, a bacterium which excretes a pure cellulosic pellicle, during her current sabbatical year in Israel. The overall project involves a variety of experimental approaches including: 1) in vivo labeling experiments designed to trace the flow of carbon from glucose to cellulose and also to determine the direction of chain growth of cellulose; 2) experiments designed to protect and/or activate the apparently labile synthesizing complex in order to study the process in vitro.

Other related projects included in this task: a biochemical analysis of the process of cell wall regeneration in protoplasts, a process which represents one of the critical early steps in the genetic engineering of plants involving protoplast fusion techniques; and, a study of the process of synthesis of legume storage proteins - a major source of protein in man.

73. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

REGULATION OF PROTEIN FORMATION IN PLANTS: SIGNIFICANCE IN GROWTH REGULATION — $124,000
Philip Filner
MSU/DOE Plant Research Laboratory

One of the most common environmental perturbations experienced by plants is a change in the available concentration or chemical form of an essential nutrient. The ability of a plant to cope with such events determines its ability to survive. We are studying a novel coping mechanism which we discovered in the XD line of cultured tobacco cells. These cells have the potential for multiplying with a doubling time of 2 days, which they can realize when growing on nitrate, ammonium, or mixed amino acids for nitrogen. However, when grown on urea nitrogen, the doubling time is almost 4 days, because of insufficient urease. We found that the cells possess a mechanism for increasing urease activity, but it requires about 40 generations of growth on urea to become manifest in a cell population, when the high urease variants begin to overgrow the slower low-urease cells. This is far slower than known mechanisms for enzyme de-inhibition, activation or induction, which require seconds to hours, but it is far faster than is expected for conventional mutations, which are estimated to occur at a frequency of ca. 10^{-7}, and would require about 100 generations to become manifest.

The conditions under which the urease increase occurs and the dynamics of the phenomenon, are reminiscent of selections for gene amplification in bacteria, yeast and animal cells. These appear to be related to insertion-element mediated control of genes in bacteria and yeast. A major objective of this project is to determine if such a mechanism is responsible for the origination of high urease variants. Experiments designed to quantitate urease protein in low and high urease cells are in progress. A second objective is to determine whether the phenomenon is unique to urease or whether it is a general mechanism which operates whenever an enzyme is rate-limiting for growth. Selections for improved growth on other growth rate limiting nutrient sources are in progress. A third objective is to determine if the high urease variation, when stable in cultured cells, is expressed in shoots regenerated from such cells. Such shoots are available.

74. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

RESISTANCE OF PLANTS TO ENVIRONMENTAL STRESS — $87,000
Andrew Hanson
MSU/DOE Plant Research Laboratory

This task investigates the metabolic basis for injury from, and adaptation to, environmental stresses--especially water stress--with the long-range objective of developing novel selection methods for stress-adaptation applicable in plant breeding. Accordingly, research is conducted in collaboration with the plant-breeding program of Dr. D. A. Reicosky (Crop and Soil Science Department, MSU), and has the following aims: (1) to characterize the metabolic responses of plants elicited by stress; (2) to assess genetic variation for these responses; (3) to evaluate the adaptive value of responses by physiological and genetic experiments; (4) to estimate the bioenergetic costs of the responses. One metabolic response to water- and salt-stress in several cereals and other crops is accumulation of glycine betaine; this stress-response is being examined from the standpoint of possible adaptive value, and as a potential index of stress-injury or -resistance in plant breeding. Test objects are whole plants and mini-crops of barley, and young sugar-beet plants. The accumulation of the indole alkaloid gramine is under evaluation as a potentially adaptive response to high-temperature stress in barley.

75. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

PLANT GROWTH REGULATIONS BY HORMONES (CYTOKININS, GIBBERELLINS, ETHYLENE) — $107,000
Hans Kende
MSU/DOE Plant Research Laboratory

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

a) Regulation of stress ethylene 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-amino-cyclopropane-1-carboxylic acid (ACC) synthase. Research will mainly concentrate on the mechanism by which ACC synthase is regulated.

b) Regulation of aging in plants by ethylene and cytokinins: Of particular interest are degradative processes which affect cell membrane integrity and cellular compartmentation. Membrane breakdown leading to irreversible deterioration of the cell is promoted by ethylene and retarded by cytokinins. Investigations will also focus on the mechanism by which ethylene synthesis is regulated by ethylene (positive feedback).

The work described above is part of the Laboratory's effort to understand how stress affects plants and how plants cope with it. Work on plant senescence is also important in the context of storage and transportation of perishable agricultural commodities. Manipulation of the aging process may lead to decreased energy input in the prevention of product spoilage.

76. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

THE SACCHARIFICATION OF WOOD AND WASTE CELLULOSE — $147,000
Derek T. A. Lamport
MSU/DOE Plant Research Laboratory

A simple economic method of "cracking" cellulose to yield feedstock sugars remains elusive.

This task explores a novel method for "cracking" cellulose using gaseous or liquid anhydrous hydrogen fluoride at circumambient temperatures and pressures. The method leads to cellulose and hemicellulose saccharification and has the advantage over other proposed processes of speed and simplicity. Furthermore theory predicts that the HF can largely be recycled. Therefore we shall determine saccharide yields, and fluoride retention, as a function of time, temperature and evaporative technique.

With optimized reaction conditions we can proceed to process design and possible scale-up to pilot plant level.

77. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

REGULATION OF FLOWERING — $164,000
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, potent 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 to the lack of promoters of flowering; now we find that it may also be caused by the presence of inhibitors of flowering. The inhibitors have been shown to 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 (long-day, short-day and day-neutral plants). Current work of this task is directed primarily at the isolation and identification of antiflorigen.

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 be considered as a problem of energy partitioning in the plant. For these reasons, research on regulation of flowering, including flowering inhibitors as a new "element" in this regulation, is highly pertinent to the PRL research program.

78. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

ACQUISITION OF ENVIRONMENTAL INFORMATION THROUGH LIGHT PERCEPTION — $118,000
Kenneth L. Poff
MSU/DOE Plant Research Laboratory

This task aims 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 are studying light sensing in the cellular slime mold Dictyostelium in which we have identified several photoreceptor pigments and their subcellular localization, and are proceeding to isolate and analyze photoreceptor pigment mutants which will permit dissection of the components of the photoreceptor transduction sequence.

We are also studying the "blue light" photoreceptor system(s) which control(s) numerous light responses in plants, by using specific inhibitors as probes into the initial steps in the transduction sequence. The use of these probes has permitted us to analyze the roles of carotenoids and flavins in phototropism, and to "photoaffinity-label" the phototropism photoreceptor pigment.

Temperature perception is being studied in two systems: thermotaxis by pseudoplasmodia of Dictyostelium and the sensing of low temperature by cultured cereal cells prior to the acquisition of frost hardiness. Thermotaxis is a unique model system for the study of biothermometry because of the extreme sensitivity to temperature of the pseudoplasmodia, the narrow temperature range over which temperature is measured, and the phenomenon of adaptability -- the dependence of the thermotaxis temperature range on the previous growth temperature of the cells.

Non-visual light perception is a plant process which determines the conversion of solar energy into chemical energy through various mechanisms including control of the array of leaves to light. Temperature perception is one aspect of the resistance of plants to environmental stresses.

79. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

DEVELOPMENTAL BIOLOGY OF NITROGEN-FIXING ALGAE — $137,000
Coleman P. Wolk
MSU/DOE Plant Research Laboratory

This task is concerned with the fixation of atmospheric nitrogen (N_2) by filamentous cyanobacteria (blue-green algae), and with the closely related phenomena of cellular interactions and differentiation in these organisms. Special cells called heterocysts fix N_2, in cooperation with nearby vegetative cells, inhibit vegetative cells from becoming heterocysts, and induce them to become spores. The following projects are currently pursued: (1) Mutants affected in nitrogen fixation, development, photosynthesis, and intermediary metabolism having been isolated, genetic transfer is being attempted. (2) We are elucidating the principal distinguishing biochemical characteristics of heterocysts and spores, including those characteristics of heterocysts which make them functionally dependent upon attachment to vegetative cells. (3) We are trying to identify the interactions by which vegetative cells supply reductant for N_2 fixation in heterocysts. This entire research is related to the problem that most crop plants depend upon supply of nitrogen fertilizers, the production of which requires substantial energy expenditures. The long-range goal is to provide nitrogen for plants by photosynthesis, possibly through symbiosis with N_2-fixing cyanobacteria, rather than by use of natural gas.

80. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

PHOTOSYNTHETIC PARTITIONING OF ASSIMILATES — $45,000
Jan A. D. Zeevaart
Hans Kende
MSU/DOE Plant Research Laboratory

This task is concerned with partitioning of assimilates. The purpose is to determine, in the bean plant, (i) the role of root cytokinins in the regulation of plant senescence; (ii) the role of hormones in the flow of assimilates from source to sink; (iii) the partitioning of assimilated carbon and nitrogen at different stages of development in plants grown under two different nitrogen regimes, namely, supplied with nitrate, or dependent on biological nitrogen fixation; (iv) the energy requirements for nitrogen metabolism, in particular the energy needed for biological nitrogen fixation. Thus, this task is related to the over-all program of the Plant Research Laboratory in that it is concerned with the conversion of solar energy into biomass; ultimately the results of this research may also help to design strategies that will make it possible to reduce the energy input into crop production in the form of chemical fertilizer.

81. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

ENVIRONMENTAL CONTROL OF PLANT DEVELOPMENT AND ITS RELATION TO HORMONES — $87,000
Jan A. D. Zeevaart
MSU/DOE Plant Research Laboratory

Environmental factors such as daylength, light intensity and quality, temperature and water deficits have pronounced effects on plant growth and development. The objective of this task is to study the role that hormones play as intermediaries between the perception of an environmental factor and the morphological manifestation in the following cases:

(a) Stem and leaf growth in rosette plants as regulated by gibberellins (GAs). The goal is to determine what kind of changes the photoperiod causes in the GA status that ultimately result in stem elongation.

(b) Wilting of plants which is due to a water deficit (at the cellular level: zero turgor), and is associated with accumulation of abscisic acid (ABA) and its metabolites, as well as closure of stomata. Conversely, relief of stress results in rapid degradation of excess ABA. The aim is to determine how ABA synthesis is enhanced in stressed leaves, and how ABA - the so-called stress hormone - reduces water loss from leaves.

(c) Flower formation is induced by the flower hormone. The nature of this hormone remains in question. The current approach is to compare the chemical composition of exudates from flowering and vegetative plants by physical-chemical methods.

Projects (a) and (c) are concerned with optimizing the conversion of solar energy into biomass, while (b) deals with adaptation of plants to environmental stress. Thus, all three projects are related to the over-all program of the Laboratory.

82. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

DIFFERENTIAL GENE EXPRESSION IN RHIZOBIUM — $63,000
Barry Chelm
MSU/DOE Plant Research Laboratory

The interaction of Rhizobium bacteria with a legume host to establish a symbiotic, nitrogen fixing relationship requires a series of developmental steps in both the bacterium and host; this task analyzes the pattern of gene expression in the bacterium which occurs during this process. The identity of promoter regions in the bacterial genome and detection of sequential patterns of bacterial gene expression during the nodulation process will be analyzed.
(Project to be initiated in late FY 81)

83. MICHIGAN STATE UNIVERSITY
East Lansing, Michigan 48824

INTERACTION OF NUCLEAR AND ORGANELLE GENOME — $64,000
Lee McIntosh
MSU/DOE Plant Research Laboratory

Research is conducted which will lead to the development of a model for nuclear/ organelle genome interactions. The transcriptional regulation of chloroplast genes during plastid development will be investigated by cloning genes which are under "light control". DNA sequences of promoter genes appearing before structural genes will be determined. Nuclear control of chloroplast gene expression will be studied in nuclear mutants of higher plants which are known to lack a chloroplast gene product.
(Project to be initiated in late FY 81)

84. SOLAR ENERGY RESEARCH INSTITUTE
Golden, Colorado 80401

BASIC PHOTOBIOLOGY RESEARCH $100,000
Michael Seibert
Paul Weaver
Stephen Lien

This proposal outlines a three-task research program in the general area of photobiological H_2 production. Task 1 will use microbiological, biochemical, and genetic techniques to identify the electron transport components and pathways associated with hydrogenase activity in photosynthetic bacteria. The ultimate goal is to determine the feasibility of obtaining an organism which evolves H_2 through a less energy intense, ammonia-insensitive hydrogenase reaction rather than through the currently exploited nitrogenase pathway. Task 2 will focus on the mechanism of hydrogenase activation during anaerobic incubation of algal cells and on the isolation and purification of algal hydrogenase to a state suitable for detailed biochemical and physical characterization. These studies will yield new information on the biochemical events leading to the induction of hydrogen metabolism in various hydrogenase-containing algae. A better understanding of the hydrogenase system will be needed to devise an efficient procedure for the isolation or construction (by mutational and genetic manipulation) of algal strain(s) capable of sustained H_2 evolution under oxygenic photosynthetic conditions. Task 3 will seek to understand the properties of bacterial chromatophores and reaction center complexes in monolayer and multilayer stacks. These precisely controllable assembles in conjunction with optical and electrochemical techniques will lead to improved understanding of primary photosynthetic processes and perhaps serve as biological model systems for H_2 production.

85. UNIVERSITY OF TENNESSEE
Comparative Animal Research Laboratory
1299 Bethel Valley Road
Oak Ridge, Tennessee 37830

PLANT BIOMASS AND ENERGY CONVERSION $220,000
M. J. Constantin
F. J. Ryan

A number of Wisconsin 38 tobacco cell lines that are able to grow in suspension cultures in the presence of s-aminoethylcysteine (s-AEC) will be characterized for lysine transport. Those whose lysine transport characteristics differ from that of the wild type lines will be placed on plant regeneration medium. Regenerated plants will be tested to determine their response to s-AEC, and selected ones will be hybridized with wild type plants for genetic characterization.

A systematic approach will be initiated to develop rapidly growing suspension cultures of Nicotiana sylvestris. As soon as we are able to grow suspension cultures satisfactorily, selection will be initiated for Adh^- mutant cell lines. The plan is then to regenerate plants that are Adh^- that can be used in a reversion assay where pollen is analyzed for Adh^+; i.e., results of a reverse mutation. Limited effort will be continued with Early-Early Synthetic Maize to determine the effective number of germline cells at risk during different developmental stages of the sporophyte. The Hordeum root tip assay will be used in comparison with Salmonella and Saccharomyces mutation system to complete the testing of aqueous extracts of solid wastes.

Changes in the concentrations of reduced glutathione and NADPH will be measured in stem tissue of paraquat-treated Virginia pine. Additional experiments will quantitate changes in the concentration of starch in paraquat-treated tissue and will correlate these changes with oleoresin synthesis. The data from these experiments will provide information on the flow of carbon and changes in the pools of reducing equivalents during paraquat-induced oleoresin synthesis. In addition to elucidating this important biochemical phenomenon, this information may be useful in establishing criteria for the selection and breeding programs aimed at optimizing the production of oleoresin.

INDEX OF INVESTIGATORS

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