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Annual Report and Summaries of FY 1985 Activities Supported by the Division of Biological Energy Research

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U.S. Department of Energy
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Division of Biological Energy Research

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Office of Energy Research
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Division of Biological Energy Research
Washington, D.C. 20545

PROGRAM OVERVIEW

The Biological Energy Research (BER) program of the Office of Basic Energy Sciences is devoted to discovering and describing biological mechanisms that might be employed as the basis of future energy-related biotechnologies. Clearly, the study of green plants as producers of renewable resources is a key aspect of the BER program. Plant productivity encompasses a broad array of processes that are integrated to form a complex whole, the producing plant. The comprehension of these component processes and their regulation is one of the major objectives of the program. Another major aspect of the BER program examines that portion of the microbiological world involved with conversions that yield fuels and/or chemicals. These bioconversion processes can provide a substitute for fossil energy resources directly or through energy conserving measures in which biological mechanisms displace industrial energy input requirements (e.g., biological nitrogen fixation, recovery of key resources from dilute concentrations, and conversion of noxious waste products to usable materials [carbon monoxide to organic acids]). In each instance, the research is aimed at understanding the fundamental mechanisms of conversion rather than a description or optimization of a process.

Topic areas covered in the plant science segment of the BER program include:

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

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

1. *Metabolic Regulation,* the mechanisms by which the pathways are controlled directly by affecting the levels and/or activity of the enzymes involved, e.g., feed-back inhibition, and enzyme modification.
2. *Genetic Regulation,* the mechanisms by which the genetic material controls the modulation of a pathway by turning it on or off, and other physiological genetic control mechanisms.
3. *Hormonal and Environmental Regulation,* the mechanisms by which plant growth substances and external signals (e.g., light) affect the control of activities in cells or organs and ultimately the whole plant.

C. **Stress Physiology and Adaptation,** reflecting the influence of environmental conditions on how effective and extensive the conversion of solar energy by plants can be.

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

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

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

The microbiological segment of the BER program emphasizes a number of research areas including:

G. **Lignocellulose Degradative Mechanisms.** Comprises studies on the genetic and biochemical control of

the polysaccharide-degrading enzymes and the breakdown of lignin components. These areas are aimed at understanding and eventually being able to control the microbial degradation of these most abundant renewable materials.

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

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

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

K. The molecular basis of **thermophily** or high temperature tolerance is also included.

L. **Microbial Ecology,** includes microbe–microbe interactions that are crucial to fermentations and other biotransformations as well as some microbiological interactions with plants.

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

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

Within the program, there are representative projects that generate results in any one year that attract more than usual attention and which reflect the nature of the research supported. Several of these results are mentioned below:

- A discovery has been made that the pigment, phytochrome, which is important in many photomorphogenetic developments in plants, occurs as different protein forms when the plants from which the pigment is prepared are grown with or without light. Most earlier studies had been done with pigment prepared from etiolated (dark grown) plants, an atypical growth situation.

- An analytical result from studying woody plant cells with raman microprobe spectroscopy showed that the lignin of cell walls occurs in a planar orientation (aromatic ring axis parallel to walls). Although orientation of the cellulosic portion of the walls has been known, the finding that lignin is highly ordered is a new insight.

- The pathways by which methane is produced from acetate or CO_2 in methanogens recently has been suggested to involve a cyclical set of reactions with a number of different cofactors. While not all of the factors have been identified, the scheme is a hypothesis consistent with the available data and a significant contribution to understanding the metabolism of methanogenic bacteria.

- A genetic manipulation system for the fungal plant pathogen *Pyricularia oryzae* has been developed. This achievement opens the way to studying host–pathogen relationships with fungal pathogens not previously possible using genetic approaches. This development should now permit a more rapid comprehension of recognition phenomena and the basis of resistance-susceptibility responses.

These research areas conform to BER interests in terms of understanding plant productivity and its control (photomorphogenesis, in this case), the chemical nature of the products (lignin orientation in cell walls), mechanisms of microbial bioconversions (as in methanogenesis), and lastly, plant pathogenesis as a process by which the productivity of plants is diminished in terms of efficiency in use of fertilizer and irrigation energy.

As an endeavor, science is a dynamic entity with ever changing horizons. The BER program, to keep itself at the forefront of technical development, uses workshops, and other conferences for identifying critical research related to its mission as well as to encourage dissemination and discussion of results. During FY 1985 BER provided support for the activities indicated in the following list:

Conferences and Other Activities Supported in FY 1985

1. *University of California, Riverside*—Partial support for the 8th Annual Symposium in Plant Physiology, "Regulation of Carbohydrate Partitioning in Photosynthetic Tissue," January 1985, Riverside, CA. (Proceedings published by the American Society for Plant Physiologists 1985.)

2. *University of Missouri*—Partial support for "Symposium on Current Topics Biochemistry and Physiology," April 1985, University of Missouri, Columbia. (Proceedings to be published.)

3. *University of California, Los Angeles*—Partial support for a Symposium on Molecular and Cellular Biology entitled "Plant Genetics," April 13–19, 1985, Keystone, CO. (Proceedings to be published.)

4. *American Society for Microbiology*—Partial support of Conference on "Engineered Organisms in the Environment: Scientific Issues," June 10–13, 1985, Philadelphia, Pa. (Proceedings to be published by the American Society for Microbiology.)

5. *Cornell University*—Partial support for symposium on "Biotechnology in Plant Science: Relevance to Agriculture in the Eighties," June 23–27, 1985, Cornell University, Ithaca, NY. (Proceedings to be published by Academic Press, Inc.)

6. *University of Georgia*—Partial support of a "Workshop on Microbial Interspecific Hydrogen Transfer," July 29–31, 1985, Woods Hole, Massachusetts. (Summary to be published.)

7. *University of California*—Partial support for "International Phloem Transport Conference," August 18–23, 1985, Pacific Grove, California. (Proceedings to be published.)

8. *Oak Ridge National Laboratory*—Partial support for symposium on "Coupling of Carbon, Water and Nutrient Interactions in Woody Plant Soil Systems," October 6–11, 1985, Knoxville, TN. (Proceedings to be published.)

9. *Boyce Thompson Institute*—Partial support for a "Conference on Anhydrous Biology," October 21–25, 1985, Lake Como, Italy. (Publication planned.)

10. *International Society for Plant Molecular Biology*—Partial support for "1st International Congress of Plant Molecular Biology," October 27–November 2, 1985, Savannah, Georgia.

11. *Michigan State University*—Partial support for conference on "Crop Productivity—Research Imperatives Revisited," October 14–18, 1985, Boyne Highlands, Michigan. (Conclusions to be published.)

12. *University of Illinois*—Partial support of conference on "Exploitation of Physiological and Genetic Variability to Enhance Crop Productivity," October 25–26, 1984, University of Illinois, Urbana. (Proceedings published by the American Society of Plant Physiologists 1985.)

13. *National Academy of Sciences*—Partial support for the preparation of a Guide for the Safe Handling and Disposal of Hazardous Biological Materials in Laboratories. (To be published by National Academy of Sciences.)

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

In recognition of the technical difficulties in culturing fastidious anaerobic microorganisms and the relatively small research community dealing with some of the organisms of interest, the BER program has provided partial support of a research-training session at the Marine Biological Laboratory in Woods Hole, Massachusetts, in the summer of 1985. The session was keyed to problems associated with handling anaerobic microorganisms.

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

Thus the BER program perception is that there is great need to achieve better understandings of the genetic basis of biochemical and physiological processes. Increasing emphasis on biochemical studies aimed at defining regulatory mechanisms in plants and microorganisms is going to be critical in the coming years. More preparation to deal with biochemical phenomena must be factored into training curricula. Of consider-

able importance also will be efforts to encourage the utilization of the many powerful analytical techniques of chemistry in approaching difficult biological problems (e.g., NMR spectroscopy, molecular structural studies using neutron and x-ray diffraction of crystalline biomolecules, and others). The implication is that much more interdisciplinary research activity is requisite in such approaches. All of this, of course, should proceed in parallel with the rapid progress in molecular genetics.

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

	Number of projects	FY 85 funding (in thousands of $)	Percent of total funds
University Contracts and Grants	88	7,105	57%
Michigan State University Plant Research Laboratory	11	1,800	15%
National Laboratories Brookhaven National Lab Lawrence Berkeley Lab Oak Ridge National Lab Los Alamos National Lab	13	2,595	21%
Solar Energy Research Institute	1	110	1%
Other research institutions (federal, state, industrial nonprofit)	8	502	4%
Conferences & miscellaneous	16	249	2%
	137	12,361	

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

In FY 1985 some 89 new proposals were received to be considered for funding. Some 14 new awards were made over and above on-going projects. There were numerous meritorious proposals that could not be funded because of limited resources. All projects, both new and on-going, are subjected to some form of peer review (mail reviews, panel reviews, site visit reviews) on a regular basis.

It is a matter of considerable importance to the BER program to acknowledge the most valuable cooperation and assistance of several hundred scientists, both in this country and abroad, who generously provided their time and expertise in the review of projects and proposals. To say that much of the strength of the U. S. federal system of research support derives from such participation is not an overstatement.

The BER technical program staff was increased 100% this year with the addition of Dr. Gregory L. Dilworth. It is our expectation that the staff addition will further enhance responsiveness to the research community.

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

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

Contents

ANNUAL REPORT AND SUMMARIES OF FY 1985 ACTIVITIES SUPPORTED BY THE DIVISION OF BIOLOGICAL ENERGY RESEARCH

Arizona State University
Tempe, Arizona 85287

1. *Antenna Organization in Green Photosynthetic Bacteria*
R.E. Blankenship — **$88,600**
Department of Chemistry

The photosynthetic unit of all chlorophyll-based photosynthetic organisms consists of a collection of pigments that act as an antenna, absorbing light and transferring the energy to a reaction center, where energy storage takes place by a series of chemical reactions. The green photosynthetic bacteria have an unusually large photosynthetic unit of up to 2000 Chl/reaction center. The majority of the antenna pigment molecules are contained in chlorosomes, ellipsoidal vesicles attached to the underside of the cell membrane. Additional antenna pigments and reaction centers are contained in integral membrane proteins. The project objective is to determine the molecular organization and the mechanism of excitation transfer in the antenna system of green photosynthetic bacteria. The principal strategy involves isolation of the antenna system, biochemical resolution into its constituent subassemblies, and characterization using absorption and both steady-state and transient fluorescence spectroscopies. Results have identified a sequence of at least four distinct pigment species that transfer energy from the chlorosome into the membrane and eventually to the reaction center. The 740 nm-absorbing bacteriochlorophyll *c* that is the main pigment in the chlorosome has an extremely short (< 30 ps) fluorescence lifetime. Fluorescence excitation spectra indicate that the excitation is efficiently transferred to the membrane. Model compound studies, along with a recently-published analysis of the sequence of the BChl *c*-binding peptide indicate that the pigments are probably organized by pigment–protein and direct pigment–pigment interactions into what are essentially pigment oligomers. Future work will test this model and will be aimed at understanding the factors that enable this system to avoid the efficiency loss mechanism (presumably by electron transfer quenching) observed in chlorophyll aggregates *in vitro*.

Battelle–C.F. Kettering Research Laboratory
Yellow Springs, Ohio 45387

2. *Basis of Competitiveness of Rhizobia*
W.D. Bauer, W.R. Evans — **$79,000**

Gram negative soil bacteria of the genus *Rhizobium* are able to infect and nodulate specific legumes and fix atmospheric nitrogen for their host. To improve the agricultural benefit of this symbiosis, it is necessary to get the best available strains of rhizobia to form most of the nodules on host plants in the field. It has not been possible to achieve this consistently because inoculated strains of rhizobia do not colonize and infect roots of seedlings sufficiently well to compete against the indigenous rhizobia. The project objective is to determine what factors contribute most importantly to the competitive ability of inoculated rhizobia. Recent studies have established that the relative competitiveness of two strains in mixed inocula can be predicted from their efficiencies in independent inoculum dose-response measurements. Such efficiencies, measured as the number of cells required to generate a given number of nodules in a certain region of the host root, were found to vary with culture age and medium. Dose-response studies of infection number and development indicate that infection number is proportional to the log of the inoculum dose, but that nodule number decreases above a certain dosage. The role of bacterial attachment in root colonization and competitiveness is being examined in detail. Recent studies have confirmed that only a small subpopulation of *Rhizobium* cells in a culture is capable of loose attachment to roots and that an even smaller subpopulation is capable of firm attachment. Transposon mutants with both diminished and increased attachment capabilities have been isolated and are being tested. In addition, a field isolate with diminished attachment capabilites had been tested and found to lack detectable pili, to colonize the root poorly, and to compete poorly against strains that attach well.

Boyce Thompson Institute
Ithaca, New York 14853

3. Carbon Metabolism in Legume Nodules
T.A. LaRue **$44,000**

Symbiotic nitrogen fixation in legume nodules consumes more photosynthate than can be accounted for by the known energy requirements for nitrogenase. The object of our research is to determine how the legume nodule metabolizes carbohyrate to provide energy and reductant for nitrogen fixation. Because the plant cells within the nodule contain very little free oxygen, we are investigating the possible roles of anaerobic pathways of carbohydrate metabolism. When mitochondria are isolated from nodules and incubated in an aerobic system, they behave like mitochondria isolated from other plant parts (i.e., they take up oxygen when using succinate or malate). They produce ATP, and have a P/O ratio of about 2.5. However, when the mitochondria from nodules are incubated at the microaerobic conditions that exist in the nodule, their oxygen uptake activity is reduced 10-fold, and the P/O ratio falls below one (i.e., they are producing little energy). We are examining how the mitochondria use the many carbon substrates available in the nodule, and we will identify the ATP-generating mechanisms used under microaerobic conditions.

Brandeis University
Waltham, Massachusetts 02154

4. Carbon and Hydrogen Metabolism of Green Algae in Light and Dark
M. Gibbs **$57,000**
Institute for Photobiology of Cells and Organelles

A major goal of this project is to evaluate fermentative metabolism in eukaryotic green algae adapted anaerobically to a hydrogen metabolism. The pattern of starch fermentation (mole per mole glucose in starch consumed) in light vs. dark is: ethanol 0.1, 0.9; acetate 0.0, 1.1; formate 0.9, 2.1; CO_2 2.5, 0; and H_2 6.9, 0.4. Acetate is formed when an uncoupler is present indicating reutilization. Fermentation of exogenous acetate does not occur in the dark. In the light, the stoichiometry of CO_2 and H_2 per mole of acetate is indicative of a glyoxylate cycle supported by the presence of soluble malate synthase and citritase and inhibition of gas evolution by fluoroacetate. About 80% of the radioactivity from 2-^{14}C-acetate under N_2 is found equally divided between lipid and starch; a distribution not affected by the presence of DCMU indicating that acetate is not only the source of carbon but also of reductant. An atmosphere of H_2 changes the ratio favoring lipid synthesis. Isotopic analysis indicated the presence of a low level of pyruvate synthase. Enzymic profile studies have been initiated to determine the cellular location of the fermentative sequences since the glyoxylate cycle is not particulate. Photosynthesis and photoreduction (H_2, CO_2) in the isolated chloroplast appear to react differently to inorganic phosphate, sugar phosphates, and inhibitors of electron flow. The algal thylakoids catalyze O_2 uptake with NADH as donor, a process which may provide ATP for the dark oxyhydrogen reaction. *Chlorella vulgaris* ferments its starch and glucose to lactate (1.75 mole per mole) with smaller amounts of CO_2 and ethanol. Current work evaluates the importance of chloroplast respiration during fermentation and respiration of starch and of exogenous glucose in *Chlorella*.

Brookhaven National Laboratory
Upton, Long Island, New York 11973

Biology Department

5. Chlorophyll–Protein Complexes: Photoregulation of Transcription, Stability, and Phosphorylation
J. Bennett **$220,000**

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

6. Plant Molecular Genetics
B. Burr, F.A. Burr **$240,000**

The appearance of mutations in plants regenerated from tissue cultures has generated hope among plant breeders for a new method of inducing useful mutations. We have used the maize plant to measure the rate of mutation and examine their nature at the DNA level. At the *Sh1* locus, mutation increases ten-fold over the spontaneous rate when regenerated plants are used as pollen parents. Five mutant alleles have been cloned and compared with progenitor alleles by restriction mapping. No changes were observed at this level; we assume that some sort of point mutations have occurred. Two spontaneously-occurring mutations contain rearrangements. Due to the very small size of the mutant sectors on regenerated plants, we assume that the majority of mutations occur after the plant has been removed from tissue culture. This work is being extended to other loci. Attempts are being made to isolate and characterize two regula-

tory genes from maize. The *02* gene appears to be a positive transcriptional regulator of zein gene storage protein synthesis. Presumed *Spm*- and *Ac*-induced mutations of the gene have been made, and these transposable elements are being used as molecular tags to isolate the gene. The *C1* gene is required for the expression of enzymes in the last stages of anthocyanin pigment biosynthesis. *Spm*- and *Sc*-induced mutations of the gene exist, and these elements are being used as tags to clone the gene. Structural genes controlled by both of these regulatory genes have already been cloned so that the interaction of regulatory gene product with target DNA can be examined.

7. *Mechanisms of Energy Conversion in Photosynthesis*

G. Hind **$300,000**

The project goal is to discover how energy is transformed from electron potential to chemiosmotic potential in photosynthetic systems. Processes involving cyclic electron transport are of prime concern and are studied in (1) intact chloroplasts of the C-3 plant, *Spinacia oleracea*, (2) bundle sheath cells of the C-4 plant, *Zea mays*, and (3) isolated heterocysts from the filamentous cyanobacterium, *Anabaena 7120*. In these systems, electron flow from reduced plastoquinone to the photochemically-generated oxidant, $P700^+$, is mediated by a cytochrome *b–f* complex. The polypeptide compositions of complexes from these organisms are being compared and their activities *in situ* or following detergent extraction are being studied using flash, steady-state, and low-temperature spectrophotometry. In cyclic flow, electrons return to the cytochrome complex via ferredoxin-$NADP^+$ reductase, which is non-covalently bound to its stromal surface. The structures of the complexes and binding of reductase are studied using cross-linking reagents. The protein kinase activity of reductase is being evaluated for its possible *in vivo* role in state transitions, which control apportionment of excitation energy, from light-harvesting chlorophyll, between photosystems. Passage of electrons through the complex is coupled to vectorial H^+ transport, possibly by a form of Q-cycle (as postulated by Mitchell). The stoichiometry of this coupling and its dependence on ambient redox poise are being studied to elucidate the coupling mechanism. These investigations will provide knowledge of the factors limiting photosynthetic reduction of CO_2 and N_2. In showing how the cytochrome complex serves as an efficient energy transformer, they are also of presumed relevance to future design of biomimetic energy conversion devices.

8. *Analysis and Uses of T-DNA*

D. Sciaky **$140,000**

Analysis of T-DNA has aided in the construction of plant-cloning vectors for genetic engineering in plants. T-DNA is used in this laboratory in two different ways. We are studying what effect the distance and the orientation of the direct repeats flanking T-DNA have on the insertion of foreign DNA into the plant genome. We are generating random insertion events of the right direct repeat flanked by the transformation markers, octopine and nopaline, of pTiA6 into a plasmid that contains a fixed left direct repeat of pTiA6. These insertions will be mapped and the plasmids used to transform *N. plumbaginifolia*. Transformation frequency will be correlated with the presence of the opines. We are also using T-DNA to aid in understanding the molecular mechanism of somaclonal variation in plants. A kanamycin resistance gene expressed in plants is being modified in a specific manner so that it no longer is capable of conferring kan^R in plants. Using cloning vectors constructed in this laboratory, we will transfer these modified genes into *N. plumbaginifolia*, and (after several generations) place mesophyll protoplasts under the conditions of somaclonal variation and select for kan^R cells. We will then examine the kan gene of these resistant cells and determine what sort of mutation led to the expression of kan^R.

9. *The Physiology and Biochemistry of Cyanobacteria*

H.W. Siegelman **$200,000**

Cyanobacteria are cosmopolitan organisms of soils, marine, and freshwaters. They are frequently responsible for water blooms and can cause serious water management problems in rivers, lakes, and reservoirs. The success of cyanobacteria in nature is probably associated with an efficient photosynthetic mechanism, frequent possession of gas vacuoles permitting vertical migration in the water column, and ready adaptability to varying environmental factors. The molecular structure and composition of the photosynthetic energy collection systems including phycobilisomes, chlorophyll proteins, and carotenoids are being characterized in several cyanobacterial species. We examine the steady-state photoadaptation of selected cyanobacteria to light intensity and quality. Cell division rates are closely related to the total amount of light energy received and apparently not light quality dependent. The phycobilisome composition of these cyanobacteria is strongly regulated by light intensity and quality. Phycobilisomes from these cells are being purified by hydrophobic-interaction chromatography; their mass and structure are being characterized by electron microscopy, gel permeation, and absorbance spectroscopy. HPLC and diode-array spectrophotometry are used to characterize phycobiliprotein stoichiometry of the phycobilisomes. Antibodies to the phycobiliproteins are raised to initiate a molecular biology examination of the control mechanisms regulating phycobilisome composition.

University of California
Berkeley, California 94720

10. *Transcription Control Elements and Manipulation of Chloroplast Genes*

W. Gruissem **$70,000**
Department of Botany

Attempts to change traits in crop plants by introducing desirable genes into plant cells or by transformation of chloroplasts require understanding (1) why genes are expressed only at certain developmental stages or levels and (2) factors controlling and components involved in their expression. This project focuses on the molecular mechanism of chloroplast gene expression. Much speculation has occurred regarding the promoter sequences required for transcription initiation of plastid genes by RNA polymerase(s). DNA sequences for several chloroplast genes are available, and comparison of 5′ flanking regions in higher plant chloroplast genomes has revealed DNA sequences with good or partial homology to prokaryotic promoter elements. We have developed a spinach chloroplast *in vitro* transcription system, which allows the more detailed characterization of chloroplast promoter sequences. The genes for which

promoter and regulatory DNA regions are currently being examined include several tRNA genes (trnM2, trnVl, trnRl, trnHl, and trnSl), and the genes for the 32 kilodalton polypeptide of photosystem II (psbA), the β-subunit of the ATPase complex (atpB), and the large subunit of ribulose-1,5-bisphosphate carboxylase (rbcL). The double-stranded exonuclease Ba131 is used to establish the 5′ boundaries of promoter elements. The 5′ upstream regions are then restored with synthetic oligonucleotides. This allows the introduction of any desired mutations into DNA sequences, which can be assayed for their effect on transcription. We will attempt to further characterize the different RNA polymerase activities involved in the expression of ribosomal RNA, tRNA, and protein-coding genes. Our understanding of gene regulatory processes in chloroplast should be helpful for evaluating future attempts to manipulate and optimize the photosynthetic apparatus.

11. *The Regulation of Enzyme Secretion in Plant Cells by Calcium*

R.L. Jones **$94,500**
Department of Botany

The synthesis of barley α-amylase isoenzymes is controlled in part by both gibberellic acid (GA) and Ca^{2+}. Four isoenzymes of α-amylase (numbered 1 to 4) can be characterized by agar gel electrophoresis. These fall into two distinct immunological groups designated A (isoenzymes 1 and 2) and B (isoenzymes 3 and 4). Isoenzyme 2 is synthesized under all conditions of aleurone incubation, while isoenzyme 1 is synthesized only when GA is present. The synthesis of isoenzymes 3 and 4 requires the presence of both GA and Ca^{2+}. We have prepared a cDNA clone complementary to RNAs coding for B-group α-amylases and are using it to determine whether the expression of the genes controlling the synthesis of α-amylase isoenzymes requires GA, Ca^{2+}, or both. We will also establish whether GA or Ca^{2+} influence the turnover of these mRNAs. Work is in progress to prepare monoclonal antibodies to all four α-amylase isoenzymes at the translational level. We are studying the intracellular route of α-amylase secretion in aleurone layers incubated in medium containing the ionophore monensin. Monensin inhibits the secretion of α-amylase isoenzymes 3 and 4 in particular and causes these enzymes to accumulate in the cells of the aleurone layer. We will investigate the possibility that monensin inhibits α-amylase secretion by interfering with the glycosylation of these proteins. Our studies on the metabolism of Ca^{2+} will focus on the localization of this and other ions using x-ray microanalysis and metallochromic dyes.

12. *Heterozygosity, Growth Rate, and Stability Among Inbred and Crossbred Trees of Knobcone Pine*

S.H. Strauss, W.J. Libby **$29,200**
Department of Forestry and Resource Management

The influence of the mating system on expression of heterozygote superiority is important to understanding its causes, but it has received limited attention. Analysis of the relation of heterozygosity to stability for fitness components has often produced conflicting results. This project will elucidate these issues by examining how inbreeding, crossbreeding, and climatic variability affect heterosis at allozyme loci. The relationships of individual heterozygosity to growth rate and reproduction were studied in 10-year-old trees of *Pinus attenuata* Lemm. that were the products of controlled self- and cross-pollination. Heterozygosity was determined at 25 polymorphic allozyme loci and related to the rate of trunk growth, cone production, and the degree of climatically-induced variability in annual growth. Within the crossbred progeny, heterozygosity was positively related to trunk growth, but negatively correlated with cone production. Within the inbred progeny, heterozygosity was correlated with only the rate of trunk growth. The crossbreds showed substantially greater responsiveness to climate, whether measured on absolute or relative scales. Within the inbred and crossbred groups, there was no significant relationship of heterozygosity and stability, though the more heterozygous trees showed slightly lower stability. These results suggest the following: (1) allozyme heterozygosity may be a useful predictor of growth rate in crossbred populations; (2) the very weak, though statistically significant, strength of allozyme-related heterosis within the inbreds (where about 10% of the genome was marked for heterozygosity) suggests that single-locus overdominance would be exceedingly difficult to detect in natural populations; and (3) greater responsiveness of the more heterozygous individuals to some kinds of environmental variation should be viewed as a manifestation of, rather than a departure from, heterosis.

University of California
Davis, California 95616

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

R.W. Breidenbach, **$60,000**
D.W. Rains, M.J. Saxton
Plant Growth Laboratory

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

14. *Restriction of Virus Infection by Plants*

G. Bruening **$73,000**
Department of Plant Pathology

The productivity of a particular cultivar, in terms of biomass, food and/or fiber, often is limited by the action of plant patho-

gens. An obvious and direct approach to limiting the deleterious effects of a pathogen is to develop a cultivar that is tolerant or resistant to the pathogen. Although sources of resistance to viruses are widely used by plant breeders, they often are not available for economically important viruses, and very little is known about the biochemical mechanisms by which a plant can restrict the replication of a plant virus. The objective of this research is to identify the mechanisms by which certain cowpeas resist cowpea mosaic virus (CPMV) and to isolate and analyze the corresponding resistance gene(s). A long-term goal is to engineer (in plants) resistance to plant viruses. Previously we have obtained evidence for a resistance mechanism in which one line of cowpea produces an inhibitor that specifically inhibits a proteinase. The proteinase is CPMV-specified, essential to CPMV replication, and difficult to assay. The proteinase inhibitor activity has been recovered from cowpea protoplasts. We now have improved the proteinase assay and are using it to detect the inhibitor in extracts of cowpea leaves. We will continue our efforts to purify the proteinase inhibitor to begin a search for its gene. Preliminary tests of another possible mechanism of resistance to CPMV also are in progress. Understanding these mechanisms will allow resistance to virus infection to be more widely available in cultivars as an economical and ecologically-sound plant virus control measure.

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

J.L. Ingraham **$58,000**
Department of Bacteriology

Denitrification is the biological process by which certain bacteria reduce fixed nitrogen in the form of nitrate or nitrite ion to the gaseous species N_2O or N_2 and thereby deplete the available nitrogen in terrestrial or aquatic environments. Many aspects of this process, including the proteins that catalyze it and the mechanism by which expression of denitrification genes is controlled, remain to be elucidated. We study these aspects using the techniques of physiological genetics. Mutant strains of *Pseudomonas stutzeri* (an active denitrifier capable of natural genetic transformation) that are blocked in various steps of the process will be isolated and characterized. Emphasis will be on obtaining mutants blocked in the last two steps of the process: the reduction of nitrite ion and nitrous oxide gas. Mutagenesis will be accomplished by inserting transposons into the chromosome of *P. stutzeri* using a Tn10-carrying suicide plasmid we constructed and one carrying Tn5 constructed by Puhler. Specific denitrification genes will be isolated by complementing mutant strains with recombinant plasmids from a gene bank of the *P. stutzeri* chromosome, which we have constructed in *Escherichia coli*. These plasmids can be mobilized at high frequency into mutant strains of *P. stutzeri* using a triparental mating system. Identification of complementing plasmids will provide information about the relative location of denitrification-encoding genes on the chromosome of *P. stutzeri*. Another objective is to obtain fusions between denitrification promoters and β-galactosidase. Such strains should be valuable tools for studying the control of expression of denitrification genes (temporally and in response to environmental factors) due to the much simpler assay of β-galactosidase as compared with the assay of proteins effecting denitrification.

University of California
Irvine, California 92717

16. *Bioenergetics of Salt Tolerance*

J.K. Lanyi **$148,000**
Department of Physiology and Biophysics
and
L. Packer
University of California, Berkeley, CA
Department of Physiology and Anatomy

The bioenergetic aspects of cellular salt tolerance include ion transport across membranes, redirection of metabolic pathways for the increased synthesis of osmoregulatory compounds, replacement of some salt-sensitive components with salt-resistant ones, modification of the photosynthetic apparatus for increased efficiency, and the signals that turn these processes on and off during salt stress. We have developed and adapted methods to study these phenomena in the extreme halophile, *Halobacterium halobium*, and in the facultative halophiles cyanobacterium *Synechococcus* 6301 and Ba-1 from the Dead Sea. In *Synechococcus* we explore the membraneous and cytoplasmic events that occur in sequence during short-term adaptation to salt. In the thylakoids of this organism we are attempting to describe features of the photosynthetic apparatus that are changed by salt stress. In the halophilic bacteria, as well as in *Synechococcus*, we are establishing pathways and mechanisms of cation and anion transport, which result in the characteristically asymmetric distribution of ions (and salt) across the cytoplasmic membrane. Results with these systems so far have yielded interesting and promising results, particularly in suggesting general mechanisms shared by halotolerant cells. Using this broad approach, we intend to develop the conceptual basis for a refined description of the physiology of cellular adaptation to salinity.

University of California
Los Angeles, California 90024

17. *Energy Capture and Use in Plants and Bacteria*

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

Our studies focus on the mechanism of formation of ATP by plants and bacteria as catalyzed by the membrane-bound ATP synthase complex. Hypotheses under study are: (1) energy serves to promote the binding of ADP and P_i and release of ATP by the synthase, (2) multiple catalytic subunits on the enzyme participate sequentially with prominent catalytic cooperativity, and (3) during catalysis there is a rotational change in position of the catalytic subunits in relation to a noncatalytic core. With the purified chloroplast F_1 ATPase, these hypotheses are being explored by derivitization with the analog 2-azido-ATP and with dicyclohexylcarbodiimide (DCCD) using isoelectric focusing to identify labeled β subunits. Preliminary experiments with these probes, indicating that catalytic turnover causes change in positions of β subunits on the enzyme complex, will be explored further. We expect to develop cleavable crosslinking reagents that will tether the γ subunit to a catalytic β subunit. If rotational catalysis occurs, this should block such catalysis, but catalytic capacity may be regained when the crosslinker is cleaved. In another approach, individual β subunits are being

labeled with fluorescent probes to find if catalysis changes the properties of the probe on the β subunit and its steric relation with probes on other subunits. Studies with *E. coli* enzyme will include tests of possible ATP modulation of oxygen exchanges to assess catalytic cooperativity between subunits, use of mutant forms of the enzyme, or specific chemical modification (together with dissociation and reconstitution) to probe subunit function.

18. *Methanogenesis from Acetate: A Key Intermediate in Nature*

R.A. Mah **$76,000**
School of Public Health

Complete biological degradation of organic matter yields methane and carbon dioxide as terminal products. This conversion is accomplished by several groups of interacting bacteria, including methanogens. Acetate is an intermediate in two-thirds of the methane produced. The project objective is to study organisms that decarboxylate acetate to produce methane. We are also searching for new acetate-degrading bacteria by examining unusual environments such as sediments of lakes with high salt concentrations or pH. We have not yet found acetate-degrading bacteria under such conditions, although we have found other unique methylotrophic and hydrogen-using methanogens, which are being characterized. One of these isolates, an alkalinophilic, H_2-oxidizing methanogen, was obtained from the Wadi el Natrun in Egypt. The cells of this isolate were rod-shaped, stained gram negative, and grew only on H_2 and CO_2 as substrate; trimethylamine, methanol, acetate, and formate were not methanogenic substrates. Its most distinctive characteristic is the broad pH range of 7.6 to 9.7 which supported growth; a pH range of 8.1 to 9.1 was required for optimum growth. Growth occurred between 25 and 37°C, with optimum growth occurring at 37°C.

University of California
Riverside, California 92521

19. *Catalytic Mechanism of Hydrogenase from Aerobic N_2-Fixing Microorganisms*

D.J. Arp **$47,000**
Department of Biochemistry

This project aims at elucidating the catalytic mechanism of hydrogenase from aerobic N_2-fixing organisms. This enzyme efficiently recycles the H_2 evolved by nitrogenase. Several properties of these hydrogenases make them ideal to function in an environment in which all of the available substrate is generated *in situ* (e.g., a very low rate of the back reaction, hydrogen evolution, and a low K_m for H_2). We are particularly interested in the enzyme from *Rhizobium*-induced root nodules because of the potential for increased legume productivity in symbioses expressing this enzyme. The specific goals of the project are: (1) to determine the mechanism of the isotope exchange reaction of rhizobial hydrogenase; (2) to investigate rhizobial hydrogenase-catalyzed para-H_2 to ortho-H_2 conversion; (3) to purify and characterize component 559-H_2 from soybean bacteroids and investigate its ability to couple and form a complex with rhizobial hydrogenase; and (4) to purify and characterize the hydrogenase of *Azotobacter vinelandii*. Hydrogenase is of particular interest because it is one of a limited number of enzymes that contain nickel. The project will provide insight into the mechanism of hydrogen uptake by rhizobial and *Azotobacter* hydrogenase that, in turn, will lead to a better understanding of H_2 cycling in these organisms. This information is important to genetic engineering of new strains and may lead to improved techniques for selecting the best strains of *Rhizobium* for inoculation.

University of California
Santa Cruz, California 95064

20. *Tonoplast Transport and Salt-Tolerance in Plants*

L. Taiz **$94,700**
Department of Biology

One of the distinguishing features of salt-tolerant plants is their ability to take up large quantities of sodium chloride into vacuoles. Recent investigations with isolated membranes have shown that ion transport across the tonoplast is driven by electrogenic proton-pumping ATPases. The objective of this project is to determine whether the tonoplast proton pumps of halophytes differ qualitatively or quantitatively from those of glycophytes. We have been characterizing the effect of sodium chloride on the activity of the tonoplast proton pump of maize, a glycophyte. Maize roots are divided into three separate regions: tip, cortex, and stele. After homogenization the membrane fractions are separated on sucrose density gradients and used for *in vitro* proton transport. Our results indicate that 100 mM sodium chloride causes a 3- to 4-fold increase in the activity of the tonoplast ATP-driven proton pumps in the tip and stele region of maize roots. There is also an increase in Golgi proton pump activity, which may contribute to the vacuolation process. We will investigate the mechanism of this salt stimulation, since it appears to play a central role in salt adaptation in maize. The hybrid maize variety is being compared with a moderately salt-resistant strain of maize, blue corn, as well as a halophytic grass, *Distichlis*. Antibodies to the tonoplast ATPase have been prepared that can be used to isolate, characterize, and quantify the enzyme. These studies may indicate what factors set the upper limit on salt accumulation in halophytes and glycophytes.

University of California/San Diego
La Jolla, California 92093

21. *Identification and Manipulation of* Rhizobium *Phytohormone Genes*

G. Ditta **$72,000**
Department of Biology

Nodule development during *Rhizobium*–legume symbiosis involves specific changes in the growth pattern of root cortical cells. It has been known for many years that *Rhizobium* species can produce both auxin (indoleacetic acid; IAA) and cytokinin during vegetative (asymbiotic) growth, but it is not known whether phytohormones produced by the invading bacteria play a role in this process. The project objectives are (1) to investigate the endogenous pattern of IAA production by *Rhizobium meliloti* (the species that infects alfalfa) and (2) to manipulate elevated levels of auxin and cytokinin production by this organism during symbiosis. Preliminary experiments have

established that *R. meliloti* has one or more aromatic amino acid transferases capable of converting tryptophan to indole pyruvic acid, an IAA precursor. We will obtain mutants defective in such enzymes to assay their symbiotic phenotypes. Since bacteria frequently have several aromatic amino transferases with overlapping specificities, multiple mutations may be necessary to completely eliminate IAA production by *R. meliloti*. An alternative way of investigating symbiotic effects of bacterially-produced phytohormones is to have *R. meliloti* overproduce these substances. For this purpose, we are constructing plasmids that place auxin and cytokinin biosynthetic genes derived from other organisms under the control of known *R. meliloti* symbiotic promoters. Changes in the pattern of symbiosis should reveal aspects of development that are hormone-regulated.

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

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

S.L. Dellaporta — **$75,000**
Banbury Center

We will extend our characterization of the *R* locus by a series of molecular experiments designed to isolate the tissue-specific *R* components using our *R* probe. We will concentrate on isolating the plant, seed, and leaf color components of *R*. This information will be complementary to the data from collaborations at the University of Wisconsin (Kermicle) and the University of Georgia (Wessler). A combination of genomic blot analysis, genomic cloning, and DNA sequencing will be used to isolate and characterize the differences in each *R* component. Preliminary data indicate a high degree of sequence conservation in each component, which will be isolated and the primary structure determined. With the transcriptional data generated in the Wessler laboratory, and the mapping of the tissue-specific region of an *R* component in the Kermicle laboratory, we are hopeful that the differences in primary sequence observed from our molecular characterization will identify the region responsible for tissue-specific gene regulation. This information will provide us with the conceptual framework to begin understanding how the *R* locus controls the tissue-specific expression of anthocyanins in maize.

University of Colorado
Boulder, Colorado 80309

23. *Studies on Oligosaccharins: Carbohydrates Possessing Biological Regulatory Activities*

A.G. Darvill, P. Albersheim — **$164,800**
Department of Chemistry — **(9.5 mo. U. Co., 2.5 mo. U. Ga.)**

This project identifies, isolates, and characterizes oligosaccharins, naturally-occurring complex carbohydrates that possess biological regulatory activities. We hypothesize that oligosaccharins, when released from the complex carbohydrates of plant cell walls, regulate various physiological functions within the plant. We are studying or searching for the following oligosaccharins: (1) an oligosaccharin isolated from polygalacturonic acid and soybean cell walls that elicits phytoalexin (antibiotic) accumulation in plant tissues and that acts synergistically with a hepta-β-glucoside phytoalexin elicitor of fungal cell wall origin; (2) an oligosaccharin that inhibits 2,4-D promoted growth in pea epicotyls, and the enzymes involved in the metabolism of this oligosaccharin (this oligosaccharin may be involved in the control of auxin-stimulated plant growth); (3) an oligosaccharin that may be a trigger of the hypersensitive resistance response (a fungal enzyme that releases this oligosaccharin from plant cell walls has been highly purified); (4) an oligosaccharin that inhibits flowering in duckweed (*Lemna gibba* G3) and tobacco cultures; (5) oligosaccharins that induce flowers, roots, vegetative buds, and callus in isolated tobacco epidermal strips; (6) oligosaccharins capable of stimulating the regeneration of plants from monocot protoplasts and cultured cells; and (7) an oligosaccharin capable of determining the sex of flowers in the dioecious plant, *Mercurialis annua*.

24. *Structural Studies of Complex Carbohydrates and Plant Cell Walls*

M. McNeil, A.G. Darvill, P. Albersheim — **$190,500**
Department of Chemistry — **(9.5 mo. U. Co., 2.5 mo. U. Ga.)**

The cell walls of plants determine their structure and morphology and are also a source of complex carbohydrates with biological regulatory properties (oligosaccharins). This project involves (1) development of methods to purify and structurally characterize complex carbohydrates and (2) isolation and structural characterization of the complex carbohydrates that constitute approximately 90% of the walls of growing plant cells. We are investigating complex carbohydrates of cell walls of suspension-cultured cells of dicots, monocots, and gymnosperms, and examining complex carbohydrates secreted by these cells (which are excellent, soluble models of the complex carbohydrates of cell walls). Our studies of the polysaccharides of monocot, dicot, and gymnosperm cell walls have indicated that although the quantities of the polysaccharides in the walls vary greatly, every cell wall contains the same array of polysaccharides; the fine structures of the corresponding polysaccharides in the cell walls of different plants differ. The studies have concentrated on three cell wall polysaccharides: xyloglucan, rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II). These polysaccharides have been shown to possess unexpectedly complicated structures. For example, RG-II contains at least 12 different glycosyl residues, including apiosyl, aceryl (3-C-carboxy-5-deoxy-L-xylosyl), and KDO (3-deoxy-D-manno-2-octulosonic acid). The structure of these complex polysaccharides is further complicated by the presence of O-acetyl esters on some of the glycosyl residues. We are developing analytical methods including techniques to identify the locations of base-labile substituents (e.g., acetyl esters). We are also developing methods to cleave uronosyl residues while leaving the neutral glycosyl residues of the complex carbohydrates intact. These methods are particularly useful in our studies of RG-I and RG-II.

25. *The Genetics of Pathogenicity of the* Pyricularia

B. Valent, F.G. Chumley — **$24,900**
Department of Chemistry — **(4.5 mo.)**

Genetic analysis of factors that govern host range and aggressiveness in rice pathogenic isolates of *Pyricularia* has not been possible because rice pathogenic field isolates are uniformly

female sterile (which makes it impossible to cross two rice pathogens, even when they are clearly of opposite mating types). Rice pathogens will mate in crosses with fertile hermaphroditic pathogens of other grasses. In these crosses, the rice pathogen always functions only as a male. We aim to develop laboratory strains that are pathogenic to rice and capable of mating as males or as females in a genetic cross. This project involves crosses between rice pathogens and fertile lab strains pathogenic to goosegrass or weeping lovegrass, with subsequent screening for rice pathogenicity and hermaphroditism among the progeny. Rcently, a cross between a Chinese rice pathogen and one of our fertile lab strains has yielded progeny with the properties we have sought. Only one percent of the progeny from this cross is viable; such poor viability is typical of crosses that involve field isolates. One-third of the viable progeny sporulate well enough to test their pathogenicity. About 50% of them cause some symptoms on rice; 10% cause severe disease on rice. This fraction of rice pathogens is high in comparison to other crosses we have analyzed. Furthermore, 10% of the viable progeny are hermaphrodites. We have identified one progeny as a virulent rice pathogen that mates well both as a male and as a female. It has the same mating type (compatibility group) as its rice pathogenic parent. These observations have been confirmed by re-isolating conidia from lesions on rice. All the re-isolated strains mate well as males or as females, and they all have successfully re-infected rice, causing severe symptoms. Results show it is possible to develop rice pathogens with full sexual competence.

Columbia University
New York, New York 10032

26. ***Regulation and Genetic Organization of Hydrogenase***
A.I. Krasna **$70,000**
Department of Biochemistry and Molecular Biophysics

Hydrogenase is an enzyme of unique biochemical interest because of the nature of its substrates, H^+ and H_2, which are the simplest stable molecules. The enzyme plays an important role in the anaerobic metabolism of many bacteria and algae (in fermentative reactions, photosynthesis, and nitrogen fixation). The objective of the research is to elucidate the regulation and genetic organization of hydrogenase in *E. coli*. Our laboratory has isolated a variety of mutant strains of *E. coli* with altered hydrogenase activity. The hydrogenase-negative mutants will be characterized to establish loci of the mutations, the number of genes responsible for the hydrogenase phenotype, and whether the mutations are in structural genes or regulatory elements. Other mutants, which are hydrogenase-positive, express different levels of hydrogenase activity under various growth conditions; the regulatory mechanisms responsible for the control of their expression will be examined. Little is known concerning the nature and structure of hydrogenase protein and its role in catalytic activity. The hydrogenase gene will be isolated by cloning in hydrogenase-negative strains and its base sequence determined, which should lead to the amino acid sequene of the protein. The same approach will be used to clone the gene from other microorganisms. Some of the hydrogenase-positive strains differ from the parental strain in reactivity with exogenous dyes as substrates. It will be established whether this is due to a requirement for other factors or to mutations in hydrogenase.

Cornell University
Ithaca, New York 14853

27. ***Effects of Freezing and Cold Acclimation on the Plasma Membrane of Isolated Cereal Protoplasts***
P.L. Steponkus **$69,600**
Department of Agronomy

The project objective is to identify the cellular and molecular bases of freezing injury and the molecular mechanisms responsible for cold acclimation in winter cereals. Three different forms of injury of isolated protoplasts and the causal stresses have been identified including: (1) expansion-induced lysis, a consequence of irreversible endocytotic vesiculation of the plasma membrane, which occurs during freeze-induced osmotic contraction; (2) loss of osmotic responsiveness associated with lamellar-to-hexagonal$_{II}$ phase transitions in the plasma membrane and subtending lamellae; and (3) intracellular ice formation, which is the result of mechanical failure of the plasma membrane and is thought to be the result of freeze-induced electrical transients. Cold acclimation increases the tolerance of the plasma membrane to the various stresses responsible for the three forms of injury. Expansion-induced lysis is precluded because of the conservation of membrane surface area during osmotic contraction due to the formation of exocytotic extrusions rather than endocytotic vesiculation of the plasma membrane. Severe freeze-induced dehydration does not result in lamellar-to-hexagonal$_{II}$ phase transitions. Intracellular ice formation occurs at much lower temperatures and is associated with a two-fold increase in the electrical stability of the plasma membrane. Because many of these differences are also observed in liposomes constructed from lipids isolated from the plasma membranes of non-acclimated and acclimated tissues, alterations in the lipid composition appear to play a significant role in the cold acclimation process. Current studies will characterize the lipid composition, including a phospholipid molecular species analysis, of the plasma membrane of non-acclimated and acclimated tissues.

28. ***Importance of Phytoalexin Tolerance and Detoxification for Pathogenicity***
H.D. Van Etten, **$66,100**
D.E. Matthews
Department of Plant Pathology

Synthesis of phytoalexins by plants in response to challenge by microorganisms is believed to be part of an active mechanism of disease resistance. Research has shown that one means by which pathogens may overcome this potential resistance mechanism is by detoxifying their hosts' phytoalexins. The fungus *Nectria haematococca* causes a root and stem rot of pea, although the infected tissue accumulates high concentrations of the phytoalexin pisatin. Virulent isolates of this fungus detoxify pisatin by a demethylation reaction. In crosses between isolates, pisatin demethylation segregates as an essential trait for virulence. Results suggest that *N. haematococca* possesses up to five independent genes for pisatin demethylation, some of which confer only low rates of demethylation and do not enhance virulence. Our objective is to determine the relationship between

these genes and the enzyme pisatin demethylase. The enzyme is a substrate-inducible microsomal monooxygenase. One of the required components, NADPH-cytochrome c reductase, has been purified to near homogeneity and the other component, cytochrome P-450, has been partially purified. Pisatin demethylase activity can be reconstituted by combining the two components. Heterologous reconstitution experiments indicated that reductases from demethylating and non-demethylating isolates of the fungus were functionally equivalent. Thus genetic control of pisatin demethylation does not appear to involve different genes for the reductase. Qualitative and quantitative differences in pisatin demethylation associated with differences in virulence, may be due to differences in cytochrome P-450 structural genes or their regulation. These possibilities will be tested by comparing physical, catalytic, and regulatory properties of the pisatin-demethylating cytochrome P-450 from genetically defined isolates of *N. haematococca.*

29. *Studies of the Genetic Regulation of the* **Thermomonospora** *Cellulase Complex*

D.B. Wilson **$32,600**
Department of Biochemistry, Molecular and Cell Biology

Thermomonospora fusca is a thermophilic, filamentous bacterium that produces active, stable cellulase and xylanase activities. The project objectives are (1) to purify all of the *T. fusca* cellulases and xylanases, characterize each of the purified enzymes, and determine the role of each cellulase in the hydrolysis of crystalline cellulose; and (2) to clone all the *T. fusca* cellulase genes and determine the mechanisms that regulate cellulase and xylanase synthesis. We have purified three different cellulases from *T. fusca* culture supernatant using hydroxyl apatite chromatography and DEAE chromatography as the final step. All three are endocellulases, but they differ in molecular weights, substrate specificities, and immunologically. We have cloned four different cellulase genes into *E. coli.* All of them appear to code for endocellulases. None of the enzymes they code for are inhibited by antisera prepared against any of the three cellulases we have purified, but all are inhibited by an antisera prepared against *T. fusca* culture supernatant. Efforts continue: (1) to fractionate the *T. fusca* culture supernatant to isolate the additional cellulases that are present; (2) to characterize the enzymes produced by the cloned genes and determine their role in the hydrolysis of crystalline cellulose; (3) to localize each cellulase gene within the cloned DNA and sequence each gene; and (4) to determine if the cloned genes are expressed in *Bacillus subtilis* and *Streptomyces lividins*, to find another host for cloning experiments to isolate the genes we have not been able to clone into *E. coli.*

30. *Microbial Ecology of Thermophilic Anaerobic Digestion*

S.H. Zinder **$83,000**
Department of Microbiology

The objective of this project is to provide an integrated understanding of the ecology of the microbial populations in a thermophilic (58°C) laboratory-scale digestor being fed a lignocellulosic waste. Special attention is being paid to the formation and breakdown of acetic acid, the precursor of two-thirds of the methane produced by the reactor. Among the methods being used to study these organisms are: (1) viable counts and culture studies using habitat and niche-simulating media; (2) direct microscopic observation of populations using phase-contrast, epifluorescence, and electron microscopy; and (3) ^{14}C-radiotracer methods to study carbon flow to methane. Results obtained thus far include: (1) isolating a thermophilic *Methanothrix*; (2) developing a defined growth medium for the *Methanothrix* culture, demonstrating a requirement for biotin, and that growth was more rapid (24 h Td at 60°C) with HS-Coenzyme M as a reductant than with cysteine; (3) characterizing alcohol catabolism by a two-membered coculture that converts acetate to methane using interspecies hydrogen transfer; (4) demonstrating that a culture of *Clostridium thermocellum* produces a factor inhibitory towards growth of a thermophilic culture of *Methanosarcina*; and (5) demonstrating, with an HPLC as a fractionating system, that ^{14}C-labeled glucose was metabolized by digestor populations directly to acetate, CO_2, and presumably H_2, without the formation of any other intermediate products. Addition of hydrogen caused a shift toward lactate production from glucose. Current research centers on comparing the kinetics of acetate catabolism in three thermophilic cultures capable of converting acetate to methane: *Methanosarcina*, *Methanothrix*, and the acetate-oxidizing coculture.

Desert Research Institute
Reno, Nevada 89506

31. *Gas-Exchange Characteristics of Leaves as Indicators of the Biochemical Reactions Limiting Photosynthesis*

T.D. Sharkey **$58,700**
Biological Sciences Center

Photosynthetic CO_2 assimilation is studied to determine the molecular processes that determine the gas-exchange characteristics of intact leaves. It has been shown that O_2-insensitive photosynthesis in C_3 plants is mediated by deactivation of the primary CO_2-fixing enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase. Experiments are conducted to determine the mechanism by which this enzyme is deactivated and the underlying metabolic imbalance that leads to this deactivation. Whether deactivation of this enzyme is a physiological adaptation to prevailing metabolic limitations or a deleterious consequence that directly limits productivity is being investigated. The photosynthetic gas-exchange behavior of intact leaves will be compared to fluorescence and light-scattering signals to enhance understanding of underlying limitations of photosynthesis in the intact leaf. Photosynthetic CO_2 assimilation and metabolites of quick-frozen leaves will be measured on plants growing in natural habitats to determine which process or groups of processes limit photosynthesis in leaves in natural conditions.

Florida State University
Tallahassee, Florida 32306-3050

32. *Guard Cell Biochemistry*

W.H. Outlaw, Jr. **$106,000**
Department of Biological Science

The aperture size of stomatal guard cells in leaves is regulated to effect a compromise between the opposing priorities of

avoiding water loss and of admitting CO_2. Pore enlargement is brought about by swelling of the subtending guard cell pair, resulting from accumulation of solutes (K^+ and, to a lesser extent, Cl^-) from the apoplast and synthesis of low MW substances (e.g., malate) from osmotically inert substances (e.g., starch). The specialized metabolism that these cells have evolved to fulfill their crucial role in the plant's physiology extends to more subtle aspects, which this project studies. Despite the presence of green plastids, which conduct linear electron transport, guard cells lack the ability to photosynthetically reduce significant quantities of CO_2. To clarify its role in guard cells the organization of PS II is investigated by fluorometric (and soon by electrophoretic) techniques. Presumably, ABA is an endogenous signal that mediates stomatal closure, but little is known regarding the presence of ABA or of any enzyme specific for its metabolism in guard cells *in situ*. Our sensitive immunological approach hopefully will replace the current conjectures with a data base. PEPCarboxylase, which catalyzes a reaction at a regulated metabolic point in anion biosynthesis, exists as various isoforms, even in C_3 leaves. Because of its central role in guard cell biochemistry, we are studying the properties of this enzyme using kinetic analysis and techniques for physical separation. Advances in these three areas will improve our understanding (and possibly control) of water loss and CO_2 uptake by leaves.

University of Georgia
Athens, Georgia 30602

33. *Development of Methods to Structurally Characterize Complex Carbohydrates*

P. Albersheim, A.G. Darvill, H. van Halbeek, W.S. York
Department of Biochemistry
$39,200
(2.5 mo.)

This research focuses on the analysis of complex carbohydrates by several techniques, principally fast atom bombardment mass spectrometry and high field nuclear magnetic resonance spectroscopy (NMR). The mass spectroscopic analyses are extremely powerful in providing critical information about main and side-chain fragments of oligosaccharides. However high resolution NMR is the method of choice for the analysis of anomeric configurations and the points of attachment of glycosyl linkages. The potential of most of the modern pulse techniques and extended two-dimensional-NMR approaches developed recently has already been explored for small organic molecules, peptides and smaller proteins, and (to a lesser extent) nucleic acid (DNA/RNA) fragments. For carbohydrates of biological importance, which often have relatively more complex (branched) structures than peptides and nucleotides, very few new methods have been tested (only on mono- and disaccharides). We will explore how effectively the newly-developed NMR techniques decipher the primary structures and conformations of oligosaccharides of biological importance. We believe it is feasible to establish the structures and solution conformations of oligosaccharides of up to 16 monosaccharides (from plant cell walls) and oligosaccharides similar or slightly larger in size (from glycoproteins, glycolipids, and glycosaminoglycans).

34. *Biosynthesis of a Microbial Biosurfactant: A Novel and Unique Product*

W.R. Finnerty
Department of Microbiology
$83,800
(FY84 funds)

The biosynthesis of a biosurfactant by a new *Arthrobacter* species was studied with respect to general physiology and development of genetic transfer mechanisms. The project objective was to develop insight into the physiological and genetic mechanisms underlying the biosynthesis of extracellular biosurfactants. We focused on taxonomic verification of the genus, establishing physiological correlates to biosurfactant production, and determining molecular mechanisms for transmission of genetic information in *Arthrobacter*. This new soil isolate is a member of the genus *Arthrobacter*, as determined by G/C composition and cell wall analyses. Esterase- and lipase-negative mutants do not release extracellular biosurfactant. Biosurfactant-negative mutants have been obtained by screening mutagenized populations against antibody prepared to pure biosurfactant. This microorganism harbors three plasmids: pMS1 (42 Kb), pMS2 (14 Kb), pMS3 (11 Kb). pMS2 and pMS3 encode arsenate resistance and have been purified and mapped by restriction endonucleases. We have developed a transformation system in *Arthrobacter* H-13A through protoplast regeneration. pMS2 and pMS3 were restricted with Hind III and ligated into pIJ30, a 20 Kb vector developed for *Streptomyces* with thiostrepton resistance. Transformants were selected by colony hybridization containing recombinant pIJ30 expressing thiostrepton and arsenate resistance. Genes encoding alkane oxidation and biosurfactant production are chromosomal determinants. A transformation system was successfully developed in *Arthrobacter* H-13A, allowing genetic approaches to the delineation of biosurfactant biosynthesis and cloning of specific genes involved in the biosynthesis and regulation and biosurfactant synthesis.

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

J.L. Key
Department of Botany
$89,100

The influence of environmental stresses on gene expression in crop plants is under investigation, focusing on heat stress and gene expression in soybean. In analysis of the transport of nuclear-encoded heat shock (HS) proteins into chloroplasts and isolation of cDNA clones to those proteins, two abundant HS proteins of 27 and 22 kD from total translation products of poly (A) RNA from soybean are transported into isolated pea chloroplasts. When pea and corn poly (A) RNAs from heat-stressed plants are translated *in vitro*, major polypeptides of 23 kD and 25 kD, respectively, are transported into pea chloroplasts. A cDNA clone to the soybean 22 kD HS protein has been isolated. Monoclonal antibodies from several hybridoma lines that react with members of the 15 to 18 kD group of soybean HS proteins were developed for use in localization of the proteins and to quantitate them under various physiological states. Genomic and cDNA clones for HS proteins 70 kD and 84 kD were isolated. Induction of these HS mRNAs, of 2,000 and 2,400 bases in length, respectively, under various physiological states has been assessed. These mRNAs are not particularly abundant relative to the 15 to 18 kD HS protein mRNAs. Genomic clones of the 70 and 84 kD HS proteins have been isolated and are being sequenced. We determined that other environmental

stresses generally do not activate some common regulatory mechanism to produce a common set of proteins that would provide physiological protection to stress in general or generate a homeostatic state. One exception is arsenite, which causes the accumulation of most HS mRNAs and proteins. Cadmium induces a weak response, but does not mimic HS as closely as arsenite.

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

R.B. Meagher **$75,000**
Department of Genetics

The ribulose bisphosphate carboxylase small subunit (RuBPCss) gene family in soybean provides a unique set of strong transcriptional control sequences and signals for light-regulated transcription. The project objective is to characterize the gene family, elucidate the mechanisms of action of the RuBPCss transcriptional signals, and make them available to control foreign genes in plant cells. DNA sequence comparisons within soybean and among a number of plants, green algae, and blue algae indicate that the RuBPCss gene family is far more highly conserved than expected from immunological studies on the proteins. Two closely related genes in soybean, SRS1 and SRS4, are responsible for 80% of the transcription in isolated nuclei and the steady-state mRNA levels in light-grown soybean leaves. The transcription of both genes is induced over 30-fold when plants grown in darkness are transferred to light or given a short exposure to light. If dark-grown plants are given a short exposure to white light followed by a short exposure to far-red light, or if fully light-grown soybean plants are subjected to far-red light, transcription is turned off within minutes. These data suggest that transcription is closely linked to phytochrome. Fusion of the promoter region from SRS1 to a bacterial drug-resistance gene, NPTII, when transformed into petunia plants, results in light-induced expression of the chimeric gene, which functions as a selectable drug marker during transformation. Future work focuses on identifying the various DNA sequences responsible for the control of transcription and the responsiveness of this gene to white and far-red light. Further understanding of the strong activity of the soybean RuBPCss promoters will make them valuable for the high-level synthesis of foreign gene products in plant cells.

37. *Microbiology and Physiology of Anaerobic Fermentations of Cellulose*

H.D. Peck, Jr., L.G. Ljungdahl **$234,000**
Department of Biochemistry

Investigations into the biochemistry and physiology of the four major groups of microorganisms (primary, ancillary, secondary, and methane bacteria) involved in the anaerobic conversion of cellulose to methane and carbon dioxide continue. The primary microorganism of major focus is *Clostridium thermocellum*. Its cellulolytic enzyme system, when active on crystalline cellulose, is a polypeptide complex with an M_r of several million. It is attached to the cellulose via a yellow affinity substance. Soluble cellulolytic proteins not able to bind to the cellulose have only β-endoglucanase activity. The roles and properties of the cellulolytic complex and the yellow substance are being determined. Enzymes of the secondary bacterium *Thermoanaerobacter ethanolicus* are studied to understand the control mechanism for ethanol production. It contains both primary and secondary alcohol dehydrogenases. *Clostridium thermoaceticum* is a secondary bacterium that converts methanol and CO_2 to acetate. The mechanism of this conversion is investigated as is an ATPase from the same bacterium. The ATPase is ATP-generating. Research on ancillary bacteria emphasizes the sulfate-reducing bacteria from the aspects of H_2 cycling, specificities of electron-transfer proteins and enzymes, the mechanism of bisulfite reductase and APS reductase, the enzymology and physiology of new genera of sulfate-reducing bacteria, and both H_2-utilizing and acetate-utilizing methanogens. The studies with H_2-utilizing methanogens will stress hydrogenase and the effect of inorganic pyrophosphate on growth. The research on the acetate-utilizing methanogens will involve the bioenergetics of sulfite reduction and the mechanism of acetate formation induced by pyrophosphate.

38. *Phytochrome in Photosynthetically Competent Plants: Characterization by Monoclonal Antibodies*

L.H. Pratt **$53,000**
Department of Botany

Green, photosynthetically competent plants respond to incident radiant energy in ways that influence their photosynthetic productivity and the partitioning of fixed carbon. Phytochrome is the chromoprotein responsible for mediating most of these photomorphogenic responses. Little is known about phytochrome in green plants. Results obtained with a panel of monoclonal antibodies to oat phytochrome from etiolated shoots indicate that phytochrome from green and etiolated oats are antigenically distinct. This antigenic dissimilarity does not arise from artifactual, post-extraction modifications. Initial experiments indicate further that similar antigenic differences also exist for phytochrome from green and etiolated peas. It is not yet possible to determine whether these antigenic differences reflect differential posttranslational modification of the same gene product or the existence of two different gene products; evidence tends to favor the latter. One monoclonal antibody does bind to a protein the size of phytochrome from all sources tested, including the green alga *Mougeotia*, the moss *Physcomitrella*, and various angiosperms, including oats. With this antibody, phytochrome from green and etiolated oats are found to be indistinguishable in size, but the former is much more susceptible to modification in crude plant extracts, making it more difficult to isolate in native form. Partial proteolytic digestion of phytochrome from green and etiolated oats yields distinctly different peptides. Attempts are made to obtain monoclonal antibodies specific to the protein isolated from green oats. Further characterization of this newly discovered phytochrome in green plant tissues will contribute to understanding of how, at a primary molecular level, photomorphogenesis is regulated in photosynthetically competent plants.

39. *Nitrogen Control of Photosynthetic Protein Synthesis*

G.W. Schmidt **$68,000**
Department of Botany

Nitrogen deficiency severely limits plant growth and productivity, largely through effects on photosynthesis. We study this effect in the green alga *Chlamydomonas*, a model system for analyses of photosynthetic protein synthesis. Nitrogen limitation dramatically decreases chlorophyll and photosynthetic proteins, but there are differential effects as determined by

immunological assays. Light-harvesting apoproteins (LHP) are reduced by 80% while 10 other photosynthetic proteins are reduced from 40 to 65%. Using the wheat germ and reticulocyte lysate systems primed with RNA from N-limited or N-sufficient cells and immunoprecipitation of the translation products, N-limited cells have comparable levels of photosynthetic transcripts (per unit RNA) as N-sufficient cells. Data are substantiated by Northern and dot blot assays using cloned DNA sequences. It appears that post-transcriptional processes are important in the accumulation of photosynthetic proteins in N-limited cells. Pulse-labeled LHPs are not recovered in photosynthetic membranes. Preliminary data indicates that LHPs may be present in the soluble form as higher-molecular-weight precursors, perhaps because their transport into chloroplasts is blocked. Analyses of pigment biosynthetic mutants indicate that chlorophyll is necessary for stabilization of LHP apoproteins after import, but not for transport or proteolytic maturation of LHP precursors. Assembly of LHPs into photosynthetic membrane complexes requires xanthophylls. The importance of xanthophylls in forming LHP complexes is also evident from *in vitro* reconstitution assays we have developed. Analyses of a chl b/neoxanthin mutant indicate these pigments are required for topological organization of LHP proteins in the lipid bilayer and the transfer of excitation energy to RCII. Nitrogen-limited cells are severely deficient in violaxanthin, which may account for their low levels of LHP accumulation.

40. *Theory on Extreme Thermophilic Anaerobes Growing Over Temperature Spans of 40°C or More*

J.K.W. Wiegel — **$40,000**
Department of Microbiology

Bacteria that grow over 70°C are considered extreme thermophiles. Among these, there are species able to grow over an extended temperature span of 40°C or more. Examples for this group are the anaerobic eubacteria *Clostridium thermohydrosulfuricum*, *Thermoanaerobium brockii*, *Thermoanaerobacter ethanolicus*, the anaerobic archaebacterium *Methanobacterium thermoautotrophicum*, and the aerobic *Bacillus stearothermophilus*. They all grow in the temperature range of approximately 35 to 78°C. For these species we have shown that they exhibit a biphasic dependence of the growth rate and the growth temperature. The corresponding Arrhenius graph has a curve with an intermediary plateau that is found between 55 and 60°C. This biphasic growth response is investigated on the enzyme (protein) level by examining the protein pattern of the organisms grown at various temperatures including T_{min}, T_{opt}, and T_{max}, below, at, and above the plateau. Initially we use two-dimensional gel electrophoresis (O'Farrell's gel) to obtain the differences in protein pattern related to growth temperatures. These experiments will be followed by temperature shifts with the employment of radioactive-labeled amino acids. These studies will reveal whether a synthesis of new proteins occurs in bacteria when shifting the growth temperature from below to above that of the plateau. This is done to evaluate our hypothesis that these organisms inherit (for some critical enzymes/proteins) two sets of proteins, one for the lower range of 35 to approximately 65°C and one for the upper temperature range from approximately 50 to 78°C. We will start to isolate temperature-sensitive mutants not able to grow at either the higher or lower temperature range.

University of Georgia
Tifton, Georgia 31793

41. *Development of Innovative Techniques That May Be Used as Models to Improve Plant Performance*

W.W. Hanna, G.W. Burton — **$39,100**
Department of Agronomy

This project develops techniques for transferring genes from wild species to cultivated plant species, to demonstrate the wealth of germplasm in the secondary and tertiary gene pools that can be transferred to cultivated species, and to develop an obligate apomictic pearl millet. Species within the genus *Pennisetum* are being used as test organisms. The approach uses plants of wild species with different genetic backgrounds and ploidy levels (chromosome numbers) crossed and backcrossed with different genotypes of pearl millet, *P. americanum*, with different ploidy levels to produce fertile interspecific hybrids and derivatives. We have shown how valuable germplasm can be masked on certain genomes by other genomes and stored in a perennial or vegetatively propagated wild species such as *Pennisetum purpureum*. We have been able to transfer this hidden or stored germplasm to cultivated pearl millet and are in the process of evaluating it. Partially fertile and apomictic BC_2 pearl millet x *P. squamulatum* hybrids and trispecific pearl millet–*P. squamulatum*–*P. purpureum* hybrids have been produced, showing the potential of transferring apomixis from the wild to cultivated species to fix hybrid vigor. Diverse cytoplasm from the wild species have been transferred to pearl millet and are in the initial stages of evaluation. The overall impact would be on increased, more efficient, and more reliable production of food, fiber, and forage.

Harvard University
Cambridge, Massachusetts 02138

42. *Unraveling Photosystems*

L. Bogorad — **$175,000**
Department of Cellular and Developmental Biology — **(2 yrs.)**

The project objective is to identify and characterize essential protein components of the energy-transducing reaction centers of photosynthesis and to understand how these components are arranged in the membrane. Such information should bring us closer to comprehending the functional architecture of this apparatus for converting light energy into electrical potential energy. The identification and characterization of protein components of the reaction centers is being advanced through a program of gene isolation, modification, and reintroduction (transformation) using cyanobacteria. The prokaryotic organisms carry out basically the same type of oxygen-evolving photosynthesis as the chloroplasts of higher plants. One of the most favorable cyanobacterial species for this work (since it can grow on an exogenous carbon source independently of photosynthesis or, alternatively, photosynthetically) has the disadvantage of not taking up foreign DNA. We have devised a new procedure that promotes such uptake and incorporation into the chromosome of the organism. This technique will be essential for studying functions of deleted genes by complementation with foreign DNA introduced into mutants. The conformation of

membrane proteins *in situ* is studied through the use of antibodies each specifically directed against a defined hydrophilic region. Thus far, the work has led to a new view of the probable disposition in the membrane of a quinone-binding protein of photosystem II. Work that will permit more detailed analysis is in progress.

Harvard University
Petersham, Massachusetts 01366

43. *Structure and Function of* Frankia *Vesicles in Dinitrogen Fixation by Actinorhizal Plants*
J.G. Torrey **$75,300**
Cabot Foundation, Harvard Forest

In the filamentous bacterium *Frankia* of the Actinomycetales the dinitrogen-fixing enzyme nitrogenase develops under specific conditions both in the root nodules of infected woody dicotyledonous plants and in the free-living bacterium grown in culture. In most *Frankia* symbiosis nitrogenase develops within specialized terminal swellings of the bacterial filaments called vesicles. Similar vesicles are induced to form in filamentous colonies grown aerobically in defined medium by witholding fixed nitrogen (NH_4Cl). The vesicles possess a specialized multilaminate envelope, providing a physical barrier to O_2 access, which would otherwise denature the nitrogenase enzyme. Our research aims at understanding the chemical and physical environments that influence vesicle formation and the development of nitrogenase within the vesicle, which are separable events. Work focuses on the red alder, *Alnus rubra* and its free-living microsymbiont *Frankia* strain HFPArI3 and the Australian she-oak *Casuarina cunninghamiana* and the *Frankia* isolate HFPCcI3. In the former, *Frankia* vesicles occur within N_2-fixing nodules and free-living culture. In the latter, vesicles occur only in free-living culture. In nodules of the host plant, *Frankia* remains filamentous. We have shown that vesicle formation as well as nitrogenase induction is subject to ambient levels of combined nitrogen and to the pO_2 of the atmosphere surrounding the *Frankia*. Work continues on the conditions influencing effective N_2-fixation in these and other actinorhizal plants.

University of Idaho
Moscow, Idaho 83843

44. *Genetics and Chemistry of Lignin Degradation by* Streptomyces
D.L. Crawford **$75,000**
Department of Bacteriology and Biochemistry

Project objectives are (1) to study the *Streptomyces*-mediated reactions causing lignin solubilization, and the pathways by which acid-precipitable polymeric lignins (APPLs) are further metabolized; (2) to develop genetic techniques for generation of APPL-overproducing *Streptomyces* strains, and to characterize the chemistry of APPLs produced by genetically altered strains; and (3) to use a plasmid vector for cloning lignin catabolism genes of *Streptomyces*, and to identify the enzymes coded by those genes. The chemistries of lignin degradation, APPL production, and APPL metabolism have been elucidated for *S. viridosporus*, *S. badius*, and *S. setoni*. Ultraviolet mutagenesis and protoplast fusion have been used to produce stable APPL-overproducing strains of *S. viridosporus*. The plasmid vector p1J385 has been used to clone a gene coding for phenylalanine ammonia lyase from *S. verticillatus* into a nonligniolytic strain, *S. lividans*. We have also transformed *S. viridosporus* with this multi-copy plasmid. APPL-overproducing strains are being screened for overproduction of lignocellulose-degrading enzymes, and the chemistry of the APPLs generated by these strains is being characterized. Plasmid p1J385 is being used to clone the *S. viridosporus* gene(s) coding for APPL production into *S. lividans*. We will also clone a known lignin depolymerization system, the β-etherase complex, from *S. viridosporus* into *S. lividans*, and we will amplify expressions of these genes in *S. viridosporus* by transforming the plasmid back into *S. viridosporus*. This will allow us to isolate specific genes coding for lignin degradation in *S. viridosporus*, identify the enzymes coded by those genes, and determine the role of each in lignin metabolism.

University of Illinois
Urbana, Illinois 61801

45. *Fatty and Aromatic Acid Catabolizing Bacteria in Methanogenic Ecosystems*
M.P. Bryant **$68,300**
Department of Dairy Sciences

The project objectives are to isolate and to determine the systematics, physiology, and catabolic biochemistry of syntrophic obligate acetate- and H_2-forming anaerobic bacteria from methanogenic ecosystems that require coculture with a hydrogenotroph (e.g., a methanogen to grow and catabolize fatty acids and monobenzenoids). Bacteria not requiring syntrophy are also of interest. We previously described those syntrophs that catabolize propionate, C_4 to C_8 fatty acids; and *Syntrophus* that catabolizes benzoate. A *Syntrophomonas* sp. *beta* that oxidizes C_4 to C_{18} fatty acids to acetate and H_2 has been isolated in coculture. A syntroph (PA-1) that degrades many monobenzenoids (e.g., benzoate, phenylacetate, phenol, resorcinol, catechol, gallate, phloroglucinol, and other compounds) has been isolated. It grows very little in pure culture on most substrates without a hydrogenetroph, but is the most versatile benzenoid-degrading syntroph yet isolated and will be studied for pathways of catabolism. Another new species, *Eubacterium oxidoreducens*, catabolizes gallate (3,4,5-trihydroxybenzoate) via decarboxylation and hydroxyl shift to phloroglucinol, NADPH-linked phloroglucinol reductase to dihydrophloroglucinol, the final products being acetate and butyrate. It requires exogenous H_2 or formate (NADP-linked formate dehydrogenase) to generate NADPH for phloroglucinol reduction. A previously unknown acyclic intermediate, 3-hydroxy-5-oxocapoate, is in the pathway. The studies provide information on (1) whether monobenzenoids containing less than three hydroxyl functions might be catabolized by bacteria in methanogenic ecosystems without necessity for obligate syntrophy and (2) biochemical ecology and pathways of catabolism of these important benzenoid compounds.

46. *Photosynthesis in Intact Plants*

A.R. Crofts **$110,000**
Department of Physiology and Biophysics

Photosynthetic electron transport and the reactions of energy coupling can be studied in intact plants by spectrophotometric and fluorescence techniques. The project objectives are (1) to develop instrumentation for the measurement of these reactions under field conditions and (2) to study (in a parallel project) similar reactions under laboratory conditions in which the experimental parameters are more easily controlled. We have completed the construction of two portable fluorescence instruments (10 to 20 lbs, microcomputer controlled, battery operated, built-in screen for graphic and alpha-numeric display, battery-backed memory for storage of experimental results); and we are building a field spectrophotometer to similar specifications. The field double-flash kinetic photometer has been used to study herbicide-resistant strains of weeds. We have identified (by laboratory comparison) the functional modification of the two-electron gate in these strains. We have also begun similar studies on resistant strains of *Anacystis* for which the DNA sequences of the modified protein are under study at the University of Chicago. Laboratory work on reactions of the donor side of PS II will elucidate the partial reactions in reaction-center preparations stripped of different sub-units to identify spectrophotometrically-measurable electron transfer reactions.

47. *Mechanism of Proton Pumping in Bacteriorhodopsin*

T.G. Ebrey **$55,300**
Department of Physiology and Biophysics

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

48. *Studies on the* E. coli *Respiratory Chain*

R.B. Gennis **$86,000**
Department of Chemistry
and
Department of Biochemistry

The aerobic respiratory chain of *E. coli* is responsible for providing the energy required by the cell for oxidative phosphorylation and for driving active transport. The respiratory chain essentially directs electron flow from the oxidation of organic substrates (e.g., succinate) to oxygen, which is reduced to water. This electron flow is coupled to proton translocation across the cytoplasmic membrane, thus generating a proton motive force. This project will identify all the biochemical components of this system and explore the mechanism of proton translocation at the molecular level. Two major components have been identified as ubiquinol oxidases: the cytochrome *o* complex and cytochrome *d* complex. They carry out the same reaction and are redundant in the cell. Our research effort focuses on the cytochrome *o* complex. The enzyme is now purified, and contains four polypeptides by SDS-PAGE analysis. The redox centers appear to be iron-protoporphyrin IX and, probably, copper. Both monoclonal and polyclonal antibodies have been raised against the enzyme, but they appear to be non-inhibiting. The pure enzyme can be reconstituted into single-walled phospholipid vesicles and appears to insert uni-directionally. Electron transfer from quinol to oxygen in the reconstituted system proceeds rapidly and generates a transmembrane potential (also observed in the cell); we study this system using genetics and recombinant DNA technology. Mutants have been obtained and mapped, and the gene coding for the enzyme has been cloned, resulting in considerable overproduction. Future efforts involve studying the gene structure, including the DNA sequence of the coding region for all the polypeptide components.

49. *Genetics of the Methanogenic Bacterium* Methanococcus voltae

J. Konisky **$55,000**
Department of Microbiology

The overall goal of this project is to develop a genetic system and understand mechanisms of gene expression for the methanogen, *Methanococcus voltae*. The project involves isolation of mutants, search for plasmids, and characterization of methanogen *hisA* gene expression. We have followed up our cloning of the gene that encodes the methanogen *hisA* enzyme by determining its nucleotide sequence. Analysis of the gene structure allows us to propose those methanogen sequences that are used as promoters by *E. coli*. We are now examining *hisA* transcription in the methanogen strain by isolating and characterizing methanogen *hisA* mRNA. We intend to use S1 nuclease mapping to determine *hisA* methanogen mRNA in cultures grown under a variety of conditions. In collaboration with the University of Georgia, we are isolating and characterizing plasmids from newly isolated methanococci. These are being used to construct potential shuttle vectors for use in *Mc. voltae–E. coli* gene transfer. We are also carrying out DNA–DNA hybridization studies to determine the relatedness of new isolates. Bromoethane sulphonate (BES) is a toxic analog of methyl-CoM, a cofactor in methanogenesis. During the past year, we have made some progress in characterizing several BES-resistant mutants isolated in our laboratory. Although such mutants are resistant to this drug *in vivo*, BES is still a potent inhibitor of methanogenesis in crude extracts. We plan a major effort to determine the mechanism of BES resistance.

50. *The Roles Played by Mitochondrial DNA and Nuclear Genes in Reversions to Fertility in S-Type Male-Sterile Maize*

J.R. Laughnan **$173,200**
Department of Plant Biology **(2 yrs.)**

This project focuses on understanding cytoplasmic male sterility (CMS) in maize, where three such systems are known: *cms-C*, *cms-T*, and *cms-S*. Studies mainly concern the *cms-S* system, involving genetic and molecular characterization at the nuclear and mitrochondrial levels of spontaneously occurring cytoplasmic and nuclear reversions of *cms-S* to the male-fertile

condition. Cytoplasmic reversion is invariably associated with changes in mtDNA. In inbred lines M825 and 38-11 cytoplasmic revertants fail to replicate the plasmid-like mtDNAs S1 and S2, whereas revertants in WF9 retain them. In all cases (over 40 have been examined) cytoplasmic reversion to fertility is also associated with rearrangement of the main mtDNA genome, as judged by the use of a number of radioactively-labeled cloned mtDNA gene probes in restriction enzyme fragment analyses. The nuclear genotype controls the nature of the associated mtDNA rearrangements as well as whether or not S1 and S2 continue replication. The spontaneous nuclear revertants have been mapped to a number of different chromosomal locations, and allelism tests indicate that numerous sites are probably involved. In the four instances studied, these newly arisen *Rf* elements are shown to undergo secondary transposition; this phenomenon is under intensive investigation. The *Rf* elements appear to be another category of transposable elements in maize. Unlike the controlling elements already identified as transposable in maize, the *Rf* elements carry with them, when they move, the code for manifestation of a unique visible phenotype: male fertility in the *cms-S* background.

51. Acetoclastic Methanogenesis
R.S. Wolfe **$77,900**
Department of Microbiology

The microbial formation of methane is carried out by a unique group of bacteria known as methanogens. These strict anaerobes are widespread in nature, found in diverse habitats (the rumen caecum of herbivores, the intestine of mammals, the digestive tract of termites, sludge digesters, sanitary landfills, marine and freshwater aquatic sediments, marshes, and bogs) wherever active anaerobic biodegradation of organic matter occurs. Of the substrates used by methanogens, acetate, a major metabolic product by fermentative bacteria in these habitats, accounts for about 60% of the methane formed. Our investigations focus on (1) the interaction of methanogens that use acetate (acetophilic methanogens) with organisms that produce it and (2) the biochemistry of acetoclastic methanogenesis. We have studied the microbial components that carry out the conversion of phenol and various derivatives of phenol to methane and carbon dioxide. The project objective is to define the microbial interactions in the recovery of energy (methane) from anaerobic treatment of industrial wastes. A biocatalytic system consisting of three organisms was defined for the anaerobic conversion of phenol to methane and carbon dioxide. Of the newly discovered coenzymes (methanofuran and tetrahydromethanopterin) of methanogens, we are studying the presence and activity of these cofactors in acetate-grown methanogens. Methanofuran was found in about the same amount (2 nmol/mg dry cells) in *Methanosarcina* grown on hydrogen and carbon dioxide as on acetate. Similar results were obtained for tetrahydromethanopterin. Methyl-tetrahydromethanopterin was readily converted quantitatively to methane by extracts of acetate-grown cells.

Institute of Paper Chemistry
Appleton, Wisconsin 54912

52. Raman Microprobe Investigation of Molecular Structure and Organization in the Native State of Woody Tissue
R.H. Atalla **$49,800**
Chemical Sciences Division

The Raman Microprobe has revealed evidence of variablity of molecular structure and organization within different domains of the cell walls of tissue from loblolly pine (*Pinus taeda L*) and black spruce (*Picea mariana*). The objective of this project is to investigate the range of variation in composition and molecular orientation within individual cells, between adjacent cells, between cells from different annual rings, and between cells from different types of tissue. Results indicate that the aromatic rings of the phenyl propane units of lignin are most often oriented in the plane of the cell wall surface. However, in some regions they are preferentially oriented perpendicular to the cell wall surface or (less often) they appear to have little preferential orientation. These results suggest the existence of nodes in the organization of lignin not unlike those known to occur in many celluloses. With respect to the carbohydrate components of the cell walls, the key findings are that cellulose is also highly organized. Though such a high degree of organization was known to occur in celluloses from seed hairs and bast fibers, our observations provide the first direct evidence that a similar level of architectural complexity prevails in woody tissue. Future work will include more comprehensive mappings of molecular organization and compositional variation over a wider range of morphological features. Results will further fundamental understanding of the architecture of cell walls, and will provide a better foundation for analysis and design of industrial processes that use biomass as a primary resource.

University of Kentucky
Lexington, Kentucky 40546-0091

53. In Vivo and In Vitro Assembly and Disassembly of the Water-Oxidizing Complex of Photosynthesis
G.M. Cheniae **$77,500**
Department of Agronomy

The project objectives are (1) to determine the components necessary for the efficient coupling of photosystem II (PS II) traps to the water-oxidizing complex, (2) to determine the components of the water-oxidizing complex itself, (3) to gain insights into the reactions underlying the multiquantum process required for photoactivation of the water-oxidizing complex with ligation of manganese, and (4) to determine the molecular bases for photoinhibition of the donor side of PS II traps, which occurs rapidly when the water-oxidizing complex has been inactivated. All of these processes can be observed *in vivo* and *in vitro* (using Triton-prepared PS II membranes). Certain PS II extrinsic proteins reassemble only after the ligation of manganese, a process dependent on photoactivation. However, the ligation of manganese into membranes is independent of any of the PS II extrinsic proteins. The disassembly/reassembly markedly alters the Cl^- and Ca^{2+} requirements for PS II trap/S-State enzyme coupling and for O_2 evolution and the specifically-

bound calcium (2 Ca/200 Chl) found in functional complexes. The disassembly/reassembly also markedly alters the chemical reactivity of the Mn–S-State complex with hydrophilic/lipophilic redox reagents. Such chemical reactivity is dependent on redox potential and affected by light and Cl^-. These results suggest that the PS II trap/water-oxidizing complex is susceptible to many perturbations. Moreover, they indicate that recovery from photoinhibition of the donor side of photosystem II is dependent on resynthesis of a chloroplast DNA-encoded polypeptide, which is not the 32 kDa Q_B protein.

Lawrence Berkeley Laboratory
Berkeley, California 94720

Applied Science Division

54. Photochemical Conversion of Solar Energy
L. Packer, R.J. Melhorn, A.T. Quintanilha **$125,000**

This project objective is to identify molecular mechanisms of activity of bacteriorhodopsin (BR), a relatively stable and simple energy-transducing protein that directly couples the absorption of visible light to the production of proton and electrical gradients across purple membranes (the photoactive components of halobacteria). Our studies with BR include research on isolated purple and white membranes. The latter, obtained from retinal-deficient mutants of halobacteria, synthesize the apoprotein and are capable (upon the addition of all-trans retinal) of reforming fully functional proton pumping activity. Research focuses on structural studies to elucidate function using chemical modification and spin-labeling techniques to determine the role of specific charged amino acid residues in BR activity. Removal of the C-terminal tail by proteolytic cleavage reveals marked changes in the properties of the isolated purple membranes; they become stacked membrane sheets, piled on top of one another. Formation of these large aggregates is detected by laser light scattering and electron microscopy. This finding has an important consequence on the measured efficiencies of proton release during operation of the BR photocycle (H^+M412 ratios). Using white membranes (which are less susceptible to this stacking effect) with laser flash photolysis techniques, we are finding higher efficiencies for proton release than previously reported. We investigate the role of membrane surface charge and carboxyl groups in this process. Structural factors important for maintenance of high efficiency of proton release will be further elucidated.

Biology and Medicine Division

55. Resonance Studies in Photosynthesis
A. Bearden **$68,000**

Understanding photoconversion of light to chemical potential and the subsequent pathways of electron flow in dark electron transport in green-plant (chloroplast) photosynthesis requires careful quantitation of participating active molecules. As electron paramagnetic resonance (EPR) spectroscopy of electron-donors and electron-acceptors must necessarily be done over a wide range of temperatures and microwave powers, quantitative measurements are often difficult. By careful design and analysis of our EPR methods, we are studying quantitatively the sequence of electron-donors (including P700) and electron-acceptors (including the iron–sulfur clusters) in a series of *Chlamydomonas* mutants. These studies are made at temperatures from 4 K to room temperature with the use of well-understood EPR standards. Concomitant optical studies using a modified Aminco-Chance dual-wavelength spectrometer and optical-fiber methods will permit optical data to be obtained in the same geometry necessary for high-sensitivity EPR measurements. The use of *Chlamydomonas* mutants (for which significant genetic, biochemical, and functional data are now available) should afford a unique opportunity for comparison of the organism's biophysical structure and photosynthetic function relations. An extension of these methods to the green algae, *Dunaliella parva* is planned; the chloroplasts in this organism are of a size that permits optical-fiber spectroscopic analysis of a single chloroplast. If sufficient population differences are present, it may be possible to apply cell-sorting techniques to obtain populations for EPR examination with natural enrichment in specific photosystem components.

Chemical Biodynamics Division

56. Light-Regulated Expression of Nuclear and Chloroplast Gene Expression
J.C. Bartholomew **$60,000**

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

57. Regulation of Plant Gene Expression
J.A. Bassham, J.C. Bartholomew **$286,000**

Mechanisms of regulation of genetic expression of photosynthetic and biosynthetic carbon paths in green plants are elucidated. Expression of enzymes as a function of species, development, and environment is evaluated through measurements of

mRNA, polypeptides, enzyme activity, and so forth. Current investigations focus on expression of enzymes of the C_4 carbon metabolism shuttle mechanism, believed to be responsible for the high productivity of C_4 plants such as corn and sugar cane. Regulation of expression of these enzymes is followed in C_4 (especially maize) and in C_3 (especially wheat) plant tissues, in plant cells in tissue culture, and in isolated protoplasts and organelles. The timing of expression of key enzymes and the transition from heterotrophic to autotrophic type in relation to developmental morphology is followed. The enzymes required for the C_4 carbon shuttle are expressed to some degree in the C_3 plant (wheat), although the amounts in most cases are much less. The leaf form of pyruvate, Pi dikinase (PPDK), which is nuclear-encoded, cytoplasmically synthesized, and processed after transfer through the chloroplast membrane into the chloroplast to give mature protein, is present in wheat leaves at about 1 to 2% of the level found in maize. There may be a small amount of intracellular carbon transport in wheat, which lacks Kranz anatomy and thus does not have an intracellular transport like C_4 plant leaves. Techniques of somatic fusion (including electrofusion, sorting of fusion products by flow cytometry, culturing of hybrids, and plant regeneration) will be used to investigate control mechanisms between chloroplasts and nucleus. Results are expected to provide information needed for future genetic engineering to improve productivity.

58. Plant Hydrocarbon Biosynthesis

M. Calvin, J.A. Bassham **$80,000**

Terpenoid compounds (isoprenoids) are a major class of hydrocarbon compounds (compounds containing very little or no oxygen) found in many plant species. This diverse class of compounds includes sesquiterpenes, terpenes, carotenoids, triterpenes (including sterols), polyisoprenes (rubber), and others. All of these compounds presumably share a common biosynthetic pathway from acetyl CoA through the synthesis of the isoprene structure as isopentenyl pyrophosphate (IPP). Biosynthetic steps to sterols were earlier elucidated from work with animals and yeast, and while studies with plants show many common features with the animal pathway, there remain many incompletely studied aspects in plants. Of particular importance to understanding the control of photosynthate allocation to hydrocarbon production in plants are questions of intracellular compartmentation of enzymic steps in the pathway, identification of rate-limiting step(s) in IPP synthesis, and the purification and characterization of the enzyme(s) involved in such steps. The relative quantities of end products is controlled by enzymes at branch points in the subsequent conversion of IPP to larger molecules. Our present work is principally with *Euphorbia lathyris*, and focuses on (1) the characterization of hydroxymethylglutaryl CoA reductase (HMGR), the rate-limiting step between acetyl-CoA and IPP; (2) the nature of squalene cyclization to make sterols in this species; and (3) the characterization of subcellular organelles involved in terpenoid biosynthesis. These studies will provide purified enzymes and information essential to future investigation of control of gene expression regulating the quantity and quality of plant hydrocarbon production.

59. Photosynthetic Gene Expression in Rhodopseudomonas capsulata

J.E. Hearst **$190,000**

This project studies in depth a photosynthetic gene cluster from the non-sulfur purple bacterium *Rhodopseudomonas capsulata*. The structural genes for the two light-harvesting I polypeptides are sequenced. The genes for the three reaction center polypeptide subunits, H, M, and L are also sequenced. In addition, 30 Tn5.7 transposon mutants in the photosynthetic gene cluster are now isolated, mapped, and phenotypically characterized. A remarkable sequence homology exists between the M and L subunits of *Rps. capsulata* and the D2 and D1 polypeptides, which are coded on the chloroplast genomes of higher plants and algae. Based on this homology and chemical intuition, we propose a model for the quinone-binding sites of photosynthetic reaction centers. Using reverse transcription on isolated messenger RNA templates from synthesized oligonucleotide primers, the sequenced areas of the photosynthetic gene cluster will be mapped with respect to their operon structures and the positions and sequences of their promoter sites. The distinctions between O_2-regulated promoter and constitutive promoter sequences will be established, and ultimately, the basis for this distinction at the level of RNA polymerase will be determined. Starting with the gene map for the bacteriochlorophyll and carotenoid biosynthetic pathway provided by the transposon mutagenesis, we are associating a defined enzymatic activity with each gene locus. The operon map for these unsequenced regions of the photosynthetic gene cluster is being determined. Finally, the quinone-binding regions of the reaction center polypeptides in the photosynthetic membrane are being located by affinity labeling techniques using newly developed class of suicide quinones that are activated only by the natural quinone reductases that exist in the photosynthetic membrane.

60. Mechanism of Water Splitting and Oxygen Evolution in Photosynthesis

M.P. Klein **$127,000**

Oxygen evolution in photosystem II is thought to involve reactions through a cycle of states by which electrons are removed from H_2O and donated to the oxidized $P680^+$ reaction center. The most successful interpretation of the data regarding this cycle is Kok's S-state scheme, which postulates a series of five (S_0-S_4) states through which electrons are cycled during oxygen evolution. Manganese is thought to play a central role in the chemistry of these reactions. X-ray absorption spectroscopy using synchrotron radiation is used to determine the structural and electronic state of the manganese sites. In photosystem II particles of both spinach and the cyanobacterium *Synechococcus*, we have determined that the manganese occur as a binuclear complex with Mn–Mn separation of about 2.7 A. We have recently observed a manganese x-ray absorption edge shift upon the transition from S_1 to S_2, which implies an oxidation state change for manganese associated with the advancement of this photochemistry. These studies provide the first evidence that manganese is directly affected by the light reactions. There is a multiline EPR signal, also suggestive of a binuclear complex, which is an indicator of State S-2. We are beginning to employ ENDOR and electron spin echo spectroscopy to determine the types and numbers of atoms that are ligands of the manganese atoms, the role of halide ions, the sites of water entry into the complex, and the topology of the manganese complex relative to other redox sites in the photosynthetic apparatus.

61. *Phycobiliproteins: Structural, Synthetic, and Mechanistic Studies*

H. Rapoport **$64,000**

Phycobiliproteins are a family of macromolecules that serve as light-harvesting components of the photosynthetic systems of cyanobacteria (blue-green algae), the red algae, and the cryptomonads. Covalently attached open-chain tetrapyrrole prosthetic groups (bilins) enable these proteins to absorb visible light in the region between the blue and far-red absorption peaks of chlorophyll *a*. Four different tetrapyrroles have been identified in cyanobacterial and red algal biliproteins: phycocyanobilin (PCB), phycoerythrobilin (PEB), phycourobilin (PUB), and a phycobiliviolinoid chromophore. A single biliprotein subunit may carry different bilins (e.g., the β subunit of R-phycocyanin carried both PCB and PEB). The project objective is to establish the detailed structure and stereochemistry of these pigments. Our studies include several findings: (1) the only linkages between the bilin and polypeptide found thus far are thioether bonds through cysteinyl residues; (2) in the β subunit of C-phycocyanin, one PCB is linked through ring A, the second through ring D; (3) in the α and β subunits of B-phycoerythrin, four PEB groups are attached to the polypeptides through single thioether bonds to ring A, whereas one PEB is linked to the β-subunit of B-phycoerythrin through two thioether bonds, one to each of rings A and D of the bilin; and (4) an isolated tryptic peptide from the β subunit of R-phycoerythrin carries a PUB group linked through two thioether bonds, whereas a tryptic chromoprotein peptide derived from the γ subunit of this protein carries a PUB attached through a single thioether bond. Discovery of diversity of linkage modes and consequently of bilin structures, reveals an added level of structural complexity that must be fully understood before the spectroscopic properties of this class of proteins can be interpreted.

62. *Photosynthetic Membrane Structure and Photosynthetic Light Reactions*

K. Sauer **$185,000**

Through measurements of the nanosecond decay kinetics of fluorescence from photosynthetic membranes, we are able to monitor the dynamics of excitation transfer among the antenna pigments and of trapping in the reaction centers. A model is emerging that describes how the light-harvesting pigments are organized and how this organization changes as a consequence of the metabolic control processes. To simplify the analysis, we are examining the behavior of isolated sub-complexes associated with photosystems I and II of higher plants as well as for the cyanobacterium *Synechococcus*. Particularyly for PS II we are studying the association of particular functions (quinone binding, chlorophyll binding, manganese binding, reaction center activity) with specific proteins in the complex. To further physical measurements (e.g., optical absorption, EPR, and x-ray spectroscopies), we are preparing complexes highly enriched in the components under investigation, and we are monitoring the relevant activities to insure the integrity of the complexes. After initial identifications, we are determining N-terminal amino acid sequences. Based on the sequence information, we will construct probes to locate the genes that code for these proteins and ultimately will clone and sequence the genes. In parallel we will attempt crystallography of the important membrane sub-complexes to determine their structure by x-ray diffraction analysis. A similar study is underway for the reaction center complex of the photosynthetic bacterium *Rhodopseudomonas capsulata*. Our ultimate goal is to understand how the structure of these complexes facilitates the conversion of light into chemical energy, which is the principal role of photosynthetic organisms.

Lehigh University
Bethlehem, Pennsylvania 18015

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

B.S. Montenecourt, J.A. Sands **$82,900**
Department of Biology and Biotechnology Research Center

Microbial cellulases are important enzymes of potential industrial application in the conversion of renewable biomass to glucose syrups. The multienzyme cellulase complex secreted by the mesophilic filamentous fungus *Trichoderma reesei* has been the most highly studied. Much is known about the biochemistry of the enzymes and the structure of some of the cellulase genes; much less is known about events involved in the secretion of these glycosylated enzymes. We are using a combined genetic and biochemical approach to elucidate the nature of the process of cellulase secretion by *Trichoderma*. Durng the past year, we have made significant progress in understanding the role of glycosylation in the secretion of cellulase by wild type *T. reesei* and by hypersecretory mutants. We have shown that inhibition of N-linked glycosylation does not prevent secretion of active enzyme, but that the enzymes lacking N-linked oligosaccharides are less thermostable and less resistant to proteolytic digestion than the fully glycosylated enzymes. Current studies will lead to further understanding of the importance of glycosylation and to the nature of the intracellular (pre-secretion) forms of the cellulases. In conjunction with these biochemical studies on hypersecretory mutants, we are continuing to isolate a variety of new temperature-sensitive mutants that will be useful for genetic analyses. Among the temperature-sensitive mutants that we have isolated, several appear to show preferential loss of secretion of cellulases or proteases at the high temperature. Continued biochemical and genetic studies may lead to a thorough understanding of the crucial molecular events involved in the secretion of high levels of active cellulases by *Trichoderma*.

Los Alamos National Laboratory
Los Alamos, New Mexico 87545

Life Sciences Division

64. *Nuclear Magnetic Resonance Studies of Cell Wall Synthesis and Salt Tolerance in Cereals*

J.W. Heyser, L.O. Sillerud **$100,000**

This project uses recent advances in high-resolution nuclear magnetic resonance (NMR) spectroscopy to follow *in vivo* the dynamics of cell wall biosynthesis in cereal tissue cultures, and the gradual adaptation to sublethal concentrations of NaCl by intact plants, tissue cultures, and protoplasts. Resynthesis of cell walls by cereal protoplasts represents a limiting step in the application of single-cell biotechnologies to these major food crops. Cell wall fractions were isolated from protoplast-derived millet (*Panicum miliaceum*) cell suspensions after being grown

on [1-^{13}C] glucose, or natural-abundance sucrose or glucose. The ^{13}C label is incorporated with a minimum of chemical modification in sugars derived from glucose, as all classes of polysaccharides, pectins, starch, hemicelluloses, and non-cellulosic -glucans show strong C-1 NMR signals. Thus the NMR signals for each C-1 carbon of the sugars present in the various cell wall fractions indicate their relative concentrations. Short-term *in vivo* labeling experiments using ^{13}C-arabinose synthesized at Los Alamos National Laboratory will show the dynamics of cell wall incorporation of this major cell wall sugar. Chemical composition of the millet cell wall varies little throughout the cell culture cycle and is similar to that of embryonic corn coleoptiles. Sodium-23 NMR, in combination with dysprosium-based anionic shift reagents, has been used to monitor sodium transport *in vivo*. The uptake and efflux of ^{23}Na by NaCl-adapted and nonadapted wheat (*Triticum aestivum*) and saltgrass (*Distichlis spicata*) plants show different kinetics, which vary depending on the degree of adaptation. An energy dependence for sodium transport has been shown for intact wheat plants. Similar results have been obtained for NaCl-adapted versus nonadapted cultures of proso millet.

Martin Marietta Laboratories
Baltimore, Maryland 21227

65. Studies of Photosystem II Using Artificial Donors
R.J. Radmer **$95,000**

The objective of this project is to study the mechanism of photosynthetic H_2O oxidation by the use of artificial donors. The approach uses specialized mass spectrometry and flash-kinetic spectrophotometry techniques developed in-house. Many of these studies make use of competitive inhibitors of H_2O oxidation that have the ability to compete with and override H_2O oxidation without destroying the O_2 system. Experiments involving the interactions of NH_2OH and NH_2NH_2 derivatives with the intact O_2-evolving and Tris-extracted systems allowed us to map the intact O_2-evolving site. These data led to a model of the H_2O-binding site that is consistent with requirements of H_2O binding, and rationalizes some of the apparent anomalies observed for the O_2 system. An interesting consequence of our spectrophotometric studies is the finding that the kinetics of TMPD oxidation can be used to distinguish active, fully-competent O_2 centers from inactive (e.g., Tris-treated) centers simultaneously in the same preparation. We are currently using these techniques to characterize PS II preparations modified by the removal of specific polypeptides. Other studies in progress make use of labeled H_2O and H_2O_2 to characterize the O_2-evolving site. These studies will aid in our understanding of the chemistry of photosynthetic water oxidation.

University of Maryland
College Park, Maryland 20742

66. Energy-Dependent Calcium Transport Mechanisms in Plant Membranes
H. Sze **$63,600**
Department of Botany **(FY84 funds)**

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

University of Massachusetts
Amherst, Massachusetts 01003

67. Conversion of Cellulose to Ethanol by Mesophilic Bacteria
E. Canale-Parola, **$78,000**
C.E. Dowell, S.B. Leschine
Department of Microbiology

The project objective is to study the genetics of mesophilic anaerobic bacteria that ferment cellulose to ethanol. Bacteria used in this investigation are strains of *Clostridium* that (1) convert to ethanol not only cellulose, but also components of the hemicellulosic portion of biomass and (2) are relatively ethanol tolerant. Studies include plasmid analysis and fine-structure mapping of genes involved in the breakdown of cellulose to soluble sugars. These genes code for cellulase system enzymes such as endo-1,4-β-glucanases, exo-1,4-β-glucanases, β-glucosidases, and cellobiose phosphorylase. The research includes characterization of cellulase system enzymes present in ethanol-producing mesophilic clostridia as well as enzymes used by these bacteria in the breakdown of hemicellulosic components. This work will provide fundamental information on the genetics of cellulose-fermenting, ethanol-producing clostridia. This information will be valuable for understanding metabolic processes used by anaerobic bacteria to degrade cellulose, and

for developing clostridial strains that can be used in the industrial conversion of biomass to ethanol. Finally, the research will elucidate basic aspects of the genetics of clostridia. At present, very little is known about the genetic systems of this large group of anaerobic bacteria.

Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

68. ***Antibody Analysis of the* Rhizobium meliloti *Surface***
E.R. Signer **$85,000**
Department of Biology

The interaction of rhizobia with the roots of legumes to form nitrogen-fixing nodules shows considerable species-specificity, and is therefore likely to depend on the interaction between the surface of the two symbiotic partners. We use monoclonal antibodies to probe the surface of alfalfa symbiont *Rhizobium meliloti* SU47 to identify surface antigens involved in nodulation. Certain mutants selected as insensitive to monoclonal Ab6 have an altered surface, as shown by concomitant acquisition of resistance to several phages. These mutants form nodules on alfalfa, but the nodules fail to fix nitrogen. Light and electron microscopy show that these mutant nodules lack the root-hair curling and the intracellular infection threads characteristic of wild-type nodules. The mutants appear to invade directly through the epidermis, and within the nodule are found only between cells in superficial root tissue layers. Moreover, these mutants have been found identical to one class of mutants lacking an extracellular acidic heteropolysaccharide, isolated as unable to bind the fluorescent stain calcofluor-white. We have shown that at least some of the genes for expression of this polysaccharide are located on a previously uncharacterized megaplasmid that is distinct from a megaplasmid of similar size known to carry genes for nodulation and fixation. We are characterizing the genes for this polysaccharide and its physiological role with respect to the various stages of formation of the nodule. This is the first time a molecular species of known structure has been implicated in the nodulation process. Elucidation of its function should ultimately help the genetic manipulaton of *R. meliloti* for increased efficiency of nodulation and/or fixation of nitrogen.

Meharry Medical College
Nashville, Tennessee 37208

69. ***Respiratory Enzymes of* Thiobacillus ferooxidans**
R.C. Blake, Jr. **$81,200**
Biochemistry Department

Certain chemolithotropic bacteria inhabit ore-bearing geological formations exposed to the atmosphere and obtain all of their energy for growth from the dissolution and oxidation of minerals within the ore. Despite the environmental and economic importance of these organisms, very little basic information is available concerning the identity and disposition of the respiratory enzymes responsible for these activities. The aim of this research is to initiate the systematic isolation and characterization of the respiratory enzymes expressed by these chemolithotropic bacteria when grown on both reduced metal substrates and reduced inorganic sulfur compounds. We will examine the species, *Thiobacillus ferrooxidans*. Two proteins thought to be involved in the oxidation of ferrous ion (rusticyanin and cytochrome *c*) and one enzyme involved in the oxidative metabolism of reduced sulfur (rhodanese) will be isolated and characterized in detail. Four major experimental goals are proposed: (1) the kinetic characterization of the electron-transfer reaction between ferrous ion and purified rusticyanin (an acid-stable blue copper protein); (2) the kinetic characterization of the subsequent electron-transfer reaction between rusticyanin and a purified, acid-stable cytochrome *c* (both goals to be accomplished by stopped-flow spectrophotometric measurements); (3) the demonstration in extracts of *T. ferrooxidans* of all eight sulfur-oxidation enzymes currently known; and (4) the isolation of rhodanese (thiosulfate sulfur transferase), and the detailed description of its substrate specificity by steady-state kinetic measurements. The project will eventually contribute to a basic understanding of biological energy transduction. It can also provide useful information toward manipulating *T. ferrooxidans* and related organisms for commercial use.

Michigan State University
East Lansing, Michigan 48824-1101

70. ***Molecular Cloning of the Genes Encoding Certain Key Enzymes Involved in Lignin Biodegradation by* Phanerochaete chrysosporium**
C.A. Reddy **$58,400**
Department of Microbiology and Public Health

Lignin is a unique, heterogeneous, aromatic polymer and is the second most abundant renewable organic resource in the biosphere. Lignin degradation plays a central role in the biospheric carbon cycle and there is considerable potential for biotransformation of lignin into aromatic chemical feedstock. The project objective is to clone and characterize various genes (and their products) involved in lignin degradation by *P. chrysosporium* and to understand the regulation of expression of these genes. Initially, we will focus on isolating glucose oxidase-negative (gox^-) and ligninase-negative (lig^-) mutants and characterize these for their ability to degrade ^{14}C lignin to $^{14}CO_2$, and various other secondary metabolic characteristics. One of our goals is isolation of *LIG* and *GOX* genes by complementation of the above lig^- and gox^- mutants, respectively, with a *P. chrysosporium* genome library to be constructed in the later part of this study. Another area of focus will be to construct a cDNA library in yeast and *E. coli* expression vectors using the poly(A)$^+$ RNA isolated from idiophasic cells. Lig^+ yeast and *E. coli* clones will be detected by immunological screening procedure using purified ligninase antibodies. Work on production of specific antibodies to purified ligninase is in progress. Subsequently we plan to determine the complete structure of the cloned *LIG* gene(s). This research will contribute to a better understanding of the basic biology of lignin degradation and to the eventual commercial exploitation of biomass as a source of fuels and chemicals.

71. *One-Carbon Metabolism in Anaerobic Bacteria: Organic Acid and Methane Production*

J.G. Zeikus **$89,900**
Department of Biochemistry

This project seeks to understand one- and multi-carbon metabolism by acetogenic and methanogenic bacteria. Our studies with the acetogen, *Butyribacterium methylotrophicum*, focus on elucidating enzymes, carbon flow, and electron and carbon carriers of the pathways of acetate and butyrate synthesis during growth on substrates (e.g., glucose, pyruvate, methanol, formate, carbon monoxide, and hydrogen-carbon dioxide). Enzymes dealing with one-carbon oxidation–reduction reactions, glycolysis, and electron transfer are assayed in the organism after growth on various substrates. Mutants blocked in particular metabolic steps are isolated to confirm hypothetical pathways. Carbon flow into end products is traced using ^{13}C nuclear magnetic resonance spectroscopy. In our studies with the methanogen, *Methanosarcina barkeri*, we investigate the catabolism of acetate to methane to confirm a proposed pathway for this conversion process. Isotope tracer studies and other biochemical approaches are used in these studies. We also conduct electron flow and electron transport phosphorylation studies on *B. methylotrophicum* and *M. barkeri*.

Michigan State University/DOE Plant Research Laboratory
East Lansing, Michigan 48824

72. *Differential Gene Expression in* Bradyrhizobium

B.K. Chelm **$187,000**

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

73. *Resistance of Crop Plants to Environmental Stress*

A.D. Hanson **$181,000**

Plants have biochemical or metabolic adaptations to environmental stresses, as well as adaptations expressed at higher levels of organization. Biochemical and genetic understanding of metabolic adaptations to stress would permit their exploitation in crop improvement for stress environments, via conventional or recombinant DNA technologies. The project objectives are (1) to identify adaptive metabolic responses of plants to stress, (2) to find the enzymes and genes involved, and (3) to explore the effect on the whole plant of genetically modifying metabolic adaptations. We are investigating two metabolic responses to stress: betaine accumulation and lactate glycolysis. During water- and salt-stress, certain plants accumulate betaine. Indirect evidence indicates that betaine acts as a non-toxic cytoplasmic osmoticum during stress. We have found that betaine is synthesized in the chloroplast by a two-step oxidation: choline → betaine aldehyde → betaine. The oxidation reactions are stimulated by light. We are now studying the mechanisms of these reactions as a step towards isolating the enzymes involved. We have shown that moderate hypoxia induces lactate dehydrogenase (LDH) activity in cereal roots, apparently as a prelude to the onset of lactate glycolysis as more severely hypoxic conditions develop. We are now purifying barley LDH and raising antibodies against it to explore the induction process. Antibodies will also permit us to screen for LDH clones using an anaerobic cDNA library available in an expression vector.

74. *Action and Synthesis of Plant Hormones*

H. Kende **$226,000**

The project objective is to gain knowledge on the regulation of synthesis and mode of action of the plant hormones ethylene and cytokinin. These hormones regulate plant senescence and responses of the plant to stress. We have studied the enzymes that mediate the synthesis of ethylene from S-adenosylmethionine. The first enzyme in this pathway, 1-aminocyclopropane-1-carboxylate (ACC) synthase, is usually the limiting enzyme in ethylene synthesis. It can be induced by a variety of chemicals and conditions, including stress. We have purified this very important enzyme in plant development and are currently attempting to produce antibodies against it, enabling us to study its regulation at the molecular level. We are also investigating the enzyme responsible for ethylene formation from ACC. We have characterized some of its properties in isolated vacuoles and found that it requires membrane integrity and probably a transmembrane ion gradient. We are attempting to reconstitute it after breakage of membranes. The role of stress ethylene is being investigated in deepwater rice where low-oxygen stress occurring during submergence induces ethylene biosynthesis. Ethylene, in turn, mediates the growth response of submerged plants. We are attempting to localize the site of action of cytokinins using a cytokinin photoaffinity probe. We have developed several methods to synthesize such a probe, namely 8-azido-benzyladenine ($8N_3BA$). We are now radioactively labeling this compound. Preliminary experiments using [^3H]$8N_3BA$ indicate that this method could lead to the identification of the cytokinin receptor. Identification of such receptors will be attempted in cultured tobacco cells and in protonemata of mosses, both of which respond to cytokinins.

75. *Cell Wall Proteins*
D.T.A. Lamport **$139,000**

Recent work with tomato cell suspension cultures shows that during rapid growth there is a surprisingly large precursor pool of monomeric extensin bound ionically to pectin *in muro* but rapidly eluted from intact cells by dilute salt solutions. Facile extraction of these flexible rod-like macromolecules (visualized via EM) suggests their anticlinal (radial) orientation *in muro*. Thus the long axes of cellulose (warp) and extensin (weft) may be in perpendicular planes. The presence of the cross-linked amino acid isodityrosine in covalently bound extensin implies that cross-linkage of extensin monomers occurs *in muro*. We suggest that extensin networks of defined porosity form around microfibrils, thereby mechanically coupling the load-bearing polymers. Current work deals with the two extensin precursors P1 and P2 (i.e., their primary structure, EM visualization, and immunochemistry). P1, P2, and their HF-deglycosylated polypeptides dP1 and dP2 were antigenic, eliciting four sets of rabbit polyclonal antibodies that cross-react specifically with the glycosylated and non-glycosylated epitopes whose primary structure was further elucidated. After HF-deglycosylation, and tryptic degradation of dP1 and dP2, a relatively few major peptides dominate each peptide map: (1) P1/H5 Ser-Hyp-Hyp-Hyp-Hyp-Thr-Hyp-Val-Tyr-Lys; (2) P1/H20 Ser-Hyp-Hyp-Hyp-Hyp-Val-Lys-Pro-Tyr-His-Pro-Thr-Hyp-Val-Tyr- Lys; and (3) P2/H3 Tyr-Lys, P2/H4 Ser-Hyp-Hyp-Hyp-Hyp-Val-Tyr-Lys. Tryptic degradation was essentially complete; these major peptides represent extensively repeated units, hence a highly periodic polypeptide. The hexapeptide Val-Lys-Pro-Tyr-His-Pro domain of the P1 hexadecapeptide H20 is the prime candidate for intermolecular cross-linkage. If so the average intermolecular cross-link frequency corresponds remarkably to that predicted for a network penetrated by cellulose microfibrils.

76. *Regulation of Flowering*
A. Lang **$95,000**

This project studies promotive and inhibitory factors in flower formation, particularly graft-transmissible, hormone-like materials (florigen and antflorigen). The evidence for florigen and antflorigen is so far physiological, based primarily on grafting experiments between plants maintained in flowering and nonflowering conditions. The next obvious step would be to isolate the responsible chemical compounds and determine their structures. For florigen, this has been repeatedly attempted but so far without success. Our major effort studies isolation and identification of antflorigen; solution of this problem may aid in the search for florigen. A smaller related project concerns the problem (previously not resolved) whether a short-day plant, the Maryland Mammoth cultivar of tobacco, contained antflorigen as two related long-day plants, *Nicotiana silvestris* and *Hyoscyamus niger*. A separate project examines previous work showing that in many long-day and cold-requiring plants, treatment with the plant hormone gibberellin could substitute for the specific environmental condition normally required for flower formation. At least in one plant (the long-short-day plant *Bryophyllum daigremonatianum*) evidence has been obtained (by means of growth retardants, chemicals that inhibit gibberelin synthesis in plants) that gibberellin is an essential native factor for flower initiation. In other plants, however, growth retardants have not been found to have a specific effect on flower formation. With the recent advent of more potent growth retardants, we have resumed this line of work to determine whether or not gibberellin is more generally involved in the regulation of flower formation.

77. *Interaction of Nuclear and Organelle Genomes*
L. McIntosh **$187,000**

The development of energy-capturing complexes and carbon assimilation pathways in photosynthetic organisms requires sets of genes (located in separate genomes within a cell) to be regulated. We use two model systems to pursue gene-directed modification of these pathways. First, we study the genes encoding photosystem II (PS II) polypeptides in the photoheterotrophic, transformable cyanobacterium *Synechocystis* 6803. Several families of genes involved in PS II are characterized including, *psbA*, *psbD*, and the gene(s) encoding the extrinsic 33 kilodalton polypeptide. The promoter from *psbA-1* is used in a cyanobacterial expression vector for foreign genes in *Synechocystis*. Altered clones of *psbA* will be used to assign functional domains to the 32 kilodalton protein encoded by this gene. Secondly, we investigate the molecular basis of catalysis for ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) by expression of the *rbcL* and *rbcS* genes from *Anabaena* 7120 in *E. coli*. Both genes are expressed and give a fully functional Rubisco holoenzyme, which is approximately 0.6% of the soluble bacterial protein. Because the small subunit is found only in the higher molecular weight soluble form of Rubisco, we propose that the small subunit promotes assembly of the holoenzyme via heterodimers of large and small subunits.

78. *Sensory Transduction in Plants*
K.L. Poff **$139,000**

The project objective is to understand the mechanisms of acquisition of environmental information via light reception. We study the blue light photoreceptor pigment system(s), which control(s) numerous light responses such as phototropism in flowering plants. We use specific inhibitors and mutants as probes to dissect the initial steps in the transduction sequence. Work in progress with *Arabidopsis* demonstrates that phototropic bending can be increased using pulsed light separated by dark periods of about 15 minutes. This (1) permits the response to be increased without increasing the variability in response, (2) provides access into a specific step in the transduction sequence (the kinetic limitation), and (3) provides insight into the primarily time-dependent response known as second positive phototropism. Using multiple flashes of light to induce the response, we are selecting genetic mutants with altered phototropism. These mutants should permit positive identification of the photoreceptor pigment, access into the transduction sequence, and eventual understanding at the molecular level of the events from photoreception to the bending response.

79. *Physiological and Molecular Genetics of* **Arabidopsis**
C. Somerville **$195,000**

The overall project objective is to develop genetic methods for the analysis and modification of specific physiological processes in higher plants using the small crucifer *Arabidopsis thaliana* (L.) Heynh. as a model system. We use this conceptual framework to investigate several aspects of plant biology in which single gene variation may be a strong determinant of plant productivity. Due to the central role of RuBP carboxylase/oxygenase in both photosynthesis and photorespiration, we

attempt to create useful or informative variation in this enzyme by *in vitro* modification of several cloned RuBP carboxylase genes. We also attempt to more clearly define the role of several enzymes associated with photorespiratory metabolism by identifying mutants of *Arabidopsis* affecting these enzymes. We evaluate the applicability of theories that attribute chilling sensitivity or resistance of plant species to membrane composition by the isolation and characterization of single gene mutations that alter the leaf fatty acid composition of *Arabidopsis*. We explore the possibility that the alterations of photosynthetic rate, which often accompany experimentally induced alterations in the source/sink ratio, can be mimicked by mutations that alter the ability of the plant to partition photosynthate into starch or sucrose, respectively. Preliminary results suggest a connection between starch metabolism and the amount of photosynthate lost as respiration. We explore the possibility that genetic reduction of excess respiratory loss may be feasible.

80. Molecular Basis of Disease Resistance in Barley
S.C. Somerville **$126,000**

Plant diseases are considered a major limitation to crop yields. However little is known of the molecular basis of disease development or host resistance mechanisms. The long term goal of this project is to develop a description of the biochemical events of the infection process in compatible and incompatible combinations of barley cultivars and *Erysiphe graminis* f.sp *hordei* races. *E. graminis* is the causal agent of powdery mildew disease. The gene-for-gene hypothesis, which states that incompatible relationships between hosts and pathogens are established when a host resistance gene product interacts with a fungal gene product to trigger the host defensive mechanisms, forms the conceptual framework directing our studies of plant disease. Our approach is to identify a host resistance gene product, the *Reg 1* gene product, using monoclonal antibodies as probes for antigenic differences between resistant and susceptible congenic barley lines. Hybridoma supernatants are being screened by ELISA and immunofluorescence miscroscopy for antibodies reactive with (1) resistant barley tissue, (2) plasma membrane, or (3) epidermis. In addition, barley mutants suseptible to *E. graminis* race CR3 are being isolated from an M2 population of CI-16137 (carries the *Reg 1a1* resistance allele) to identify a null mutant. Such a mutant would facilitate the screen for the *Reg 1* gene product with monoclonal antibodies. Identification of the *Reg 1* gene product will permit cloning of the *Reg 1* gene, providing insight into the biochemical mechanisms of resistance.

81. Developmental Biology of Nitrogen-Fixing Algae
C.P. Wolk **$187,000**

Certain cyanobacteria (blue-green algae) trap solar energy via photosynthesis and use the resulting chemical-reducing power to fix atmospheric nitrogen gas (N_2). They thereby produce ammonia, which is used as the nitrogen source for cellular growth. The initial steps of N_2 assimilation take place in specialized cells called heterocysts. The project objective is to understand the supply of energy and electrons to, and the oxygen protection of, nitrogen fixation within heterocysts. Our approach makes use of our recently developed methodology for introducing cloned genes into nitrogen-fixing cyanobacteria (e.g., *Anabaena* PCC 7120) by conjugation from *Escherichia coli*. Conjugating, nitrogen-fixing isolates of *Nostoc* (ATCC 27896, 29133, and 29150) capable of heterotrophic growth may be suitable for genetic analysis of photosynthesis. Derivatives of *Anabaena* 7120 and *Nostoc* 29150 unable to fix nitrogen under aerobic conditions have been isolated. Analysis of these mutants is in progress. We have begun to map the chromosomal DNA of *Anabaena* ATCC 29413 by use of overlapping cosmid clones. In this strain, *nif* (nitrogenase) and *rbc* (ribulose bisphosphate carboxylase) genes, presumptively identified by hybridization with heterologous probes, are closely linked. Upon transfer to strains of *Anabaena* of *lux* genes from *Vibrio fischeri* and *Vibrio harveyi*, the cyanobacteria become capable of light production; *lux* genes therefore have potential for transcriptional reporting from cyanobacteria. We are developing tools for genetic analysis of photoautotrophic, nitrogen-fixing cyanobacteria. This work will increase understanding of cellular differentiation and construction of modified strains particularly suitable for commercial, biological conversion of solar energy.

82. Environmental Control of Plant Development and Its Relation to Plant Hormones
J.A.D. Zeevaart **$139,000**

Plant growth and development are affected by environmental factors such as day length, temperature, and moisture. Hormones often function as chemical messengers between the perception of an environmental factor and the morphological expression. The project objective is to characterize the hormones involved, how their synthesis and catabolism are regulated by the environment, and how these hormones act. Our studies of stem growth and flowering in rosette plants as regulated by gibberellins (GAs) have demonstrated with a cell-free system that photoperiodic induction of growth is based on the activity of enzymes that convert inactive GA precursors to active GAs (specifically the conversion GA_{19} to GA_{20}). This is a first step in the elucidation of the mechanisms that form the basis of photoperiodic control of plant growth. The objective is to determine how this enzyme activity is regulated by day length. In single-gene dwarf mutants of tomato and *Arabidopsis* we have tentatively determined, by feeding GAs and precursors, which steps in the GA biosynthetic pathway are blocked. GAs in the wild type of both species have been identified by GC-MS. We now can apply radioactive GAs and precursors to the mutants and use cell-free systems to determine the precise biochemical defects in the GA pathway. Our earlier work in regulation of abscisic acid (ABA) synthesis and catabolism suggests that stress-induced ABA is not synthesized directly from mevalonic acid. We are investigating the possibility that ABA is a breakdown product of xanthophylls with xanthoxin as intermediate. When turgor is restored, ABA is rapidly converted to phaseic acid. The objective is to prepare a cell-free system that catalyzes this step and then to determine how the activity of this enzyme is regulated by the water status of leaves.

University of Minnesota
Minneapolis, Minnesota 55455

83. The Mechanism of Switching from an Acidogenic to a Butanol–Acetone Fermentation by **Clostridium acetobutylicum**
P. Rogers **$74,500**
Department of Microbiology, Medical School

Clostridium acetobutylicum is an obligate anaerobic bacteria that ferments sugars to butyric and acetic acid early in vegetative growth, but switches to form only butanol, acetone, and ethanol during later phases of growth. The objective of this research is to elucidate the molecular mechanism used by these bacteria to regulate the synthesis and activity of key enzymes catalyzing reactions in this fermentation pathway. We will study the synthesis and degradation of enzymes directly involved in butanol formaton vs. butyric acid formation from butyryl–CoA. We find a 100-fold rise in two butanol-forming enzymes that correlate with butanol production dependent upon protein synthesis. Phosphotransbutyrylase and butyrate kinase activities change slightly, suggesting regulation of these enzymes at the level of activity; the enzymology of this system is under study. Allyl alcohol-resistant mutants have been isolated that show altered dehydrogenases and make new fermentation products such as butyraldehyde; these mutants are under study. We are developing a protoplast fusion system and DNA transformation system for *Cl. acetobutylicum* to study the genetic factors involved in expression and regulation of the fermentation enzymes. Using rifampicin-resistant and streptomycin-resistant mutants, we are developing protoplast-formation, protoplast-regeneration, and protoplast-fusion methods for *Cl. acetobutylicum*. The method for protoplast regeneration after treatment with polyethylene glycol has been accomplished, and protoplast fusion is under study. Using a *Staphylococcus* plasmid, PUB110, Km^R, we are studying DNA transformation of protoplasts of *Cl. acetobutylicum*, strain B643.

University of Minnesota
Navarre, Minnesota 55392

84. Genetics of Bacteria That Utilize Carbon Compounds
R.S. Hanson **$65,400**
Gray Freshwater Biological Institute

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

University of Minnesota
St. Paul, Minnesota 55108

85. Molecular Approaches to Genomic Organization
I. Rubenstein **$160,300**
Department of Genetics and Cell Biology **(2 yrs.)**
and
R.L. Phillips
Department of Agronomy and Plant Genetics

The classical genetic map of the maize genome is one of the most extensive of any eukaryotic organism. However, it is deficient compared with the potential that exists for its enhancement by the coupling of molecular biology with classical genetics and cytogenetics to identify and map additional chromosomal markers. The approach seeks to identify physical chromosomal markers using hybridization probes consisting of cDNA and genomic clones of known function (e.g., zein) and cDNA clones prepared from the mRNAs of a maize suspension cell culture and their corresponding genomic clones. These should be represented in our complete cosmid genomic library. The chromosomal location of each of these probes will be determined using quantitative hybridization to DNAs from aneuploid plants. The probes will also be used to detect restriction fragment length polymorphisms at given loci to serve as additional physical markers for inbred maize lines. The long-range goal is to construct a comprehensive physical map of the macroorganization of maize chromosomes by the mapping of these physically identifiable regions relative to one another and to the classical genetic map. Initial studies will utilize repeated sequences; methods for localizing unique gene sequences by *in situ* hybridization is a long-term goal, initiated after the technology for low copy number genes is further developed. Studies will be initiated to use these markers in testing backcross recovery rates.

University of Missouri
Columbia, Missouri 65211

86. Photosynthesis, Cloning, and Bioconversion of Solar Energy in Cyanobacteria
L.A. Sherman **$68,200**
Biological Sciences Division

This project identifies and clones the genes coding for membrane proteins involved in cyanobacterial photosynthesis. Cyanobacteria perform an aerobic photosynthesis nearly iden-

tical to green plants, and are suitable for genetic, biochemical, and biophysical studies in photosynthesis. The strains used for these studies, *Anacystis nidulans* R2 and *Aphanocapsa* sp., are both transformable; *Aphanocapsa* can be grown heterotrophically and photoheterotrophically, and is thus ideal for the isolation of photoshythesis mutants. We investigate three areas related to membrane structure and genetics in *Aphanocapsa*: (1) membrane protein composition, conjugation; (2) transposon mutagenesis; and (3) mutant isolation. We determine the protein composition of the photosynthetic complexes, including PS I, PS II, cyt b_6/f, and the phycobilisomes. We analyze many major proteins with antibodies produced against related proteins from chloroplasts, and now have a thorough knowledge of the protein composition of the membrane. In addition to the 51 kDa proteins found in chloroplasts, the *Aphanocapsa* complex contains a large quantity of a 36 kDa protein. This protein is considered important for overall PS II photochemistry. We also develop a conjugation system (patterned after Wolk) for the random insertion of transposons into the chromosome. The conjugative plasmid, pMD100, contains transfer functions, a kanamycin resistance gene, and Tn*501*, a transposon coding for mercury resistance. This plasmid can transfer from *E. coli* to *Aphanocapsa*, and transposition of Tn*501* occurs at low frequency. We detect random transposition of one copy of Tn*501* per chromosome, indicating that this system is capable of generating many independent lesions. Because of the growth conditions, this system is amenable to the isolation of PS II and O_2-evolving mutants.

Mount Sinai School of Medicine
New York, New York 10029

87. *The Respiratory Chain of Alkalophilic Bacteria*
T.A. Krulwich **$76,900**
Department of Biochemistry

The respiratory chain of alkalophilic bacteria is present in very high concentration in the membranes of those bacilli and appears to effectively transduce energy via proton pumping. Current studies of the respiratory chains of alkalophilic *Bacillus firmus* RAB focus on the purification, characterization, and reconstitution of specific respiratory chain complexes. A genetic approach to problems of the regulation of cytochrome levels is being initiated. The overall goals of the study are to understand the special structual/functional adaptions that these bacteria may possess and to probe the basis for apparent ph-dependent synthesis of cytochromes. The cytochrome *c* oxidase (a cytochrome aa_3) has been purified to homogeneity after extraction from the membrane. This complex contains major subunits with M_r values of 56,000 and 40,000, 2 moles of heme *a*, 2 moles of copper, and 1 mole of bound cytochrome *c*. A second cytochrome *c*, distinguishable by α-band but not redox potential, has been partially purified from salt washes of the membranes. Studies of the cytochrome oxidase in a reconstituted system are underway, and studies of complex III are planned. Mutants and genetic transformants that may illumiate regulatory questions are being isolated.

National Institutes of Health
Bethesda, Maryland 20205

88. *Partial Support of GenBank: The Genetic Sequence Data Bank*
C. Carrico **$40,000**
National Institute of General Medical Science

GenBank, the Genetic Sequence Data Bank, is an internationally available repository of all reported nucleotide sequences greater than 50 nucleotides in length, annotated for sites of biological interest, and checked for accuracy. As of May 1, 1985, GenBank contained 4.31 million bases, comprising 4,954 sequences. The data bank is operated under contract to Bolt Beranek and Newman Inc. (BBN) of Cambridge, MA. Data collection, verification, entry, and annotation is performed under the direction of Dr. Walter Goad at Los Alamos National Laboratory; while distribution, user support services, and overall data bank management are performed by BBN. This resource, administered by the National Institute of General Medical Sciences, and co-sponsored by five institutes of NIH, two divisions of DOE, the National Science Foundation, and the Department of Defense, is of particular interest to geneticists and molecular biologists. A copy of the data base is available for a modest fee on computer-readable magnetic tape to anyone requesting it, and will be available in late 1985 on IBM-compatible floppie disks. Dial-up on-line access is also available to anyone, but only a limited number of users can be accommodated at any one time. A software clearinghouse is provided on-line to supply information only on sequence analysis software available worldwide. The second yearly hard copy edition of the data base was available in April 1985, as a four-volume supplement to *Nucleic Acids Research*.

University of Nebraska
Lincoln, Nebraska 68588

89. *Developmental Regulation of Plant Plasma Membrane Antigens*
D.W. Galbraith **$73,000**
School of Biological Sciences

Understanding the molecular architecture and developmental behavior of the plant plasma membrane is central to understanding plant growth and development and the interactions of plants with the environment. This project centers around a molecular characterization of those proteins and glycoproteins specifically located at the *Nicotiana* plasma membrane. We have employed protoplasts as immunogens for the production of monoclonal antibodies. Using enzyme-linked and indirect immunofluorescence screening, we have identified and isolated hybridomas secreting antibodies directed against antigens located on the protoplast surface. We have determined that the expression of some of these antigens is under developmental control: antigens found on the plasma membranes of heterotrophic but not photoautotrophic cells. We are characterizing the ultrastructural location of these antigens in both the isolated protoplasts and intact plant organs. We are purifying the antigens and their precursors through immunoprecipitation and gel electrophoresis under denaturing conditions following either *in vivo* and *in vitro* labeling with radioactive protein precursors, or conventional cell surface iodination. We are also constructing

cDNA libraries in plasmid and phage expression vectors to permit eventual identification and isolation of the relevant genes, as well as an analysis of the mechanism of developmental control. Finally, we are using flow cytometry and cell sorting of protoplasts *in vivo* to allow the isolation of mutant cell lines deficient in the production of the plasma membrane antigens. In this way, we will provide the basis for further understanding of the mechanisms controlling cell growth and division in higher plants.

90. *Viruses of Eukaryotic Green Algae*

J.L. Van Etten **$70,000**
Department of Plant Pathology

PBCV-1 is a large dsDNA-containing (ca. 300 kbp) plaque-forming virus that replicates synchronously in a unicellular *Chlorella*-like green alga, strain NC64A. The PBCV-1–*Chlorella* system is the first example of a virus infecting a eukaryotic photosynthetic organism that can be studied with procedures directly adapted from those used to study bacteriophages. *In vivo* and *in vitro* studies have established that PBCV-1 encodes for an enzyme with restriction endonuclease-like activity and an enzyme(s) with methyltransferase activity. The PBCV-1-encoded methyltransferase transfers methyl groups to adenyl groups in the sequence GATC. The PBCV-1 encoded restriction enzyme recognizes and cleaves the sequence GATC but will not cleave G^{m}ATC. Host DNA contains GATC sequences but PBCV-1 DNA contains G^{m}ATC. Thus PBCV-1 apparently encodes a restriction and modification system that may be analogus to similar systems in bacteria. The project objectives are (1) to isolate, characterize, and clone the PBCV-1-encoded restriction modification enzymes and (2) to compare them to bacterial enzymes that recognize the same sequences. We also will examine numerous new and distinct viruses (which we have isolated and that infect the same host) for restriction-modification systems.

New York Department of Health
Albany, New York 12201

91. *Methane-Producing Bacteria: Immunological Characterization*

E. Conway de Macario, **$58,000**
A.J.L. Macario
Laboratory of Immunology
and
M.J. Wolin
Laboratory of Environmental Biology and Field Services

The long-term goal of this research is to establish which methanogens occur in complex microbial communities of biotechnologic importance. Current efforts focus on development of methodology for immunologic identification with rabbit antisera. The project comprises standardization of procedures (1) for sampling bacterial cultures, organic wastes, and digesters; and (2) for examining the materials obtained directly, avoiding enrichment-isolation. The purpose is to obtain first-hand information on the methanogenic population as it occurs prior to manipulations that might distort its profile. Antigenic fingerprinting with polyclonal antibody probes is applied to characterize the bacteria. Antigens released by the latter into their fluid environment are being measured by inhibition-blocking methods to evaluate the usefulness of these methods using antisera. Immunologic information from bacteria and fluid-phase analyses is compared with microbiologic, chemical, and engineering data. These studies are yielding novel methodology, reagents (polyclonal antibody probes), and data that should help in designing bioreactors; in their on-site monitoring when in operation; and in guiding genetic engineering for strain improvement.

State University of New York/ Binghamton
Binghamton, New York 13901

92. *Gene–Enzyme Relationships in Somatic Cells and Their Organismal Derivatives in Higher Plants*

R.A. Jensen **$95,000**
Center for Somatic-Cell Genetics and Biochemistry

The aromatic amino acid pathway is of central importance to higher plant metabolism because it generates growth regulators, a diverse array of secondary metabolites, and medically-significant drugs in addition to the three aromatic amino acids and a number of essential vitamins. *Nicotiana silvestris* has been studied at both the organismal and tissue-culture levels, and the enzymatic steps of the entire pathway have now been identified. Prephenate aminotransferase, arogenate dehydrogenase, and arogenate dehydratase have been characterized; results indicate that *L*-arogenate is probably the sole or major precursor of *L*-tyrosine and *L*-phenylalanine. Results obtained with spinach, buckwheat, duckweed, corn, and mungbean indicate that higher plants (in general) employ *L*-arogenate as a major precursor of numerous natural products. 3-Deoxy-*D-arabino*-heptulosonate 7-phosphate (DAHP) synthase, shikimate dehydrogenase, 5-enolpyruvylshikimate 3-phosphate synthase, chorismate mutase, and anthranilate synthase have also been characterized, emphasizing expression of isozyme forms, allosteric regulation, and subcellular localization. Approaches of differential centrifugation of mesophyll protoplasts and cultured-cell protoplasts, as well as enzymatic analysis of washed, intact chloroplasts show that a complete pathway network of enzymes exists within the plastid compartment. This pathway is tightly regulated by allosteric control of DAHP synthase and other branchpoint enzymes. Evidence exists that a separate, unregulated network of enzymes is located in the cytosol. This pathway may supply precursors for secondary metabolism via a simple overflow mechanism.

Northwestern University
Evanston, Illinois 60201

93. *Genetics of Thermophilic Bacteria*

N.E. Welker **$60,100**
Department of Biochemistry, Molecular Biology and Cell Biology

The ability to genetically manipulate thermophilic bacilli will profoundly affect investigations of the biochemical mechanisms of thermophily, and facilitate studies of other thermophiles without a reliable genetic exchange system or with growth char-

acteristics unsuitable for research. This project focuses on *Bacillus steorothermophilus*. A protoplast fusion system has been developed for strain NUB 36. Analysis of recombinants from two-, three-, and four-factor (auxotrophic markers) fusion experiments revealed that they are stable haploids, the exchange of genetic information is random and bidirectional, the coinheritance frequency of unselected marker pairs is reproducible, and biparentals occur with a relatively low frequency. The relative genetic map of the linkage group *his-1 gly-1 pur-1* (responds to hypoxanthine or guanine) *pur-2* (responds to adenine) has been established by protoplast fusion. The *his gly* and *pur-2* markers seem to be arranged similarly to analogous markers on the *Bacillus subtilis* chromosome (*his* A *gly* C *pur A*), with no analogous *pur-1* marker in *B. subtilis*. Strain NUB 36 contains a cryptic plasmid (approximately 30 Kd) and a restriction endonuclease. Nonrestricting mutants have been isolated and several unsuccessful attempts made to transfer thermophilic plasmid pTB90 (Tc^rKm^r) into a nonrestrictive mutant of NUB 36 by protoplast fusion. Since plasmid incompatibility with the resident plasmid may explain these results, we are in the process of curing this strain of its plasmid. A nonrestricting mutant cured of the cryptic plasmid can be used for plasmid DNA transformation and cloning and genetic analysis of specific genes associated with the maintenance of cell growth at elevated temperature.

Oak Ridge National Laboratory
Oak Ridge, Tennessee 37831

Environmental Sciences Division

94. ***Energy and Nutrient Utilization Efficiency in Intensive Forest Biomass Production***
S.B. McLaughlin, **$200,000**
D.W. Johnson, N.T. Edwards, R.J. Luxmoore, R.F. Walker

This study addresses plant physiological and soil–plant nutrient processes that are important considerations in using marginal land for silvicultural energy production. The objective is (1) to provide physiological criteria for quantifying yield potential of biomass candidate species and (2) to determine the role of soil–plant nutrient dynamics on productive potential. Yellow poplar (*Liriodendron tulipfera*), loblolly pine (*Pinus taeda*), and black locust (*Robinea pseudoacacia*) are studied in a 20 ha managed population. Treatments examined include mycorrhizal preconditioning, variable rate and timing of nitrogen supply, and mulching. Parameters measured include whole-tree allocation of energy between metabolic pools, plant water status, leaf photosynthetic rate, photosynthate allocation by foliage, and the kinetics of nitrogen mobilization and movement through the soil–plant nutrient cycles. Results on nitrogen utilization by poplar and pine indicate that total nitrogen uptake and within-plant allocation are influenced by the timing of fertilizer application. Nitrogen uptake efficiency was decreased by distributing the 100 kg N Ha^{-1} y^{-1} in four 25 kg quarterly applications, which enhance microbial nitrification and loss of nitrogen to groundwater. A single 100 kg application resulted in increased shoot:root nitrogen allocation in poplar but not pine. Analyses of samples collected to characterize seasonal cycles of tissue biochemistry, differences in photosynthetic capacity of foliage, and patterns of photosynthate allocation are continuing. These data will elucidate the interactions involved in seasonal allocation of carbon, water, and nutrients for representative biomass candidate species.

Ohio State University
Columbus, Ohio 43210

95. ***Characterization of the Organization of the Genome of Methanogens and Development of Genetic Exchange Systems for* Methanococcus vannielii**
J.N. Reeve, J.I. Frea **$70,000**
Department of Microbiology

The project objectives are (1) to determine the structure of methanogen genes, (2) to develop laboratory procedures to facilitate the introduction of DNA into methanogens, and (3) to use genetic engineering techniques to manipulate genes involved in methane biogenesis. We have cloned and sequenced DNA fragments from *Methanobrevibacter smithii*, *M. thermoautotrophicum*, and *M. vannielii*. Genes in these species are organized into multi-gene transcriptional units. Protein synthesis is apparently initiated on polycistronic mRNA (as in eubacteria) via ribosome binding sequences. The physical structure of methanogen mRNAs resembles the structure of mRNA in eubacteria (i.e., unstable molecules with only short 3′ polyadenylation tracts and no 5′ capped nucleotides). Putative methanogen promoter sequences have been identified by comparison of sequences preceding cloned genes. DNA-dependent RNA polymerase, purified from methanogens, is used to identify RNA polymerase binding sequences. *In vivo* sites of transcription initiation are determined using S1 protection analysis. Development of a genetic exchange system for methanogens requires the availability of selected genetic traits, a procedure to introduce DNA into methanogens, and a mechanism to ensure the stable replication and expression of DNA introduced into a methanogen. Screening of antibiotics has identified five compounds as possible selective agents. Neomycin resistance is used as a selective trait with the methanogen *M. vannielii*. We have constructed a wide range of plasmids with neomycin resistance determinants, which are capable of replication in many microbial species and should (if successfully introduced) replicate in methanogens. Current experiments use these plasmids as donor DNAs in a range of transformation and conjugation protocols.

University of Oklahoma
Norman, Oklahoma 73019

96. ***Metabolism and Bioenergetics of* Syntrophomonas wolfei**
M.J. McInerney **$75,000**
Department of Botany and Microbiology

The degradation of fatty acids is often the rate-limiting step in the conversion of organic matter to methane and carbon dioxide. Fatty acids are degraded in anaerobic environments by hydrogen-producing syntrophic bacteria. Little is known about the physiology of these bacteria because of their slow growth rate and the fact that they can only be grown in coculture with hydrogen-using bacteria such as methanogens. We have devel-

oped methods to mass culture the anaerobic, fatty acid degrader, *Syntrophomonas wolfei* in coculture with the hydrogen-using *Methanospirillum hungatei* so that sufficient cellular material can now be obtained for biochemical analyses. Small amounts of *S. wolfei* cells can be physically separated from *M. hungatei* cells using density-gradient centrifugation. Large amounts of cell-free extracts of *S. wolfei* free from contamination by cellular components of *M. hungatei* are obtained by lysozyme treatment. Cell-free extracts of *S. wolfei* have very high specific activities of the following beta-oxidation enzymes when assayed with short-chain acyl-coenzyme A derivatives: acyl-coenzyme A dehydrogenase, enoyl-coenzyme A hydratase, L-3-hydroxyacyl-coenzyme A dehydrogenase, and thiolase. Increasing the amount of acetate added to the medium from 0 to 65 millimolar decreases the amount of growth but not the amount of butyrate degraded by the *S. wolfei–M. hungatei* coculture. Tricultures containing *S. wolfei*, *M. hungatei*, and *Methanosarcina barkeri* have faster specific growth rates and higher yields of *S. wolfei* per mole of butyrate degraded than cocultures without *M. barkeri*. These data provide indirect evidence that acetate excretion is important in the bioenergetics of *S. wolfei*.

Oregon State University
Corvallis, Oregon 97331

97. Genomic Variation in Maize
C. Rivin **$60,000**
Department of Botany and Plant Pathology

The repetitive portion of the maize genome may be highly plastic. In a comparison of nuclear DNA from different inbred lines, we have shown that many repetitive sequences are quantitatively variable while others are constant. Quantitative polymorphisms are stable within inbred lines, but unexpected copy numbers are observed in the progeny of specific outcrosses and in tissue culture cells. The regularity of the genomic changes suggests that the variation may be a regulated process in maize. The project objective is to investigate the molecular and genetic basis for repeated sequence modulation. Two approaches will be taken using a collection of cloned maize repeated sequences. First, we will try to identify features of molecular organization, function, and DNA modification that characterize those repeats that are subject to variation in contrast to those of sequences that are invariant. Second, we will examine the genomic changes themselves by quantitative DNA hybridization and Southern blotting to determine when in the development of the F1 plant the modulation occurs, how it affects the restriction and modification patterns of specific sequences, and whether novel multiplicities that appear in F1 plants are stable in succeeding generations. Pedigree analysis of this data may reveal whether the process is genetically regulated. The same techniques will be applied to cells in culture to learn how the genotype and cell environment influence genome stability. These experiments will provide new and more comprehensive information on genome plasticity in maize and its implications for the genetic manipulation and molecular evaluation of this major crop plant.

University of Pennsylvania
Philadelphia, Pennsylvania 19104

98. Factors Governing Light-Driven Electron and Proton Translocation in Protons Across Membranes
P.L. Dutton **$83,000**
Department of Biochemistry and Biophysics

Membrane redox proteins (e.g., photosynthetic reaction centers, ubiquinol-cytochrome *c* oxidoreductases, and various terminal oxidases) separate charge across the membrane coupled to electron transfer. These kinds of enzymes represent the primary battery of energy conversion systems in virtually all forms of life. The goal of this research is to develop experimental methods to study these enzyme systems in planar arrays so that the individual redox-linked charge-separating steps are resolved and can be studied individually. It is important that the planar arrays can be placed between electrodes and the electrical responses measured directly rather than using the traditional indirect methods. Planar arrays can be quantitatively and systematically manipulated by applying electric fields. We have concentrated on reaction centers from photosynthetic bacteria to develop strategies for deposition of the protein on solid electrode supports in ordered arrays. Flash-activation of such films followed by measurement of current or voltage in the presence of applied fields is opening up new and promising views of the early events in the reaction center. A further complementary line of work is based on the use of modified enzymes: all of the above enzymes contain quinones functional in key positions, and methods are being developed to replace them with alternatives that are electrochemically and systematically varied. We intend to bring (to all the enzymes) the invaluable capability to activate via light flashes or voltage pushes.

Pennsylvania State University
University Park, Pennsylvania 16802

99. The Role of Turgor Pressure in Plant Growth
D.J. Cosgrove **$75,600**
Department of Biology

Water availability plays a key role in plant growth. At the cellular level, water stress is thought to inhibit growth by reducing the cell hydrostatic pressure (turgor pressure) necessary for irreversible expansion of cell walls. It is also possible that water stress makes the cell walls less extensible, such that they expand more slowly at any given turgor pressure. This project investigates the relationship between turgor pressure and plant growth by defining the physical factors that control growth when water is plentiful and when it is lacking. We are characterizing stem growth of young pea seedlings (*Pisum sativum* L.) as a function of water stress, which is imposed on the plants by transplanting them to vermiculite containing various amounts of water. Direct measurements of turgor pressure in individual growing cells are made using the pressure microprobe. Improvements to the pressure microprobe are being made by placing some of its functions under computer control. To measure the wall properties relevant to growth (wall extensibility and wall yield threshold), we use a novel *in vivo* stress relaxation method. Recent results show that the rate of relaxation depends directly on the value of the wall extensibility,

whereas the yield threshold determines the final relaxation state. Stress relaxation experiments are carried out using various parts of the growing region of the stem, to examine the basis for the gradient in growth rate that occurs along the stem axis. Tissue hydraulic conductance is estimated from the kinetics of tissue swelling or shrinkage when the tissue is challenged with a lower or higher water potential. These studies will identify the physical basis for reduced growth when water is limiting, and may provide insight into the nature of the wall properties that control growth.

Purdue University
West Lafayette, Indiana 47907

100. Regulation of Protein and mRNA Metabolism in Salt Tolerant and Intolerant Cultured Higher Plant Cells

R.A. Bressan, A.K. Handa, **$80,000**
P.M. Hasegawa
Department of Horticulture
and
T.H. Ulrich, G.J. King, T. Helentjaris
Native Plants, Inc., Salt Lake City, UT

We have characterized the 26 kD polypeptide associated with salt and water stress adaptation in tobacco cells. Using anti 26 kD purified IgG we have demonstrated a quantitative correlation between the levels of adaptation and accumulation of 26 kD protein. ABA-induced 26 kD polypeptide is immunologically similar to the 26 kD protein synthesized by adapted cells, these proteins produce similar peptides upon partial proteolysis. Several monocot and dicot species produce immunologically cross-reactive 26 kD polypeptide in response to ABA. There is a close association between the onset of increase of endogenous ABA and time of synthesis of 26 kD polypeptide. Tissues of plants regenerated from adapted cells produce more 26 kD polypeptide than tissues of plants regenerated from unadapted cells. An aqueous and a detergent-soluble form of 26 kD protein are present in adapted cells. The first 34 N-terminal amino acids of these polypeptides are identical except at position 23 where glycine is replaced by threonine in the detergent-soluble form. Differences in the proteins of *in vitro*-translated mRNAs from adapted and ±ABA-treated unadapted cells are greatly enhanced when mRNA is *in vitro* translated in the presence of 80 mM NaCl (a close estimate of the cytoplasmic NaCl concentration of adapted cells). We have also made antibody against three other major polypeptides associated with NaCl adaptation. An oligonucleotide probe has been made using the sequence of the 26 kD protein, which we use to screen a tobacco genomic library and will use to screen a cDNA library we are constructing in λgt10. We have purified cyanogen bromide and lys-C cleavage products of the 26 kD protein for extending the amino acid sequence determination. A cDNA library was constructed in pUC-8; three cDNA clones have been isolated by differential screening with mRNA from adapted and unadapted cells.

Rockefeller University
New York, New York 10021

101. Nuclear Genes from Nicotiana *Encoding the Small Subunit of Ribulose-1,5-Bisphosphate Carboxylase*

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

A major product of leaf nuclear gene expression is the small subunit (rbcS) of RuBP carboxylase. rbcS is made as a precursor polypeptide on cytoplasmic ribosomes. This precursor is characterized by an amino terminal extension known as the transit peptide, which mediates posttranslational import into chloroplasts. In collaboration with the laboratory of Drs. Van Montagu and Schell in Belgium, we have demonstated that the rbcS transit peptide can mediate the import into chloroplasts of a foreign polypeptide. We have introduced into tobacco cells a chimeric gene encoding a fusion protein consisting of the rbcS transit peptide linked to the bacterial protein neomycin phosphotransferase (NPT) using *Agrobacterium*-mediated transformation. Chloroplasts isolated from transformed tissue were shown to contain NPT activity. The experiments demonstrate the feasibility of introducing into chloroplasts polypeptides that have potential agricultural interest and are not normally encoded into the nucleus. The expression of the rbcS gene is induced by light; using *Agrobacterium*-mediated transformation of tobacco cells, we previously demonstrated that an rbcS promoter fragment confers light-inducibility to a chimeric gene. We have now extended these studies to examine the expression of chimeric genes containing truncated promoters, by demonstrating that sequences at least 722 bp 5′ from the rbcS cap site are required for maximum levels of expression. We have shown that these upstream regulatory sequences have characteristics similar to the enhancer sequences of certain animal viral and cellular genes. Upon inversion, these sequences still confer light-inducibility and (when ligated to the promoter of the constitutively expressed nopaline synthase gene) they promote light-inducibility. These and related enhancer-like sequences are likely to play an important role in the future genetic manipulation of plant species.

Rutgers University
New Brunswick, New Jersey 08903

102. Development of Cellulase Monoclonal Antibodies

D.E. Eveleigh, J.D. Macmillan **$63,900**
Department of Biochemistry and Microbiology

Cellulose is a major component of biomass and a potential energy resource. It can be hydrolyzed via acid or enzyme to glucose, and then fermented to a variety of energy-sparing products. Cellulases that facilitate hydrolysis are comprised of several components, which results in complexities in their study. The focus of this project is to utilize immunologic approaches to facilitate this purification and characterization of cellulases. The cellulase of *Trichoderma reesei* is used as a model system. Cellobiohydrolase is a major component of the cellulolytic complex produced by *Trichoderma reesei*, and since there are no direct methods to assay for it, we initially developed hybridomas that yield monoclonal antibodies to this enzyme. Antibodies from cell line 8B3 were purified using Protein

A-Agarose, then used to prepare immunoaffinity columns for direct isolation of cellobiohydrolase from crude culture broth. These monoclonal antibodies have also been used successfully to monitor *in vitro* synthesis of cellobiohydrolase via Western blotting. Current studies focus on developing an ELISA for this enzyme. A positive system has been developed using monoclonal antibody-horseradish peroxidase components, but as yet not quantitatively. Monoclonal antibodies have also been prepared towards *Trichoderma reesei* endoglucanase by fusing mouse spleen cells with myeloma cells (FOX–NY). The monoclonal antibodies are being similarly developed for the detection and purification of endoglucanases. These immunologic approaches vastly simplify the purification, detection, and analysis of the multi-component cellulase system, and will facilitate morphologic studies of synthesis and action of cellulase in the degradation of cellulose.

Rutgers University
Piscataway, New Jersey 08854-0759

103. Corn Storage Protein: A Molecular Genetic Model

J. Messing **$100,000**
Waksman Institute of Microbiology

We are studying the zein multigene family in maize on the molecular level to investigate regulatory circuits that are specific for the development of maize endosperm. These genes are only expressed in endosperm tissue and raise to about 50% of total protein in the corn kernel during a fixed developmental window; due to their abundance they determine the nutritional quality of corn. A smaller component of this zein fraction (a protein of 10,000 dalton (dt) molecular weight) is very high in methionine, an essential amino acid that is limiting in animal feed. Some variants of maize that have a high methionine content in their kernel also have a higher level of the 10,000 dt zein. We have purified the protein and obtained a partial amino acid sequence that reflects the high methionine content. We are using an oligonucleotide designed from this amino acid sequence to screen our cDNA library for a cDNA clone. We will use this approach to analyze the nature of the overproduction of the 10,000 dt zein.

Smithsonian Institution
Rockville, Maryland 20852-1773

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

E. Gantt **$60,900**
Environmental Research Center

Phycobilisomes are the primary photosynthetic antennae systems in red algae and cyanobacteria. Their association with photosystem II, previously expected from energy transfer measurements, has now been established in our laboratory by isolation of photosystem II phycobilisome particles. In particles from *Porphyridium cruentum* green light, absorbed phycoerythrin, was most effective in driving O_2 evolution and 2,6-dichlorophenol indophenol reduction. The chlorophyll composition, ca. 90 Chl/particles, is consistent with the small PS II Chl-antennae size. Enrichment of a 50 kD polypeptide was obtained by removal of phycobiliproteins. This peptide is considered the putative PS II reaction center. Phycoerythrin-deficient mutants of a red alga are studied to determine if (and how) the phycobilisome composition, size, and number affects the photosynthetic capacity. A comparison was made of phycobilisome size and composition in two cyanobacteria that are capable of chromatic adaptation. In *Tolypothrix*, the size remained constant by an apparent substitution of phycoerythrin for phycocyanin. In *Nostoc*, the phycobilisome size decreased with the decrease of phycoerythrin (in red light). The high molecular weight phycobilisome terminal pigment protein, with a fluorescence emission of ca. 675 to 683 nm, has been identified in numerous red algal and cyanobacterial species. Although there is considerable heterogeneity in the size (76 to 120 kD) of this protein, immunocrossreactivity has been established. At least one common immunoreactive site is present on each peptide, probably in a highly conserved sequence region. Results support the hypothesis that cyanobacteria are progenitors of red algal chloroplasts.

Solar Energy Research Institute
Golden, Colorado 80401

Photoconversion Research Branch

105. The Water-Splitting Apparatus of Photosystem II

M. Seibert **$110,000**

The water-oxidizing (or O_2-evolving) supramolecular complex of photosystem II (PS II) supplies reductant to algae and plant cells during photosynthesis. We use biophysical, biochemical, and biological approaches to identify the components of the complex, to determine how the components are related structurally, and to understand the mechanism of O_2 evolution. Experimental materials are thylakoids, O_2-evolving PS II membranes, and PS II reaction center core complexes from *Scenedesmus* and spinach. Current work has distinguished a 33 kDa extrinsic protein from a 34 kDa intrinsic protein, both of which are required for O_2 evolution but which were thought to be a single protein. Neither should be confused with the 32 kDa, herbicide-binding protein functioning on the reducing side of PS II. Mutant studies have identified the 34 kDa protein as part of the PS II core complex in *Scenedesmus* and demonstrated that this protein, not the herbicide-binding protein, is tagged by azido[^{14}C]atrazine using the normal photoaffinity labeling technique. We have also demonstrated the first successful use of surface-enhanced Raman scattering (SERS) spectroscopy to probe the surface of a biological membrane. This work identified a SERS signal related to half the manganese (required for O_2 evolution) found in PS II membranes, and the source of the signal was localized between the 33 kDa extrinsic and 34 kDa intrinsic proteins.

Southern Illinois University
Carbondale, Illinois 62901

106. Regulation of Alcohol Fermentation by Escherichia coli

D.P. Clark **$70,000**
Department of Microbiology

The purpose of this project is to elucidate the way in which the fermentative synthesis of ethanol is regulated in the facultative anaerobe *Escherichia coli*. Focus is on the two final steps in alcohol synthesis, which are catalyzed by alcohol dehydrogenase and acetaldehyde CoA dehydrogenase. We have isolated a series of mutations affecting the expression of these enzymes. Some of these mutations are in the structural genes for these enzymes; others affect the regulation of the *adh* operon. We are investigating the properties of a gene fusion to the *adh* operon in order to understand the regulation of these genes. We have also isolated a comprehensive collection of operon fusions in which the *lacZ* structural gene is fused to promoters that are inactive aerobically but active anaerobically. Although these genes (like *adh*) are only expressed under anaerobic conditions, the level of induction varies from two-fold to nearly 100-fold. The nitrogen source, medium pH, nature of the buffer, presence of alternative electron acceptors (e.g., nitrate), and other factors exert a great effect on the expression of many of these genes. In the near future we will investigate control mechanisms common to the *adh* operon and other anaerobically regulated genes.

Stanford University
Stanford, California 94305

107. Genetic Engineering of Corn and Other Higher Plants

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

The project objective is to apply to higher plants the recent developments of molecular biology in mammals and microorganisms by the development of DNA transformation systems for higher plants. Our goal is to develop a technology that will allow the transformation of an entire plant. Technologies that will allow only the transformation of tissue culture or callus culture are less important. We currently focus on two plants: maize and carrots. In the case of maize, we are trying to transform the germ line directly without going through tissue culture. Carrots were chosen as a system because they are readily regenerated from tissue culture under conditions that allow DNA transformation. All vectors have used the bacterial CAT gene connected between a maize promoter and a maize polyadenylation site or carrot promoter and carrot polyadenylation site. Each potential vector had a variety of other DNA sequences, including a maize sequence repeated approximately 10,000 times in the maize genome that functions as an ARS (autonomously replicating sequence) in yeast. This sequence is highly conserved among all maize lines and may function as an autonomously replicating sequence in maize. We focus primarily on development of the new electroporation techniques. Present experiments test for transient gene expression; we have demonstrated initial success in introducing the vectors described above.

108. Genetic and In Vivo *Spectroscopic Studies of Mechanisms of Flooding Tolerance in Plants*

O. Jardetzky, J.K.M. Roberts, J. Callis **$75,000**
Magnetic Resonance Laboratory

This project examines critically the role that alcohol dehydrogenase (ADH) plays in determining the tolerance of maize root tips to anaerobiosis, focusing on the root tip as the part of the root most sensitive to anaerobiosis. Our approach is to vary the activity of ADH in root tips to see precisely how survival during anaerobiosis is affected. The activity of ADH in root tips is varied by genetic or environmental manipulation. Survival of root tips is monitored in a viability (recovery) assay. We also measure the rate of ethanol production by the anaerobic root tips and their cytoplasmic pH. We know that cytoplasmic acidosis is a determinant of flooding tolerance in crop plants, and that ethanol production (via ADH) permits synthesis of energy (ATP) without acid production (unlike lactic fermentation). To date we have generated a number of paired F3 progeny; each pair is homogeneous in genetic constitution except that one member exhibits a different level of alcohol dehydrogenase activity (ADH). We have a number of such pairs in which ADH activity varies by more than two orders of magnitude. Most of our efforts and progress concerns propagating and screening tissues (prop roots, scutella, and pollen) from many different lines, enabling us to assess the role that ADH levels play in survival of maize roots under severe hypoxia.

109. Cloning and Mapping of Rhizobium meliloti *Symbiotic Genes*

S.R. Long **$99,900**
Department of Biological Sciences

Improved biological nitrogen fixation is a possible source of supplemental or replacement nitrogen nutritution for plants, which could spare energy resources currently spent in ammonia synthesis. The *Rhizobium*–legume symbiosis is the major agronomic biological nitrogen fixation system. Within the legume family, different plants are nodulated by particular strains or species of *Rhizobium*. We study the bacterium *Rhizobium meliloti*, which invades alfalfa (*Medicago sativa*) and a few other plants. We cloned, determined the map position, and completed the DNA sequence of four common (conserved) nodulation (*nod*) genes. These genes are used by more than one *Rhizobium* species for the invasion of their various plant hosts. We identified two other bacterial gene regions used for invasion of plants. One DNA segment, borne in plasmid pRmJT5, is closely linked to the known common *nod* genes. We conducted a transposon mutagenesis of pRmJT5 and used these to generate site-specific mutations in *Rhizobium*. The loci identified through these mutations appear to be involved in host-specificity of the infection, and are likely the same as the documented *hsn* loci. Further characterization of these *hsn* loci by deletion analysis is underway. A second putative *nod* gene DNA segment, cloned in plasmid pRmRF1, is being mutagenized to examine its function in more detail. In addition to these *nod* genes, we identified several loci involved in metabolism and symbiotic effectiveness. We isolated mutants and cloned genes for dicarborylate transport (*dct*) and recently for membrane ATPase. These genes appear to be required for normal nodule function.

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

E. Zeiger **$85,000**
Department of Biological Sciences

Stomatal control of leaf gas exchange has an impact on plant productivity and the water balance of land covered by vegetation. This research project studies the stomatal responses to CO_2 and their interactions with other factors modulating stomatal movements. Despite prevailing paradigms on the inability of guard cells to fix CO_2 photosynthetically, data from our laboratory point to a CO_2 reactivity of guard cell chloroplasts: (1) like their mesophyll counterparts, guard cell chloroplasts show fluorescence transients associated with modulation of photophosphorylation by CO_2; (2) achlorophyllous stomata from the orchid *Paphiopedilum* show a reduced CO_2 sensitivity; and (3) guard cell chloroplasts from *Vicia* and *Pisum* bind antibodies against RuBPCarboxylase. More recently, we measured a DCMU-sensitive alkalinization of a suspension medium of guard cell protoplasts irradiated with red light, which correlated with oxygen evolution, clearly pointing to photosynthesis-dependent CO_2 uptake. Ongoing research is testing the hypothesis that, under appropriate experimental conditions, guard cell chloroplasts fix CO_2. A conclusive demonstration of this hypothesis will enhance our understanding of the CO_2 response of stomata and the coupling of stomatal conductance and photosynthesis in the intact leaf. Possible applications of these aspects of stomatal function to agronomic practices are under investigation.

Texas A and M University
College Station, Texas 77843

111. Metabolic Mechanisms of Plant Growth at Low Water Potentials

J.S. Boyer **$74,000**
Department of Soil and Crop Sciences

In higher plants, growth is more frequently limited by water than any other environmental resource. The project objective is to identify the underlying molecular mechanisms causing the limitations, by focusing on the processes of photosynthesis and cell enlargement. The inhibition of photosynthesis by low leaf water potential was caused primarily by losses in chloroplast activity in sunflower (*Helianthus*). Many of the effects could be simulated by preincubating chloroplasts at Mg^{2+} concentrations expected to be present at low water potentials. Plants growing at differing Mg^{2+} concentrations were inhibited earlier at high Mg^{2+} than at low Mg^{2+} as water potentials decreased, suggesting a central role of Mg^{2+} in the photosynthetic response. We are further investigating chloroplast responses *in vivo*, where Mg^{2+} regulation probably occurs. Our studies of cell enlargement show that, in localized growing regions, photosynthate accumulates and can maintain turgor completely at moderately low water potentials. The primary signal causing decreased growth was a loss in the gradient in water potential required for water entry into the growing cells. We will explore the subsequent role of several other factors in the growth response using hormone deletion mutants and other approaches. This work will further understanding of the mechanisms of growth inhibition with limited water and may indicate ways to reduce the limitation.

Virginia Polytechnic Institute and State University
Blacksburg, Virginia 24061

112. Enzymology of Acetone–Butanol–Isopropanol Formation

J.-S. Chen **$66,900**
Department of Anaerobic Microbiology

Four alcohols (C_2 to C_4) have been suggested as feedstocks that can support a major fraction of the U.S. chemical industry using currently available technology and facilities. These alcohols are products of microbial fermentation. Two of the alcohols, isopropanol and n-butanol, plus acetone are products of the anerobe *Clostridium beijerinckii* (formerly known as *Clostridium butylicum*), the organism currently under study. DNA homology studies show that *C. beijerinckii* is distinct from the acetone/butanol-producing *Clostridium acetobutylicum*. Despite the industrial use of these anaerobes, there are unsolved biological problems: (1) the organism easily loses its producing capacity (strain degeneration) and (2) the organism does not always undergo the necessary metabolic transition to initiate alcohol/solvent production. An essential step toward understanding the nature of these problems is to elucidate the enzymology of the normal fermentative process. The project objective is to study the property of the key enzymes for the formation of acetone, butanol, and isopropanol. Emphasis is on (1) characterization of the enzyme converting acetoacetyl CoA to acetoacetate (the first enzyme of the acetone/isopropanol pathway) and (2) comparison of isopropanol dehydrogenase and butanol dehydrogenase in properties pertinent to substrate and electron-carrier specificities. We believe this work will facilitate the use of molecular biology to study how the expression of these enzymes are controlled, helping to solve the aforementioned problems. It may also serve as a model system for studying regulation of gene expression in anaerobes.

University of Washington
Seattle, Washington 98195

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

R.E. Cleland **$85,800**
Department of Botany

This project determines how plant cell enlargement is controlled and regulated at the cellular level, emphasizing the mode of action of the hormone auxin. We identified four major control points for cellular expansion: (1) the rate of auxin-induced H^+ excretion, (2) the capacity of the walls to undergo acid-induced wall loosening, (3) the rate of osmoregulation, and (4) the value of the effective turgor (i.e., the turgor in excess of the wall yield threshold). Changes in any one of these four control points will lead to changes in the growth rate. We continue our studies on each of these control points to determine the role of each in the modulation of the rate of cell enlargement. We found that auxin can alter the rate of ATP-driven proton transport in isolated plasma membrane vesicles from pea roots, to lower the Km for MgATP without changing its Vmax. We expect to obtain a more optimal system for studying this effect, making certain that these vesicles do originate from the plasma membrane. The vesicles will then be solubilized, the proteins purified, and recon-

stituted back into synthetic vesicles, to determine which proteins are required for the auxin effect. The effect of lipid composition on this activity will also be examined. Isolated Avena coleoptile walls, when under tension, undergo a two-phase extension response to acid; first a burst of extension, followed by a long-term constant rate of extension (CE). We will examine (1) the generality of the CE extension in other auxin-sensitive tissues and (2) the sensitivity of this process to calcium ions, pH, and factors that alter wall structure.

114. Genetic Engineering of Yeasts for Fermentation of Xylose to Ethanol

B.D. Hall, C. Furlong **$70,000**
Department of Genetics

Hemicellulose is one of the three major biopolymers present in woody plant tissues. Acid hydrolysis of hemicellulose produces the pentose sugar xylose. *Saccharomyces cerevisiae* can fermentatively use the isomer xylulose, but is unable to grow on xylose. We have transformed *S. cerevisiae* with a yeast expression vector containing the *E. coli* xylose isomerase gene fused to the yeast alcohol dehydrogenase (ADH 1) promoter. The *E. coli* xylose isomerase enzyme mediates the conversion of xylose to xylulose. Yeast transformants containing the xylose isomerase gene produce the protein as detected by Western blot analysis using antibody specific to the *E. coli* xylose isomerase enzyme. The protein however is particulate and has no detectable xylose isomerase enzyme activity. The yeast protein is being purified to characterize and compare it with the protein made in *E. coli*. We are also mutagenizing the yeast strain containing the xylose isomerase gene to obtain mutants that can grow on xylose. Studies on the levels of the pentose phosphate cycle enzymes in *S. cerevisiae* suggest that (with the exception of the xylulokinase enzyme) there is no significant induction of these enzymes in cells grown in medium containing xylulose as compared with cells in glucose. However xylulokinase enzyme is induced in *S. cerevisiae* cells grown in medium containing xylulose.

Washington State University
Pullman, Washington 99164

115. Regulation of Terpene Metabolism

R. Croteau **$87,000**
Institute of Biological Chemistry

Oils and resins from plants are important renewable resources. Fundamental knowledge of the biochemistry of oil and resin terpenes is needed, particularly as it applies to regulatory mechanisms at the enzyme level. The objective of this project is to provide such an understanding through the intensive investigation of two model systems: (1) camphor metabolism in *Salvia officinalis* and (2) menthone metabolism in *Mentha piperita*. This allows probing the control of both biosynthetic and catabolic processes involved in monoterpene accumulation. The pathways of biosynthesis responsible for the accumulation of both monoterpenes during development are being established. As the plant matures, both terpenoids undergo catabolism, suggesting that this process involves conversion to a glycoside and transport of this derivative to the root/rhizome. Following hydrolysis, the terpenoid undergoes oxidative degradation to acetate, which is recycled into other lipids. Terpene catabolism thus provides a carbon source to the developing root/rhizome. During the catabolic phase, the epidermal oil glands collapse as terpenes are removed from the extracellular cavity. The ultrastructural basis for this process is under investigation. Major focus is on factors that may mediate developmental changes in the rates of biosynthetic and catabolic processes. Foliar application of growth regulators and bioregulators is effective in increasing terpene yield by influencing development and altering levels of individual cyclases responsible for construction of various monoterpene types. Aditional treatments alter the mixture of terpene stereoisomers and the extent of terpene oxidation/reduction. Results will have important consequences for yield and composition of terpenoid oils and resins that can be made available for industrial exploitation.

Washington University
St. Louis, Missouri 63130

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

R.N. Beachy **$77,700**
Department of Biology

Seed storage proteins provide the bulk of dietary protein for animals, including humans. Recent research in a number of laboratories has led to a better, but not complete, understanding of seed proteins of legume and cereal grains. We are studying the expression of genes that encode the β-conglycinins, soybean storage proteins. A gene encoding the α'-subunit of β-conglycinin was previously isolated and characterized. This gene, Gmg 17.1, was introduced into petunia chromosomes via a disarmed Ti-plasmid of *Agrobacterium tumefaciens*. The α'-gene was correctly regulated in plants regenerated from transformed petunia cells; it was not expressed in leaves, but was expressed at high levels in embryos following self-pollination of flowers of the transformed plants. The soybean protein accumulates to the level of about 1% of the total petunia seed protein. The soybean α'-subunit assembled into a multimeric protein in petunia seeds in a manner similar to the assembly of β-conglycinin in soybean seeds. To identify the DNA sequences in (or flanking) the soybean gene that control its expression, we created a series of mutations that removed sequences in front of the gene. Each mutant gene was introduced by transformation into petunia as before, and the expression of the soybean gene is being monitored. Results will help to identify the DNA sequences that control the expression of this gene. This information will enable us to introduce and express other genes (in addition to seed storage protein genes) in seeds of a variety of transformed plants. We hope to use this regulatory region to introduce modified seed storage proteins back into soybean plants as a way to alter the quantity and/or quality of soybean seed proteins.

117. Hydroxyproline-Rich Glycoproteins of the Plant Cell Wall

J.E. Varner **$100,000**
Department of Biology

The cell walls of plants, particularly of dicots, characteristically contain glycoproteins rich in hydroxyproline. The project objective is to test the presumption that, in analogy with the collagen matrix of animal tissues, the plant glycoprotein(s)-extensin(s)

has an important structural/developmental role. We have (1) isolated and purified to homogeneity one hydroxyproline-rich glycoprotein from aerated carrot slices, (2) characterized the conformation of this glycoprotein as an extended polyproline II helix (it is a rod 80 nm long and visible by electron microscopy), (3) isolated and purified the mRNA for carrot extensin, (4) made cDNA against the mRNA, and (5) used the cDNA to isolate the gene for a carrot extensin. This gene has been sequenced and the amino acid sequence derived from the base sequence. The sequence, Ser Pro Pro Pro Pro, occurs 25 times; Ser Pro Pro Pro Pro Thr Pro Val occurs eight times; Tyr Lys Tyr Lys and His His Tyr Lys are common repeats. The transcripts from this gene increase in abundance following wounding (slicing and aeration of the carrot root). Another, non-homologous transcript coding for a 33 kilodalton peptide rich in proline and poor in leucine also accumulates following wounding. This second transcript encodes several repeats of His Lys Pro Pro Val and Tyr Thr Pro Pro Val. The biology of this peptide is as yet unknown. Current study includes (1) the control of the expression of the extensin gene, (2) the specificity of the prolylhydroxylase, which converts selected peptidyl hydroxyproline residues, and (3) the chemistry of the reactions responsible for insolubilizing the extensins in the cell wall.

University of Wisconsin
Madison, Wisconsin 53706

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

J.L. Kermicle **$47,000**
Department of Genetics

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

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

O.E. Nelson **$59,700**
Department of Genetics

Investigations of oligosaccharide synthases in developing maize endosperms reveal that the phosphate groups in the phosphooligosaccharides, which form a portion of the reaction products, are largely (if not entirely) linked to C6 of the glucose moiety. There is an interesting acceleration of the synthase reaction by catalytic amounts of Glc-1, 6-bisP, which is apparently not accounted for by phosphoglucomutase activity. In addition, we have found that the *brittle-1* mutant, which synthesizes comparatively little starch, has little starch granule-bound oligosaccharide synthase activity. It is not yet clear whether this enzyme deficiency is a primary effect of the *bt1* mutation, although this observation could explain the greatly reduced starch synthesis characteristic of the mutant. It has been reported previously that the *sugary-1* mutants of maize are deficient in debranching enzyme activity. Of the three peaks of activity observed after column chromatography, fraction III is loosely associated with amyloplasts, while the major portion of fraction II activity is tightly bound to or in these organelles and is freed only after glucoamylase digestion. The *sugary-1* mutants have very low starch granule-bound debranching activity.

120. Carbon Isotope Fractionation in Plants

M.H. O'Leary **$75,000**
Department of Chemistry

Plants fractionate carbon isotopes during photosynthesis in ways that reflect photosynthetic pathway and environment. The project objective is to develop an understanding and methods for using this isotope fractionation to learn about details of carbon dioxide fixation during photosynthesis. We have measured isotope fractionations for various components of the carbon dioxide fixation process (diffusion, carboxylation, and so forth), and developed quantitative models for carbon isotope fractionation *in vivo* that describe the overall carbon dioxide fixation process in terms of the relative rates and isotope fractionations of its components. We have developed experimental approaches that focus on the initial events in carbon dioxide fixation in CAM plants. We are currently developing rapid methods for studying carbon isotope fractionation occurring during short periods in C3 and C4 plants. We have developed methods for studying the activity of phosphoenolpyruvate carboxylase, ribulose bisphosphate carboxylase, fumarase, carbonic anhydrase, and other enzymes *in vivo* by means of isotope techniques.

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

P.H. Quail **$83,400**
Department of Botany

The project objectives are to purify and characterize phytochrome from fully-green, light-grown tissue. Previously we developed a simple method (implementable in any minimally euipped biochemical laboratory) for rapid extraction and assay of phytochrome in crude extracts using spectroscopic or immunochemical procedures. Initial experiments show that the phytochrome molecule predominant in such tissue is distinct from the better-characterized etiolated-tissue molecule regarding spectral properties, immunochemical characteristics, apparent molecular mass, and proteolytic fragmentation pattern. Recent efforts focus on attempts to purify this new phytochrome species. Purification by immunoaffinity column chromatography has thus far been unsuccessful, apparently due to insufficient cross-reactivity between green-tissue phytochrome and our antibodies prepared against etiolated-tissue phytochrome. Polyethylene glycol precipitation, together with hydrophobic and hydroxyapatite chromatography, have provided a 50-fold purification. A major problem is the considerable instability of the green-tissue phytochrome under condi-

tions optimal for etiolated-tissue phytochrome purification. However, initial screening has identified protease inhibitors that appear to be effective in enhancing the stability of the green-tissue molecule.

122. Molecular Mechanism of Energy Transduction by Plant Membrane Proteins

M.R. Sussman **$58,300**
Department of Horticulture

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

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123. Genetics in Methylotrophic Bacteria

M.E. Lidstrom **$106,200**
Center for Great Lakes Studies **(5 mo. U.Wa., 12 mo. U. Wi.)**

The purpose of this project is to use genetic techniques to study the transcriptional regulation of C-1 specific functions in methylotrophic bacteria. The approach involves analyzing C-1 specific genes in facultative methanol utilizers and using these organisms as hosts to study genes encoding similar functions in methane-utilizers. For the past year, we have concentrated on the methanol-utilizers. We have cloned and characterized several C-1 genes from the facultative methanol utilizer *Pseudomonas* AM1, including the structural genes for the serine pathway enzymes PEP carboxylase and malyl CoA lyase, and a series of nine genes necessary for methanol oxidation. We are currently identifying the functions and regulatory units for each of these genes. We also are studying promoters in *Pseudomonas* AM1. We have constructed a promoter-cloning vehicle to clone both methanol-regulated and constitutive promoters. These studies provide important information concerning future manipulation of methylotrophic bacteria for commercial chemical production.

Yale University
New Haven, Connecticut 06511

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

M.H.M. Goldsmith **$70,400**
Department of Biology

The auxin, indoleacetic acid (IAA), plays a central role in regulating the growth and transport of plant cells and is transported through plant tissues by an unusual polar process. IAA stimulates the electrogenic secretion of protons, which acidifies the cell wall leading to growth and increases the pH and electrical gradients at the plasma membrane that are important for the polar transport of IAA as well as accumulation of metabolites and inorganic ions by cells. Membrane vesicles are a potentially useful system for investigating the relationship between driving forces and transmembrane transport processes. Our experiments demonstrate that some of the basic properties of auxin uptake by cells are also exhibited by membrane vesicles: the accumulation depends on the pH gradient, is specific for auxins, and is stimulated by naphthylphthalamic acid and (NPA) triiodobenzoic acid. We use ^{14}C-labeled butyric acid to monitor the ΔpH during uptake of ^3H-IAA. Microsomal vesicles prepared from zucchini hypocotyls hold an artifically imposed pH gradient of about 1.2 units, alkaline inside, when suspended in pH 6 buffer. We are finding that IAA accumulation in the presence of NPA exceeds that predicted for a weak acid with a completely impermeable anion, and are exploring the possibility that a specific saturable binding of auxin contributes to the ΔpH-dependent accumulation of IAA by zucchini microsomal vesicles. We will also compare the properties of vesicles enriched for either plasmalemma or tonoplast, from tissues showing different developmental capacities for auxin transport, and from species displaying different tolerances to low temperatures. We are investigating the role of various membrane proteins in auxin accumulation including ATPases (which generate pH and voltage gradients), auxin anion efflux sites that appear to be modified by NPA, and specific IAA-binding sites.

125. Mechanisms of Potassium Transport in Plants and Fungi

C.L. Slayman **$93,000**
School of Medicine, Physiology Department

Potassium transport and accumulation are crucial to the control of growth, turgor, diurnal movements, transpiration, and many other physiological functions of plants. At least three distinct mechanisms of K^+ transport have been implicated by experiments on different plant species: channels (allowing passive K^+ movements), ATP-dependent K^+ pumping, and K^+–H^+ counterport. However, despite more than two decades of research, no dominant element or coherent picture of potassium transport has emerged. The purpose of this project is to explore a potential new mechanism that may accommodate many of the observations already made on potassium transport in plants: a high-

affinity, Ca^{++}-dependent K^+–H^+ symport, recently described in the fungus *Neurospora*. Specific research objectives are (1) to complete the electrophysiological and transport-phenomenological description of the symport system in *Neurospora*; (2) to determine the mode by which calcium ions activate or control the system; (3) to search for a similar transport system in higher plant tissues; and (4) to characterize the system in isolation (or near isolation) as a purified protein or carrier reconstituted in artificial lipid membranes. The strong electrogenicity of the K^+–H^+ symport permits direct electrophysiological measurement of membrane potentials (ΔPsi), membrane current-voltage curves (IVC), and K^+-activated changes of ΔPsi and IVC, in intact cells and tissues. Standard isotope (^{42}K, ^{86}Rb, ^{45}Ca) flux techniques will be used to obtain the complementary chemical kinetic data. The primary characterization experiments will be conducted on hyphae and spheroplasts of *Neurospora crassa*, and the search for a related symport system in higher plants will test coleoptiles and roots of oats and maize.

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