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DIVISION OF ENERGY BIOSCIENCES

ABSTRACTS OF PROJECTS SUPPORTED IN FY 1998

The Division of Energy Biosciences was provided approximately twenty-eight million dollars in fiscal year 1998. The breakdown of how the resources were distributed is indicated in the following table.

	Number of Projects	FY 98 funding (in thousands)	Percent of total funds
University and Non-Profit Institutions	193	20,527	75
Michigan State University Plant Research Laboratory	14	3,000	11
National Laboratories Brookhaven National Lab Lawrence Berkeley Lab Los Alamos National Lab National Renewable Energy Lab.	11	2,382	9
Small Business Innovation Research (SBIR) contribution, and Miscellaneous		1,209	4
Conferences, Educational Activities	5	389	1
	223	$27,507	

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DIVISION OF ENERGY BIOSCIENCES
ABSTRACTS OF PROJECTS SUPPORTED IN FY 1998

1. Metabolic Regulation of the Plant Hormone Indole-3-acetic Acid
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Funding: $92,000 12 months

The phytohormone indole-3-acetic acid (IAA, auxin) is important for many aspects of plant growth, development and responses to the environment. Our understanding of the biosynthesis, conjugation and degradation of auxin in plants, and the mechanisms by which plants regulate auxin levels has changed remarkably within the last few years. We showed that mutant plants that cannot make the amino acid tryptophan still make IAA, and in very high amounts. We also showed that both the traditional tryptophan pathway and a non-tryptophan pathway to IAA occur in plants, and sometimes both are used by the same plant at different times of development. An additional source of IAA in plants is the relatively large pool of IAA stored within plant cells in conjugated form. We are working on three fundamental problems related to how plants get their IAA: 1) Characterizing the non-tryptophan pathway to auxin using an *in vitro* system from maize seedlings; 2) Determination of how a plant decides to use one or the other pathway, or both, during various developmental stages or in response to environmental stresses and stimuli; 3) Isolation and characterization of bacterial and plant enzymes that hydrolyze conjugates that could be useful for altering IAA metabolism in specific plant tissues. Recent studies have shown that IAA levels change by two orders of magnitude following fertilization and remain high during the early stages of carrot zygotic embryogenesis, but return to lower levels as organized structures begin to develop. Carrot somatic and zygotic embryogenesis are being studied to determine how pathways to IAA are controlled during development and the consequences of activation of these pathways on IAA levels and embryo development.

2. Role and Regulation of SNF-1-like Protein Kinases in Plant Carbohydrate Metabolism
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We have continued our studies to elucidate the control by phosphorylation of sucrose-phosphate synthase (SPS) and sucrose synthase (SuSy) - important enzymes of sucrose (Suc) synthesis and degradation, respectively. With SPS, we have focused on the phosphorylation of the enzyme's two regulatory sites: Ser158, responsible for dark-inactivation of the enzyme, and Ser424, which appears to be responsible for "osmotic-stress activation" of the enzyme in darkened leaves. The amino acid residues surrounding Ser158 and Ser424 are similar and both conform to the motif targeted by SPS-kinase(s): Basic-Hydrophobic-X-Basic-X(2)-Ser-. However, the two sites are phosphorylated by distinct kinases. Ser158 is phosphorylated by PK_m, a likely member of the sucrose non-fermenting (SNF1) family of kinases that is itself controlled by phosphorylation. In contrast, Ser424 is phosphorylated by PK_N, a unique stress-stimulated kinase. Understanding those processes may uncover new information about how plants cope with stress, and could provide new avenues to increase stress tolerance of crop plants. With SuSy, our efforts have focused on identifying the physiological significance of Ser158 phosphorylation. Phosphorylation results in a small activation of the cleavage reaction that is probably not significant in vivo. However, phosphorylation appears to be part of the mechanism that controls the membrane association of SuSy. Our current working model is that phosphorylation causes a conformation change that reduces exposure of surface hydrophobic residues, thereby causing release of SuSy from the membrane. The localization of SuSy may directly control whether imported assimilates are utilized in cell wall biosynthesis or other biosynthetic pathways.

3. Consequences of Altering Rubisco Regulation
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Rubisco initiates photosynthetic carbon acquisition and its activity can be limiting for photosynthesis. Even when Rubisco activity is not limiting (e.g. low light, high CO_2, limited sinks), the activation state in the enzyme is often reduced such that its activity is no longer explicitly limited by the steady state level of the RuBP substrate. Several hypothesis have been proposed which attempt to account for this response. The activation state of Rubisco is determined by the activity of its regulatory protein, Rubisco activase. Rubisco activase is usually present as two isoforms, differing at the carboxyl terminus, generated by alternative splicing of the pre-mRNA, and of unknown significance. Activase activity requires ATP hydrolysis which responds to the ATP/ADP ratio and appears to be influenced by light intensity via the thylakoid membranes by an unknown mechanism.
The goal of this project is to examine the consequences of altering Rubisco regulation by examining the properties of *Arabidopsis* plants expressing different forms of Rubisco activase and achieved via transformation of either the wild type or the *rca*⁻ mutant (containing no Rubisco activase). Plants containing only one of the two isoforms and plants which have mutant forms of Rubisco activase less inhibited by ADP are currently being characterized. Plants overexpressing each form of activase or forms with no catalytic activity will also be created. The information gained by this research will directly address the significance of Rubisco regulation as a determinant of plant productivity and in the response of plants to their environment.

4. Structure, Function and Regulation of Antenna Complexes of Green Photosynthetic Bacteria
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All photosynthetic organisms contain chlorophyll pigments that function as an antenna, absorbing light and transferring excitations to a photochemical reaction center where energy storage takes place by a series of chemical reactions. The green photosynthetic bacteria are characterized by large antenna complexes known as chlorosomes. The overall objective of this project is to determine the molecular organization of pigments in chlorosome antennas, as well as the mechanisms of excitation transfer and regulation of this unique antenna system. The chlorosome pigments are organized *in vivo* into pigment oligomers in which direct pigment-pigment interactions are of dominant importance. Time-resolved spectroscopy has given insight into the pathway and kinetics of excitation flow from the peripheral region of the chlorosome to the reaction center. Ultrafast absorbance measurements have indicated that the pigments are very strongly coupled, leading to subpicosecond energy transfer. Green sulfur bacteria contain a redox-activated quenching mechanism for control of energy transfer efficiency. The quenching effect may be a control mechanism that protects the cell from damage during conditions where light and oxygen are present simultaneously. We have recently determined the X-ray structure (PDB ID code 1KSA) of the bacteriochlorophyll *a* antenna protein from the green sulfur bacterium *Chlorobium tepidum*. This protein is an intermediate in the energy transfer pathway from chlorosome to reaction center. Efforts to develop a system for site-directed mutagenesis of this protein are underway.

5. Chlorophyll-Binding Proteins in the Cyanobacterium Synechocystis sp. PCC 6803
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Chlorophyll synthesis is coupled tightly to biogenesis of chlorophyll-binding proteins in photosynthetic systems. This tight coupling is important to avoid accumulation of free chlorophyll, which is toxic in aerobic environments in the light. One way to address the *in vivo* mechanisms of this coupling is by genetic modification of the rate of chlorophyll synthesis and the conditions under which it occurs, followed by analysis of the effects of these modifications on synthesis of chlorophyll-binding proteins. For these studies the cyanobacterium *Synechocystis* sp. PCC 6803 is used because this organism is very suitable for gene replacement studies and because its genome has been sequenced. By deletion of the light-independent pathway of chlorophyll synthesis in this organism, a mutant has been generated that does not make chlorophyll in darkness and that starts synthesizing chlorophyll when exposed to light. Interestingly, the synthesis rate of a chlorophyll-binding photosystem II protein, D1, is very low at early times of illumination, and increases as chlorophyll accumulates. Transcript levels for this protein are high at all times, implying a translational control

involving chlorophyll. Indeed, in another mutant with a lower capacity for chlorophyll synthesis, D1 translation is even more impaired. This observation has further increased our interest in identifying potential chlorophyll-binding proteins, and determining their role in synthesis, assembly, and function of the photosynthetic apparatus. Several potential chlorophyll-binding proteins in *Synechocystis* sp. PCC 6803 have been identified on the basis of either sequence homologies or biochemical evidence, and analysis of the role of several of these proteins is in progress.

6. Restructuring Metabolism for Photosynthesis Protection
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The long-term goal of this project is the engineering increased whole-plant tolerance to drought and salinity stresses by the modification of metabolic pathways in carbon allocation. We explore the roles of accumulating substances, polyols in particular, and also proline, trehalose, ectoine, and glycine betaine. Several laboratories reported that the transgenic overexpression of enzymes that lead to the accumulation of these compounds provided limited stress protection, but mechanisms of action are still largely unknown. Effects of accumulating osmolytes have been measured in transgenic tobacco. Extending these studies we can now pinpoint a specific function for mannitol, at approximately 100 mM in the stromal space, in the protection against hydroxyl radicals. We observe that mannitol protects enzymes of the Calvin cycle, while the water-splitting machinery and photosystems are not inhibited under the conditions used. The protective effect of mannitol has been shown *in vivo*, in isolated cells, chloroplasts and *in vitro* systems. We expect that other polyols may have a similar function. Protection is exerted in a narrow range of accumulating polyols; tobacco plants with high accumulation of, e.g., sorbitol are apparently osmotically challenged and are stunted. We suspect that either osmosensing pathways are affected or that high amounts of polyols are interfering with sugar sensing in these high accumulator lines. We are now targeting the expression of different enzymes of osmolyte production to different compartments and to different tissues by the use of tissue- and cell-specific promoters. We expect multiple osmolytes to exert different protective effects.

7. Role of Cell Wall Degrading Enzymes in the Programmed Separation of Cells from Root Caps
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Funding: $98,000 12 months

We proposed a model to describe border cell separation, based on published results describing cell wall degradation by microbial pathogens. We first tested the hypothesis that pectinesterase (PME) activity is a driving force that sets in motion steps leading to cell separation. If correct, then inhibiting PME activity in the root cap should inhibit border cell separation. Our results are consistent with that hypothesis: Transgenic hairy roots of pea expressing antisense mRNA to a root cap specific PME encoding gene (*rc-pme1*) makes border cells but the cells do not separate into suspension like normal border cells. Cell elongation also is inhibited. To our knowledge, this is the first evidence that inhibiting expression of a plant cell wall degrading enzyme can significantly alter plant growth and development. In transgenic root tips with reduced *rc-pme1* expression the pH at the cell surface is measurably higher than in normal roots, as predicted by our model. Removing border cells constitutes a signal to induce not only PME gene expression but also mitosis in the root cap meristem, and a global switch in gene expression throughout the root cap leading to border cell production and separation. An extracellular chemical, 'Factor B,' appears to act as a repressor to regulate border cell development, but the effects of this signal can be overridden by increased atmospheric CO_2 levels.

8. Transgene silencing, paramutation, and promoter-homology-based control of gene expression
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Funding: $90,000 12 months

Transgenes may interact via a paramutation-like process that depends on promoter homology, causing transcriptional changes in transgene expression patterns. This phenomenon is distinct from sense cosuppression, a post-transcriptional process that requires coding sequence homology and high level expression of a sense transcript. However, a transgene causing sense cosuppression of

an endogenous plant gene is potentially a very sensitive reporter of paramutation-like interactions between transgenes which share the same promoter, for the reason that sense cosuppression is threshold dependent. We are investigating such paramutation-like interactions using anthocyanin genes in petunias, an excellent system for monitoring gene expression changes both quantitatively and qualitatively. Our preliminary observations suggest that paramutation-like interactions between transgene copies cause qualitative changes in transgene expression that result in qualitatively new patterns of sense cosuppression in petunia flowers. The experiments proposed here are designed to test this hypothesis, to investigate epigenetic properties of paramutant transgenes, and to investigate the DNA sequences in the transgene promoter that are necessary for inducing qualitative changes in cosuppression patterns.

9. Dissection of Molecular Mechanisms Regulating Protein Body Formation in Maize Endosperm
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Endosperm texture is an important quality trait in maize, as it influences the shipping and handling characteristics of the grain, its susceptibility to boring insects, the yield of grits from dry milling, energy costs during wet milling and baking properties of the flour. Factors contributing to texture, i.e. hardness and vitreousness, are poorly understood. However, there appears to be a causal relationship between kernel hardness and protein body formation in the endosperm, because mutations that affect protein body size, number and organization give rise to soft, starchy kernels. To better understand the relationship between protein body formation and the vitreous phenotype, we have conducted experiments to investigate the molecular mechanisms regulating protein body formation. Associations were found between protein bodies, zein polysomes, actin and EF-1 *in vitro*. These observations led us to speculate that the cytoskeleton plays a role in the synthesis of storage proteins in maize endosperm. To approach this question, we have begun experiments to visualize the cytoskeleton in intact maize endosperm cells and determine its relationship to protein bodies, determine whether zein mRNAs are targeted to specific sites on the rough endoplasmic reticulum (RER), and determine whether zein mRNAs localize to the RER membrane in a cytoskeletal-dependent manner. We have been able to document dramatic changes in the distribution of actin filaments, microtubules and EF-1 accompanying the accumulation of storage proteins and starch in endosperm cells. Microtubules become juxtaposed with protein bodies, which are enmeshed in EF-1 and actin. The EF-1 and actin appear to exist in a complex, as pretreatment of the tissue with cytochalasin D causes the redistribution of both proteins. Results from recent experiments in which we have localized zein mRNAs in fixed tissue and microinjected fluorescently labeled zein mRNAs into living endosperm cells, support the hypothesis that these mRNAs are associated with, and perhaps trafficked by, this cytoskeletal network.

10. Regulation of DNA Endoreduplication in Maize Endosperm
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Funding: $100,001 12 months

Nuclear DNA endoreduplication occurs widely in the metabolically active tissues of plants and animals. While a normal cell cycle has S, G2, M and G1 phases, the endoreduplication cycle has only DNA synthesis and gap phases. Maize endosperm cells engage in multiple rounds of endoreduplication from 10 to 25 DAP. Though the biological significance of this process is not understood, one suggestion is that an increase in gene copy number can increase mRNA transcript levels in a developmentally regulated manner. Investigations in our laboratory suggest that endoreduplication in maize endosperm is associated with the inhibition of mitotic cyclin-dependent protein kinases (CDKs) and enhancement of S-phase CDKs. Inhibition of mitotic CDKs is achieved via the production of an inhibitor present throughout endoreduplication. Unlike regulators of CDKs in other eukaryotes, neither covalent modification nor stable binding is required for the inhibition. The inhibitor is associated with the microsomal fraction and its activity increases significantly between 9 to 13 days after pollination. To learn more about this inhibitor, we have established a purification protocol that allowed us to purify the protein component of this inhibitor to near homogeneity. Maize endosperm contains a high level of CDK-like kinase activity which can be detected as soon as the kinase is separated away from inhibitory factors. This kinase possesses characteristics expected for the S-phase CDK of higher plants. Partially purified activity from 16 DAP maize endosperm cofractionates with electrophoretically distinct polypeptides immunologically related to *Zea mays* $p34^{cdc2}$.

11. Molecular Characterization of the Role of a Calcium Channel in Plant Development
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During their development, plants convert the information from environmental and physiological cues into specific growth responses. Transduction of this information into the appropriate cellular compartment often involves changes in energy coupling across cellular membranes. In several species of moss, changes in cellular calcium levels have been implicated in the regulation of growth habit. During the progression from filamentous to meristematic growth, cells undergo a dramatic alteration in the pattern of development involving a reorientation in cellular polarity and subsequent hormone-induced changes in cell proliferation. Calcium influx has been implicated in both of these processes: in selection of the site of asymmetry and in hormone-induced changes in cell expansion, elongation, and division.

Using calcium channel modulators (agonists and antagonists), whole plant studies have implicated control of calcium regulation during moss development to a dihydropyridine-sensitive calcium channel. Our studies of calcium influx into moss protoplasts and binding of a calcium channel antagonist to moss plasma membranes have provided a biochemical and molecular characterization of this moss calcium channel. We have shown that cytokinin, changes in voltage, and heterotrimeric guanine nucleotide binding proteins can regulate channel activity in vitro. We have identified two proteins in the moss plasma membrane that bind specifically to the channel antagonist, and studies are underway to isolate these putative channel proteins. Our studies continue to focus on the expression and regulation of the channel during development. To localize the channel spatially and temporally during development, in vivo assays using a fluorescently-labeled channel antagonist are in progress. To extend our studies of channel regulation to stages of development, specific cell types, and specific regions of the cells involved, we are performing electrophysiological measurements to characterize channel activity. Understanding the properties, expression, and regulation of the channel will help us determine the molecular mechanisms underlying calcium regulation and calcium's role in plant development.

12. Osmoregulation in Methanogens
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This project is aimed at understanding how methanogens deal with osmotic stress and to use these insights for increasing the salt tolerance of other cells. Present studies are focused in four different areas: (1) *in vivo* ^{13}C-edited ^{1}H NMR studies of *Methanococcus thermolithotrophicus* and *Methanohalophilus portucalensis* using soluble ^{13}C-labeled substrates for methanogenesis to monitor organic solute production, uptake, or loss upon alteration of external NaCl, (2) ^{39}K NMR and atomic absorption studies of these organisms upon salt stress to characterize changes in intracellular K^+, (3) defining biosynthetic pathways (e.g., identifying and characterizing key enzymatic activities) for several unique osmolytes including N-acetyl–lysine and DIP (di-*myo*-inositol-1,1'-phosphate), an unusual osmolyte that occurs in hyperthermophiles, and (4) quantifying how other external perturbations (e.g., high external pressure or moderate molecular weight PEGs) affect osmolyte accumulation in *M. thermolithotrophicus* and other bacteria known to adapt to high pressure. The first of these provides a continuous assay of how the cells alter their intracellular solutes in response to different medium conditions. The second has allowed us to explore how K^+ ion fluxes are related to organic osmolyte accumulation. The third area has provided us with several candidate proteins that may be regulated by changes in external NaCl. The fourth study will provide information on the role of Na^+ in osmolyte production and balance.

13. Differential Regulation of Plastid mRNA Stability
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Chloroplast gene expression is often regulated at the level of mRNA processing and stability. This regulation is required for the coordinated synthesis and assembly of the electron-transport chain macromolecular complexes. We have focused on the chloroplast genes *petA*, *petB* and *petD*, which encode subunits of the cytochrome *b6/f* complex. Using biochemical methods, we have characterized a 41 kDa protein (CSP41) which recognizes a conserved sequence in the 3' untranslated region of *petD* mRNA.

This protein also exhibits endoribonuclease activity. When the CSP41 binding site was altered in chimeric reporter genes introduced into tobacco chloroplasts by biolistic transformation, alterations in mRNA accumulation were seen consistent with a role of CSP41 in regulating petD 3' end maturation and/or RNA stability. The Arabidopsis csp41 gene was isolated and used to construct antisense plants with no detectable CSP41. These plants are viable, but preliminary results suggest that the accumulation of several chloroplast mRNAs may be reduced. In a related project, we have used tobacco chloroplast transformation to create disruptions of the *petA*, *petB* and *petD* genes. As expected, these plants exhibit high chlorophyll fluorescence and require sucrose. RNA accumulation patterns and polysome loading are being studied to investigate possible crosstalk between the genes encoding cytochrome *b6/f* complex subunits. Our hypothesis, based on findings for other chloroplast and mitochondrial macromolecular complexes, is that one or more subunits of the cytochrome *b6/f* complex will be under translational autorepression in these disruption plants. If so, it will illustrate that higher plant chloroplasts, like *Chlamydomonas* chloroplasts and yeast mitochondria, utilize this intricate mechanism to ensure stoichiometric accumulation of photosynthetic protein subunits.

14. Molecular Plant Genetics
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Simple sequence repeats (SSRs) for the basis for a new generation of molecular markers for higher plants. By their nature, these co-dominant markers detect higher levels of polymorphism than other single locus markers. SSRs have the further advantage that they can be detected with a simple PCR-based assay. We have modified an enrichment protocol that has allowed us to describe and map 409 SSR loci for maize and 225 for cotton. The maize loci were mapped in two recombinant inbred populations we maintain that are used by the maize genetics community for rapid gene mapping. The database for these populations has over 2250 mapped loci and is available over the internet (http://burr.bio.bnl.gov:80).

New milling methods require enhanced properties of cotton fiber. We are sequencing cDNAs from developing cotton fiber with a view toward finding genes that control cotton fiber properties such as length strength and thickness.

We are interested in the regulation of carotenoid and anthocyanin pigmentation. We have characterized in1, a negative regulator of anthocyanin biosynthesis in maize and are now focusing on its interaction with r1, the positive regulatory element that is its apparent target.

15. Molecular Bases and Photobiological Consequences of Light Intensity Adaptation in Photosynthetic Organisms
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This project addresses the basic molecular mechanisms responsible for the acclimation of the photosynthetic apparatus to changes in irradiance. We have recently identified that the redox status of the plastoquinone pool is a sensor that affects nuclear gene transcription in a eucaryotic green alga, *Dunaliella tertiolecta* (Escoubas, et al., Proc. Nat. Acad. Sci. 92:10237-41). The research builds on that discovery by analyzing the signal transduction cascade and the cue/response functions. The effect of redox Modulation in the photosynthetic electron transport chain on the expression of a variety of nuclear genes is under investigation. The research goals are to characterize the key DNA binding factors, follow the effects of redox control on the activation of the binding factors, and examine how redox poise is related to environmental cues such as irradiance, temperature and CO_2. The research has broad implication for understanding how environmental information is transduced to biochemical information with photosynthetic organisms, and how that information, in turn, affects nuclear gene expression.

16. Regulation of Energy Conversion in Photosynthesis
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The efficiency of photosystem II and the distribution of excitation energy from photosystem II to photosystem I are dynamically controlled by thylakoid-bound enzymes that modify pigments and pigment-protein complexes. Reversible protein phosphorylation on threonine residues is an important component in this regulation, and involves many photosystem II proteins. Evidence from mutant and inhibitor studies indicates that multiple protein kinases are involved.

We have described a 64-kDa enzyme, which can be solubilized with octylglucoside and partially characterized. However, enzyme activity is consistently detected in the unsolubilized fraction. Established membrane-fractionation protocols were used to explore the origin of this residual activity, which remained in tight association with core complexes containing photosystem II reaction centers. An entirely novel 58-kDa protein kinase was identified in core complexes by renaturing a Western blot and probing for autophosphorylation and histone-phosphorylation activities. This enzyme catalyzes phosphorylation of the intrinsic CP43 component of photosystem II cores, and light-harvesting chlorophyll a/b protein supplied exogenously. Its possible interaction with minor cytochrome components in the cores is under study. Future work will explore the substrate preferences of the 64- and 58-kDa kinases, and possible interaction between these enzymes. Bulk fractionation of core complexes toward obtaining 58-kDa kinase for sequencing and cloning is in progress.

Latent polyphenol oxidase (PPO) is released from thylakoids by octylglucoside. An active form of PPO forms a complex with Rubisco: the possible significance of this for Rubisco turnover is being assessed owing to the presence in PPO of a putative metalloproteinase functionality.

17. Modification of Plant Lipids
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Fatty acids perform essential roles in plants both as structural components of membranes and as a form of fixed carbon in triacylglycerols. Their properties are defined by the presence of double bonds and other substituents in the acyl chain. As a model to study how fatty acids are modified we are using the soluble acyl-ACP desaturase system. By integrating information from primary sequences with three-dimensional crystallographic structural information we have identified the molecular determinants of substrate and regiospecificity. This model of the structure-function relationships of residues within the desaturase has allowed us to rationally design new enzymes with unique properties. We are currently attempting to express these enzymes in the model system *Arabidopsis* to evaluate their utility in transgenic plants. If successful these enzymes will be introduced into crop plants which will accumulate oils that contain desired fatty acids for particular industrial applications.<

In a separate line of investigation we are probing the structure-function relationships of members of the predominant class of lipid modification enzymes that are integral membrane proteins. The alkane -hydroxylase system from *Pseudomonas oleovorans* is being developed as a model system for these experiments because it shares biochemical and structural similarities with the entire class of lipid modification enzymes. Mössbauer spectroscopy of this enzyme suggests that it has a diiron active site with properties similar to the soluble acyl-ACP desaturase active site. Experiments are also in progress to identify the molecular determinants of reaction outcome for the integral membrane desaturases and hydroxylase class of enzymes.

18. The Magnesium Branch of the Chlorophyll Biosynthetic Pathway
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Tetrapyrrole pigments function as essential components in the processes of respiration and photosynthesis, and also exist as cofactors in other important enzyme reactions. The tetrapyrroles arise from a branched biosynthetic pathway having -aminolevulinic acid (ALA) as its first committed member. ALA is known to be formed by two distinct routes: by condensation of glycine and succinyl-CoA in animal, fungal, and some bacterial cells, and by transformation of the intact carbon skeleton of glutamate in plants, algae and other bacterial cells. It has become apparent that the five-carbon pathway from glutamate is more widely distributed than was previously thought, and it may be the usual route of ALA formation in phototrophic organisms in addition to being the universal mode in oxygenic species. The macromolecular components of the five-carbon ALA-forming system have been fractionated into three enzymes and a required tRNA component. Cell-free preparations have been obtained in our laboratory from several phototrophic prokaryotes, including oxygenic cyanobacteria and strict anaerobes, that catalyze ALA formation from glutamate by reactions similar to those occurring in plants and algae. We propose to continue the characterization of the enzymes and RNA reaction components derived from phototrophic prokaryotes and from the unicellular alga, *Chlamydomonas reinhardtii*, to compare them to their counterparts in higher plants, and to study the regulation of their activity in response to light and nutritional status. The

potential of these organisms for molecular genetic studies will be exploited by cloning and sequencing the genes encoding enzymes that catalyze steps of tetrapyrrole biosynthesis. The cloned genes will be used to generate probes to study the regulation of their expression during adaptation of the cells to light and nutritional status. The probes will also be evaluated for use in measuring expression of homologous genes in other algae and higher plants.

19. Molecular and Genetic Analysis of LEAFY, a Gene Controlling Floral Induction and Flower Development in Arabidopsis thaliana
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We are continuing two separate lines of research. One is directed to understanding the signal transduction pathway by which the plant hormone ethylene is sensed, and its presence is transduced to cause alteration in plant gene activity. We have now identified five different members of the ethylene receptor gene family, all of which can be mutated to give dominant ethylene insensitivity. One model for the function of the ethylene system is that the receptors act in air to repress the ethylene response; mutating them to ethylene non-binding forms thus causes inability to respond to the hormone. To test this model we have reverted the dominant receptor mutants to obtain loss of function alleles. While single such alleles have only subtle phenotypes, double and triple mutants, in which multiple receptors are unable to signal, have strong constitutive ethylene response phenotypes; this supports the "active in air" model.

Our second area of research is the study of LEAFY, a gene with key regulatory roles in floral induction and flower development. We have found a strong LEAFY enhancer gene, named SPLAYED, that may code for a new partner that acts with LEAFY in floral development. Work is now underway to characterize and clone this gene. We are also purifying LEAFY protein from plants; purification under native conditions seems to indicate that LEAFY is found in a protein complex. Attempts to identify the other protein components of the complex are in progress.

20. Genetically Programmed Cell Death in Maize
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The maize leaf is comprised of proximal sheath and distal blade, separated at their boundary by the ligular region. We are gaining further understanding of how the ligular region, consisting of a fringe of epidermal tissue, the ligule, and hinge-like auricles, is positioned, induced, and formed. Recessive mutations in either lg1 or lg2 result in an absence of ligule and auricle structures and a broadening of the normally distinct blade/sheath boundary. DNA sequence analysis of lg1 and lg2 suggests their protein products may act as DNA binding proteins. lg1 shares homology with squamosa promoter binding proteins (Moreno et al., Genes and Development, 1997) and lg2 with basic leucine zipper type transcription factors (Walsh et al., in preparation). SEM analysis of developing ligular regions combined with genetic evidence suggests that LG1 and LG2 act in the same pathway to establish the blade/sheath boundary and position the ligule (Walsh et al., in preparation). Interpretation and propagation of a signal to induce ligule and auricle requires LG1 activity.

The ligule of Vg1-R, a spontaneous dominant mutant, is greatly reduced. We found that Vg1-R individuals initially form a normal-appearing ligule, but that cells within this structure die before development is complete. Genetic evidence suggests ectopic expression or altered function of vg1$^+$ results in cell death (Jesaitis, unpublished). An attractive hypothesis is that Vg1-R aberrantly activates programmed cell death and to test this idea, we obtained several transposon tagged Vg1-R alleles and are working to clone the gene (Jesaitis, unpublished).

21. Cellular Integration of MVA Synthesis and Protein Prenylation
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The multi-branched isoprenoid pathway from mevalonic acid (MVA) is one of the most important pathways in plants because isoprenes are required for many cellular processes such as cell division and growth, defense, hormone signaling, and photosynthesis. Research in animals and yeast has established MVA synthesis and prenylation of growth-related signal transduction proteins as critical factors for cell cycle progression and normal cell growth. MVA synthesis is also critical during plant development, but it is unknown how isoprenoid production is coordinated with cell division and growth. Modification of select regulatory proteins by prenylation provides a potentially important link between the isoprenoid pathway and growth control. Protein prenyl transferases have been found in all eukaryotic cells, and we have shown that the conserved plant farnesyl transferase can restore the function of Ras signaling in yeast. This project uses a biochemical and genetic approach to identify targets of protein prenyl transferases in plants. We have discovered that two plant regulatory proteins, the transcription factor APETALA-1 and a new type of calmodulin, are modified by prenylation. Mutations in the prenylation recognition motif, or genetic interactions between mutations in AP-1 and farnesyl transferase, show that the isoprenoid modification is necessary for the function of the proteins. Future experiments will clarify the cellular network that integrates early steps in the isoprenoid biosynthesis pathway with the function of these regulatory proteins.

22. Protein and RNA Interactions Involved in the Pathogenesis of Tomato Bushy Stunt Virus
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This research will emphasize analysis of tomato bushy stunt virus (TBSV) determinants that are important in virus assembly, replication and pathogenicity. TBSV is a small spherical RNA virus encoding five genes. We have shown that the two 5' terminal genes encode replicase subunits which are translated from the viral genome. An internal gene which encodes the coat protein, p41, is translated from the larger of two subgenomic RNAs. The second subgenomic RNA is used to express two nested genes (p22 and p19) which are involved in local and long distance movement. During the previous funding period, we examined the evolution and recombination of defective interfering RNAs associated with TBSV infections and evaluated the basis for their ability to modulate disease symptoms. We also conducted mutagenesis experiments to determine the requirements of each of the viral genes in the infection process, and obtained information that has helped define their roles in pathogenesis. These analyses have provided us with valuable information that has helped identify virus genes that elicit host responses during infection, but we have very little information about the mechanisms underlying these responses. We now propose to extend the genetic studies to investigate molecular interactions that occur during the initial stages of virus assembly, and the virus and host associations that involve p19 and p22 during infection. For Objective 1, we will characterize the initial interactions between the coat protein and viral RNA that lead to virus assembly. Experiments designed to identify and characterize the origin of assembly (OAS) on the viral genome and characterize the coat protein domains that recognize the OAS are proposed to enable us to develop a molecular model defining the nucleation events that lead to virion assembly. Objective 2 will focus on the nature of nucleoprotein complexes between p22 and viral RNA that are involved in cell-to-cell movement. Infected cells will also be examined for potential interactions between p19 and nucleic acids that may mediate host-specific responses identified using genetic techniques. In Objective 3, a cell biological approach will be implemented to determine the colocalization of p19, p22 and the coat protein with host components, and the host responses to expression of these proteins. These studies will provide a better understanding o f biochemical functions of the TBSV proteins and the complexity of virus-host interactions established during infection processes leading to disease development.

23. Determinants of Environmental Stress Tolerance by Bacteria on Leaves
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Bacteria that live as epiphytes on the surface of healthy plants are important as plant pathogens, in causing plant frost injury by catalyzing ice formation, and in other processes. The objective of this study is to determine those genes in plant-associated bacteria that are expressed in cells while on leaves but not in culture media and to determine how these traits enable epiphytic bacteria to survive the stresses encountered on leaf surfaces. We used a random mutagenesis approach to identify several loci in *Pseudomonas syringae* that are required for the survival of desiccation stress on leaves. We are developing an *in vivo* selection assay for plant-inducible genes using the *metY* locus which is required for stress survival on plants. Plant-inducible genes are being identified by complementing a *metY*⁻ mutant of *P. syringae*, which our previous work has shown to be deficient in both methionine biosynthesis and environmental stress tolerance on plants. A MetY⁻ strain harboring a plasmid library consisting of DNA segments fused to a promoterless *metY* locus survive on dry plants only if the cloned segments harbor a plant-inducible promoter. The plant-inducible loci are being sequenced to ascertain their putative functions and inactivated by insertional mutagenesis to enable their role in epiphytic fitness and stress tolerance to be determined. Current work is directed to determining the lowest level of transcriptional activity of gene fusions that is sufficient to complement epiphytic fitness in this system. The loci identified in this selection scheme will be further characterized following isolation using inverse PCR by end-sequencing. A cassette enabling

production of transcriptional fusions to a highly efficient GFP reporter gene has been made to enable the site-specific expression of plant-inducible genes to be determined.

24. Phytochrome from Green Plants: Properties and Biological Function
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Phytochrome B (phyB) is one of a five-member family of informational photoreceptors (designated phyA to phyE) in Arabidopsis. In fully green plants, phyB has a dominant role in monitoring for the appearance of competing neighboring plants and the consequent induction of the shade avoidance response. A central focus of this laboratory is to identify molecular components responsible for transducing signals perceived by phyB to the cellular response system. We have used a genetic approach to this problem beginning with a transgenic line of Arabidopsis (designated ABO) that overexpresses phyB. Seedlings of this line display enhanced deetiolation specifically in red light. To identify genetic loci necessary for phytochrome signal transduction in red light, we mutagenized ABO seeds and screened for revertants of the enhanced deetiolation response. One recessive, red-light-specific, extragenic revertant, designated *red1*, was isolated. The mutant phenotype was expressed in the original ABO background as well as in the nontransgenic Nossen (No-O) progenitor background. *red1* was mapped to the bottom of chromosome 4 at a position distinct from all known photoreceptor loci. Together with complementation analysis, the data show that *red1* is a novel photomorphogenic mutant. The evidence suggests that *red1* may represent a phytochrome signal transduction mutant potentially specific to the phyB pathway.

25. Molecular Analysis of Pathogen Recognition and Signal Transduction Events Specifying Plant Disease Resistance
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During the past year our research has focused on the molecular events involved in the delivery of the AvrRpt2 protein to the plant cell and the genetic dissection of the defense signal transduction pathway. The construction of epitope-tagged avrRpt2 avirulence genes and the production of polyclonal antisera capable of detecting the AvrRpt2 protein has allowed us to detect AvrRpt2 in induced bacteria, inoculated plants and stable transgenic plants. The results of these studies suggest that the AvrRpt2 protein is most likely processed either during the delivery to the plant cell or once it is inside the plant cell. In addition, we are beginning to study the molecular processes involved in the type III secretion process as it relates to the delivery of AvrRpt2. We are currently determining the location of the processing site and the sub-cellular location of the protein once it is inside the plant cell. The construction of transgenic Arabidopsis plants with a glucocorticoid inducible promoter has allowed us to use this system as a conditional lethal selection for mutations in the defense signal transduction pathway. Several putative mutants have been identified in this screen and are currently being further characterized. The development of this screen should allow us to perform saturation mutagenesis and allow us to detect rare mutations in this pathway.

26. Structural, genetic, and molecular analyses of gynoecium development in Arabidopsis
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First, we continued studies on the TOUSLED protein kinase (PK) gene, with emphasis on its role in gynoecium development. Mutations in tsl cause reduced differentiation of apical gynoecial tissues and eliminate fusion of the style and septum. This morphological defect correlates with the highest expression of TSL mRNA in the developing style. Double mutant analyses suggest TSL interacts with ETTIN, and redundantly with PERIANTHIA and LEUNIG. Biochemical analyses demonstrate the TSL PK trans-autophosphorylates on serine and threonine residues. TSL oligomerizes via its N-terminal domain utilizing a region containing two alpha-helical segments predicted to form a coil-coiled structure. Database analyses reveal a highly conserved TOUSLED-like kinase (TLK) family in both plants and animals, implying these enzymes perform similar roles in the nuclei of all multicellular eukaryotes. Second, our continuing studies on the ETTIN gene revealed it is expressed throughout stage 1 floral meristems and subsequently

resolves into a complex pattern within petal, stamen and carpel primordia. ETT likely imparts regional identity in floral meristems affecting perianth organ number spacing, stamen formation, and regional differentiation in stamens and gynoecia. During stage 5, ETT expression appears in a ring at the top of the floral meristem before morphological appearance of the gynoecium, supporting that ETT prepatterns apical and basal boundaries in the gynoecium. Double mutant analyses suggest ETT requires some meristem and organ identity genes (LEAFY, APETELA1, APETELA2, and AGAMOUS) but not others (APETELA3 and PISTILLATA); and ETT acts independently of CLAVATA loci but redundantly with PERIANTHIA.

27. Cellular and Molecular Characterization of Vascular Plasmodesmata
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In angiosperms, the functional enucleate sieve tube system of the phloem appears to be maintained by the surrounding companion cells. Analysis of sap collected from the sieve tubes has established that over 100 proteins are present in the sieve tube lumen. Presumably these proteins play a central role in maintaining the functional state of the sieve tubes. Parallel experiments performed with phloem proteins obtained from squash plants, the cucumber mosaic virus movement protein and KNOTTED1, a plant homeobox protein, indicated that these proteins all display comparable capacities to interact with and move through plasmodesmata. Of equal importance, we found that these phloem proteins exhibit a high affinity for the plasmodesmal binding sites; concentrations required for cell-to-cell transport were in the 20 nM range. A number of squash phloem proteins were cloned and sequenced to allow us to further explore the molecular determinants for protein trafficking through phloem plasmodesmata. In situ and immunolocalization studies confirmed that the mRNA was restricted to the companion cell whereas protein was detected only in the sieve tubes. A search of the data base revealed homologous genes in tobacco, Arabidopsis, rice and maize. We also identified sequences in these novel phloem proteins that exhibit homology to a viral movement protein. These sequences are presently being analyzed to identify the presence of common motifs that may reflect plasmodesmal localization signals. Information of this nature will allow us to explore the evolution of the capacity of plants to function as supracellular organisms.

28. Developmental Genetics of Nectaries
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Nectaries are secretory organs often involved in offering rewards for pollinators in flowering plants. Despite extensive literatures on the morphology, ultrastructure, phylogenetic distribution, and pollination biology of nectaries, little is known about their ontogeny or the genes directing their development. In the Brassicaceae, nectaries are positioned at the base of the stamens. The nectaries consist of glands that are supplied by phloem with nectar secretion likely occurring through the stomata located at their apex. Although nectaries are associated with stamens in wild-type Arabidopsis flowers, their development does not depend on the presence of the stamens since nectaries develop in singly and multiply mutant strains that lack stamens. Genetic evidence to date suggests that the development of nectaries may be independent of the expression of the ABC genes that specify the identity of the other floral organs. The only Arabidopsis mutant that is known to alter nectary development is crabs claw (crc), mutations which result in the loss of all visible signs of nectary development. In the nectary, CRC expression commences in a small number of cells (the nectary anlagen) abaxial to the stamen primordia. Expression continues in all cells of the nectary throughout floral development. Characterization of CRC and other genes known to be expressed in nectaries will provide a foundation for the molecular and genetic description of nectary development in a model species and develop tools that may be utilized to examine whether we may be able to manipulate nectar quantity and composition.

29. The Biosynthesis of Cellulose and Callose in Developing Cotton Fibers
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The ultimate goal of this project is to elucidate the mechanism and regulation of synthesis of the cell wall polymer cellulose (beta-1,4-glucan) and the wound polymer callose (beta-1,3-glucan). Two cotton cDNA clones (CelA-1 and CelA-2) that encode homologs of the catalytic subunit of bacterial cellulose synthases represent the first genes isolated from plants that may encode subunits of a cellulose synthase. Evidence for CelA function includes: a) presence of conserved motifs believed to be involved in binding of UDP-glc and catalysis; b) recombinant protein binds UDP-glc in a Mg^{2+}-dependent manner; c) genes are highly-expressed in cotton fibers at onset of secondary wall cellulose synthesis; d) another laboratory has identified a close homolog of this gene that is mutated in Arabidopsis plants that leads to a defect in cellulose synthesis. Current goals include: 1. Catalytic activity and topology: CelA, engineered to encode a myc epitope in N-ter and two HA epitopes in a predicted extracellular domain, is being expressed in yeast and plants to study the topology of CelA in the plasma membrane, to test for functional activity in yeast, and to perform immunolocalization; 2. Structure of cellulose and callose synthase complexes: the N-ter of CelA-1 contains two zinc fingers that we find bind two moles of zinc per mole of protein and results suggest this region interacts with itself and may be important in stabilizing CelA-CelA subunits in synthase complexes. We also search for other proteins that interact with CelA or callose synthase using yeast two-hybrid as well as biochemical studies (current candidates include cytoskeletal proteins, annexins and/or sucrose synthase). 3. Cel-A-GFP fusions are being expressed in transgenic plants to see if we can study the movement process for synthase complexes in living cells using the fluorescent GFP tag as a marker for CelA.

30. Structure, Function and Assembly of the Clostridium cellulovorans Cellulosome

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We are determining the structure, function, assembly and regulation of the *Clostridium cellulovorans* cellulosome (cellulase) and non-cellulosomal cellulases. By our studies we hope to engineer a more efficient cellulase and a more efficient bacterial strain that will produce large amounts of hyperactive cellulase. Our first goal has been to characterize the three major subunits of the cellulosome. We have cloned and sequenced the genes for two major subunits, cbpA, which codes for the scaffolding protein, and exgS, which codes for the exoglucanase, ExgS. We have cloned the gene for the third major subunit, P100, which codes for an endoglucanase and are in the process of sequencing this gene. Our goal is to demonstrate that mini-CbpA containing 2-4 enzyme binding domains (EBDs), P100 and ExgS are capable of forming an active mini-cellulosome. We are characterizing the EBDs to see whether they are specific for one enzyme subunit or can bind all the enzyme subunits. In addition we have cloned a gene for a second non-cellulosomal cellulase EngF. EngF is produced in large quantities during growth on cellobiose and in much lesser amounts during growth on cellulose. EngF is an endoglucanase and appears to function primarily as an oligodextrinase, since EngF is active on pentoses, but is far less active on carboxymethylcellulose than EngD, another non-cellulosomal endoglucanase, and EngB, a cellulosomal endoglucanase. We are testing to see whether synergism exists between the cellulosome and non-cellulosomal enzymes by mixing experiments with the cellulosome and EngF and EngD. By growing the cells on cellobiose and cellulose, we have demonstrated a very different pattern of cellulase synthesis. We will examine the molecular regulation of cellulosomal and non-cellulosomal genes by analyzing the transcripts and the promoters that are expressed during different growth conditions.

31. Regulation of Embryonic Development in Higher Plants

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Many genes have been identified that are required for the completion of embryonic development in higher plants, however, relatively few have been shown to play major regulatory roles. The Arabidopsis LEAFY COTYLEDON (LEC) genes, LEC1, LEC2, and FUSCA3, play central roles in embryogenesis. These genes are required to suppress the embryonic potential of the suspensor early in embryogenesis, to specify cotyledon identity, to maintain the maturation phase, and to prevent developing seed from germinating prematurely. These numerous and varied roles suggest that LEC1 may act in coordinating diverse aspects of embryonic development. To begin to understand the function of these genes at a mechanistic level, we isolated the LEC1 gene and analyzed its expression pattern. Sequence comparisons showed that the deduced polypeptide is homologous to a conserved eukaryotic transcription factor, suggesting a direct regulatory role for LEC1. As predicted from the mutant phenotype, we showed that LEC1 mRNA is present throughout embryogenesis, including the earliest stages, and that the gene does not appear to be expressed at any other developmental stages. We also showed that LEC1 mRNA is present in specific regions of the embryo, in the suspensor, and in the endosperm. Based on these results, we hypothesize that LEC1 may play its integrative role by establishing and maintaining an embryonic state within the seed. Studies are in progress to test this hypothesis.

32. Protein Translocation and Assembly in Chloroplasts
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This project seeks to elucidate the mechanism of transport and assembly of the nuclear-encoded subunits of the photosynthetic oxygen-evolving enzyme complex (OEC). This complex, which produces essentially all the oxygen in the atmosphere, consists of three nuclear-encoded subunits working in concert with chloroplast-encoded subunits of the photosystem II reaction center. Our experiments revealed that the homeostasis of the OEC, which is complicated by the rapid turnover of the reaction center in the light, is maintained in part by cycling the subunits between the complex bound to the inner surface of the thylakoid membrane and a pool of soluble subunits located in the thylakoid lumen. In addition, our experiments support a model of stepwise assembly of the OEC with the photosystem II reaction center, with the largest of the subunits binding to the reaction center in the unstacked regions of the thylakoid membrane. The partially assembled complex is then postulated to migrate to the stacked membrane regions, where the smaller two subunits are bound and enzyme activity is manifested.

We are also investigating the mechanism of transport of these OEC subunits across the thylakoid membrane. Our experiments have recently revealed a role for an azide-sensitive component involved in the transport of the smallest OEC subunit to the lumen, a process that was previously thought to occur without azide-sensitive components. Different experiments demonstrated that this translocation process occurs without the concomitant ion leakage that would be expected if the protein traversed the membrane through an aqueous pore.

33. Membrane Bioenergetics of Salt Tolerant Organisms
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Salt tolerance in the extremely halophilic bacteria requires generation of electro-chemical ion gradients across the cytoplasmic membrane that drive sodium extrusion and chloride accumulation. We study the molecular mechanisms of the proton and chloride transporting bacterial rhodopsins in these organisms. Studies of bacteriorhodopsin and halorhodopsin, respectively, explore the thermodynamics of the transport, the chromophore and protein changes that determine the changing connectivity of the active site to the two membrane surfaces during the transport cycle, and the mechanisms of ion conduction to and from the retinal Schiff base. Site-specific mutagenesis, time-resolved optical multi-channel and infrared (FTIR) spectroscopy, Raman spectroscopy, stopped-flow measurements, and through collaborations x-ray diffraction are the principal methods used in this work. With bacteriorhodopsin the main effort is to refine a detailed mechanistic model now available, and to describe the transport in structural terms to increasing degrees of resolution. With halorhodopsin we are only beginning to establish the outlines of a mechanistic model.

34. Retention, modification, and default destination of membrane proteins along the secretory pathway
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The vacuoles in the cotyledons of leguminous seeds contain glycoproteins used for storage of nitrogen (storage proteins) and involved in plant defense against insects and mammals (lectins and enzyme inhibitors). We are studying the interaction of some of these plant defense proteins with their targets in animals. We focus on two classes of proteins, alpha amylase inhibitors (aAI) and lectins, in the common bean, *Phaseolus vulgaris*, and other leguminous plants. The bean aAI inhibits the amylase of certain insects but not of others. To understand this specificity we recently cloned the amylase of the bruchid *Zabrotes subfasciatus* and purified the expressed enzyme. We have now cloned the amylase of a second insect *Acanthoscelides obtectus*. We have found in the common bean two variants of aAI and the amino acid sequences of both inhibitors have been obtained. We are now in a good position to study the interaction of the enzyme and the inhibitors. aAI-2 inhibits the amylase of *Z. subfasciatus*, but not that of *A. obtectus*. Our goals are to crystallize aAI-2 with the amylase of *Z. subfasciatus*, and to use biopanning of a peptide library to find an inhibitor for the amylase of *A. obtectus*. Progress has allowed us to obtain a grant from a joint Industry- State of California program to clone the

amylase of the western corn root worm. We are investigating a lectin from the legume *Dolichos lablab*. The cloned lectin has been expressed in *E. coli* and the isolated protein is toxic to aphids at levels that will make it feasible to use it for genetic engineering.

35. Molecular Structure, Function, and Physiology of Potassium Uptake Channels in Plants
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Potassium uptake by higher plant cells is of central importance to plant growth, stomatal regulation, cell expansion, nutrition, tropisms, osmoregulation, enzyme homeostasis and plant membrane potential control. Elucidation of the molecular mechanisms by which higher plant cells selectively transport K^+ is important for manipulation of the above processes. Patch clamp studies on guard cells and many other plant cells have suggested that "inward-rectifying" K^+ uptake channels provide a molecular pathway for membrane potential control and for proton pump-driven K^+ uptake. Arabidopsis K^+ channel cDNAs have recently been cloned, and we are functionally characterizing the structure and function of two of these cDNAs expressed in leaf cells and guard cells. The long-term goal of this research project is to gain a quantitative understanding of the physiological functions of Arabidopsis K^+ channels for important biological functions in plant leaves. We will focus on K^+ channel functions during stomatal regulation, which is important for CO_2 exchange and water transpiration. Furthermore we will study the importance of a leaf K^+ channel cDNA for leaf growth. These studies will contribute to a molecular physiological dissection K^+ channel functions in higher plant leaves and their roles in growth, development and environmental responses of plants.

36. Suspensor Differentiation During Early Plant Embryogenesis
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The molecular processes responsible for directing cells of the early plant embryo to follow distinct differentiation pathways are not understood. In many plants, the zygote divides asymmetrically into a small apical cell and a large basal cell. The apical and basal cells are destined to become the embryo proper and suspensor regions of the embryo, respectively. It is not known what molecular events direct the apical and basal cells to follow different developmental pathways. Nor is it known what genes control apical and basal cell specification within the two-cell embryo. This project utilizes the giant suspensor of the Scarlett Runner Bean, *Phaseolus coccineus*, to gain entry into the differentiation events that occur at the earliest stages of plant embryogenesis. Experiments being carried out in my laboratory are testing the hypothesis that "morphogenetic factors" are asymmetrically distributed to the basal cell and set off a cascade of events leading to suspensor differentiation. Specific experiments include (1) identifying and sequencing suspensor-specific genes, (2) studying the spatial and temporal expression patterns of suspensor-specific genes, (3) using targeted cell ablation studies to determine whether interactions occur between suspensor cells and between the suspensor and embryo proper, and (4) identifying corresponding Arabidopsis suspensor-specific genes and searching for mutant lines that have defects in these genes. The significance of this project is that it should provide new information on the molecular processes that control cell differentiation at the earliest stages of plant development.

37. Molecular Biology and Genetics of the Acetate-Utilizing Methanogenic Bacteria
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Methane biosynthesis by the *Methanosarcina* species occurs from the full range of methanogenic substrates, and is often the rate limiting step in anaerobic habitats of natural and man-made environments. The substrates include acetate, methanol, tri-methyl, di-methyl, and methyl-amines, and in most cases, H_2/CO_2 as precursors for methane formation. The *Methanosarcina* are also the most versatile among the methanogens in their ability to adapt to different habitats that vary in osmolarity. To further explore the physiology of these organisms using molecular and biochemical approaches, we are identifying and characterizing different classes of differentially expressed genes. We plan to further examine how N-acetyl–lysine, -glutamate, betaine, and potassium levels are modulated in the cell to provide appropriate osmoregulation. We will also examine the sets of genes employed for changes in

carbon substrate utilization. We will complete a high resolution physical map of the *M. thermophila* genome to aid in further exploiting the powerful differential display methods for identifying and characterizing gene families in Archaea. The high resolution physical map and the ordered BAC library generated in this study should facilitate related studies in the other *Methanosarcina* species by this and in other laboratories. Such libraries can also be exploited to aid in genome sequencing, and to exploit genome information as it becomes available. These studies will enhance our understanding of how methanogens sense their environment and adapt physiologically to varying anaerobic habitats.

38. Role of Glycolytic Intermediates in Global Regulation and Signal Transduction
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The goal of our research is to probe the existence of signal metabolites in global regulation, such as Ntr regulon. We perturbed the metabolic pools by overexpression of related enzymes, and characterized the effects on specific regulons. One of the problems in this approach is the disturbance of physiological state caused by protein overexpression itself. To control for this adverse effect, we constructed mutants that are enzymatically inactive but are stable and soluble. These mutants showed that high-level protein overexpression per se decreases ribosome synthesis. Although the phenomenon resemble the stringent response, it is not mediated by guanidine tetraphosphate, (p)ppGpp. Fortunately, the decrease in ribosome synthesis was not significant for the level of protein overexpression used in detecting signal metabolites. Overexpression of phosphoenolpyruvate carboxykinase (Pck) was used to introduce perturbation to glycolytic metabolite pools. The overexpression of Pck in a wild-type background has no effect on *glnA* expression. However, overexpression of Pck in a *pta* strain caused a non-inducible phenotype for *glnA* operon. In addition, the adenylation of glutamate synthetase remained high during Pck overexpression even during nitrogen starvation. This result suggests that a signal metabolite is perturbed to mask the effect of nitrogen limitation. Fructose 1,6-diphosphate and pyruvate levels correlate with such an effect, although the causal relationship remains to be established.

39. Sensory Transduction of the CO2 Response of Guard Cells
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Plants need to balance the rate of water vapor loss against the rate of photosynthetic CO_2 uptake. Stomata sense environmental parameters and optimize the exchange water and CO_2 in the leaf. We have identified a growth chamber environment which greatly enhances stomatal response to CO_2 and are using plants from this environment to investigate the cellular mechanism of CO_2 sensing. Zeaxanthin is a member of the carotenoid class of chloroplast pigments that has been implicated in the sensory transduction of blue light in guard cells. In mesophyll chloroplasts, zeaxanthin formation is known to be modulated by CO_2. In growth chamber-grown *Vicia faba* leaves kept under constant light (500 μmol m^{-2} s^{-1}) and temperature, guard cell zeaxanthin tracked ambient [CO_2] and stomatal apertures. Changes in zeaxanthin and aperture were reversed when [CO_2] was lowered. Guard cell zeaxanthin was linearly correlated with stomatal apertures. In the dark, the CO_2-induced changes in stomatal aperture were much smaller, and guard cell zeaxanthin did not change with chamber [CO_2]. Guard cell zeaxanthin also tracked [CO_2] and stomatal aperture in isolated stomata. Application of dithiothreitol (DTT), an inhibitor of zeaxanthin formation, to illuminated epidermal peels eliminated CO_2-induced zeaxanthin changes in guard cells and partially inhibited the CO_2 response of stomata to the levels observed in the dark. Zeaxanthin could modulate CO_2-dependent stomatal apertures in the light, while a zeaxanthin-independent CO_2 sensing mechanism would modulate the CO_2 response in the dark.

40. Regulation of Vacuolar pH in Citrus limon
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The pH of plant vacuoles is under developmental and environmental control, but little is known about how vacuolar pH is regulated. Lemon provides an excellent system for such studies because it contains two types of vacuoles: a typical vegetative-type vacuole

with a lumenal pH of ~5.5, and a highly acidic juice sac-type vacuole in the fruit with a lumenal pH as low as 2.2. Our work to date has established that both tonoplasts contain V-type ATPases. However, we have found a number of differences as well: 1) fruit tonoplasts have a lower H^+ permeability than epicotyl tonoplasts; 2) in native membranes, the epicotyl V-ATPase is strongly inhibited by nitrate and bafilomycin, while the fruit V-ATPase is relatively insensitive to these inhibitors; 3) after reconstitution into *E.coli* or soybean phospholipids, the purified fruit V-ATPase becomes sensitive to nitrate and bafilomycin; 4) reconstituting the fruit V-ATPase into fruit tonoplast lipids restores the insensitivity to nitrate and bafilomycin, hence membrane lipids protect the fruit V-ATPase from these inhibitors. However, reconstituting the epicotyl V-ATPase into fruit lipids does not make the epicotyl V-ATPase insensitive to nitrate and bafilomycin. 5) the epicotyl V-ATPase has a lower H^+/ATP coupling ratio and, when purified and reconstituted, has a higher slip rate than the fruit V-ATPase; 6) the fruit V-ATPase contains a fruit-specific 33/34 kDa polypeptide and multiple copies of a 16 kDa polypeptide. It is proposed that these subunits may contribute to the tight coupling of the fruit V-ATPase.

41. Engineering Renewable Biomaterials
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The overall goal of the proposed research is to expand the range of biomaterials produced from higher plants and increase the efficiency of biomass production. One aspect of our work is focused on identifying plant genes that would permit production of economically significant levels of industrially useful hydroxylated and epoxidated fatty acids in transgenic plants. Toward this end we are attempting to define the structural properties of desaturases, hydroxylases and epoxidases that determine the outcome of the enzymatic reactions catalyzed by these types of enzymes. One factor that limits oil production in many plant species is that oil accumulation is accompanied by high levels of energetically expensive storage protein accumulation. To examine the feasibility of uncoupling oil and protein accumulation in plants we will identify genes encoding transcriptional factors that regulate expression of genes involved in storage lipid accumulation. We will explore the ability of these factors to induce expression of the genes involved in storage oil accumulation in tissues with comparatively low levels of protein. We are also exploring the long-term potential of producing oils and related materials in roots or tubers. In this respect, we are investigating the function of a gene (designated *pkl*) that alters cell identity during early embryo development. Finally, we are investigating the biological function of a family of genes that exhibit sequence homology to components of bacterial cellulose synthases. The principal goal of this work is to identify the role of these genes in higher plants.

42. Powdery Mildew Disease Resistance
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Our long term objective is to characterize genes conferring resistance to powdery mildew diseases, which are caused by obligate fungal pathogens of the *Erysiphe* genus. Because of the extensive molecular genetic resources available for *Arabidopsis*, we chose to isolate powdery mildew resistance genes from this plant species. In previous work, seven distinct resistance loci were found in six accessions suggesting that powdery mildew resistance in *Arabidopsis* is as diverse and complex as in crop species like barley. In a large survey, 66 of 360 accessions were found to be resistant to *E. cichoracearum* isolate UCSC. A complementation test based on the 40 most resistant accessions has been initiated. From this test, new powdery mildew resistance genes and stronger alleles of known resistance genes will be identified.

We have focused on the powdery mildew resistance locus, RPW1. Based on a preliminary QTL (quantitative trait loci) analysis, two modifier genes, in addition to RPW1, control disease resistance in the accession Kas-1, suggesting that some pyramiding of resistance genes has occurred in the weedy plant, *Arabidopsis*. A map-based cloning strategy will be used to clone RPW1. To complement the map-based cloning, we have mapped 47 R-ESTs (i.e., ESTs with sequence similarity to known resistance genes) and compared their map positions with those of known disease resistance genes. No R-EST maps near RPW1; thus, the candidate gene approach has not been helpful in cloning RPW1. However, the information generated by this project should facilitate the cloning of *Arabidopsis* resistance genes by the plant pathology community.

43. Functional description of the Rhodobacter capsulatus genome emphasizing genes for nitrogen fixation and photosynthesis
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This work has become the Rhodobacter genome project with three goals: to complete the genome sequence for the type strain, SB1003; to develop expression chips for the determination of transcript levels for all the genes at once, under different growth conditions and in different mutant strains; and to develop genetic methods for deletion strain construction to enable a complete functional analysis of the genome.
The sequencing has progressed as follows: About 200 kb have been sequenced, polished and fully annotated and published. The paper by Vlcek, Paces, Maltsev, Paces, Haselkorn and Fonstein is in press in PNAS. Another 600 kb are being annotated at Argonne National Lab by Natalia Maltsev, working in the lab of Ross Overbeek. Another 600 kb are being polished for annotation. Another 600 kb have been subcloned and sequenced. The next 600 kb are being subcloned.

Expression chips are being developed in collaboration with the group of A. Mirzabekov at ANL. The major technical problems are stability of the acrylamide matrix, reduction in the fluorescence background from the acrylamide, improving the yield of the crosslinking reactions for the oligo probes. All of these are being studied. We were awarded a small starter grant from the Argonne/U of Chicago consortium to pursue these studies.

Regarding the construction of deletions, the methods are in place for cosmid-sized deletions. For single gene deletions, the approach is based on PCR amplification of the deletion target and will be developed in collaboration with Fevzi Daldal at U. of Pennsylvania.

44. Molecular genetic analysis of biophotolytic hydrogen production in green algae
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Several species of green algae have the unique biochemical capability of producing gaseous hydrogen from water using solar energy harvested by the photosynthetic apparatus, a process called biophotolysis. An understanding of factors that promote as well as limit biophotolysis may lead to a highly efficient solar energy conversion system that directly yields hydrogen gas as the energy storage medium. We are exploiting the powerful array of genetic analysis and molecular genetic manipulation techniques that have been developed for the green alga *Chlamydomonas reinhardtii* to investigate the molecular mechanisms underlying biophotolysis. Efficient hydrogen production occurs only under anaerobic or anoxic conditions. This feature limits the utility of biophotolysis, since oxygen is a necessary co-product with hydrogen. Hydrogenase, the key enzyme that couples energy harvested by the photosynthetic light reactions in the chloroplast to hydrogen evolution is not synthesized except in the absence of oxygen. The mechanism of this regulatory effect of oxygen is being investigated as part of this project. The activity of the hydrogenase in green algae is also directly destroyed by molecular oxygen, although the detail is unknown. The enzyme from Chlamydomonas has only one subunit, making it an ideal target for exhaustive mutagenesis to test the possibility that an oxygen-insensitive form can be developed. We have initiated an effort to generate genetically altered enzymes with different degrees of oxygen sensitivity. Analysis of these mutant enzymes will help to build a picture of the mechanism of oxygen inactivation and support directed efforts to develop fully oxygen-insensitive activity.

45. Analysis of cell-cell interactions during Arabidopsis Reproduction
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Funding: $100,001 12 months

Plants have a remarkable ability to interact favorably with pollen of the appropriate species, while inhibiting foreign pollen. To clarify the molecular basis fo: this discrimination, we have identified genes that are critical for pollen communication with female pistil

tissues. We have shown that mutations in these genes can affect the abundance of proteins on the surface of pollen and pistil cells. Our work focuses on understanding these surface molecules, and these studies will likely provide valuable tools for manipulating plant resources, both in agriculture and in natural ecosystems.

We are investigating interactions between pollen tubes and ovules by characterizing a mutant with altered pollen tube guidance. This mutation affects the ability of pollen tubes to adhere to ovule cells, and we are exploring the requirements for the adhesive interactions by characterizing the mutant defect and cloning the altered genes. Importantly, the mutant has allowed us to determine that gene expression in both male and female tissues is required for guidance. In parallel, we are exploring the initial interactions between pollen and the receptive stigma cells on the pistil surface by determining the function of a family of related pollen surface proteins. In particular, we are constructing alterations in these proteins to test their roles in binding to stigma cells, in mediating communication with the stigma, or in specifying pollen identity. These studies will expand our knowledge of the unique signaling mechanisms that enable plant cells to communicate, despite their thick extracellular walls.

46. The Magnesium Chelation Step in Chlorophyll Biosynthesis
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In photosynthetic organisms, the synthesis of new energy generating membranes requires the coordinate synthesis of proteins, their associated cofactors, and various lipids. The important cofactors, chlorophyll and heme, share a common biosynthetic pathway, diverging at the point of metal ion insertion. Mg-chelatase catalyzes the insertion of magnesium into protoporphyrin-IX, the first step unique to chlorophyll synthesis. Our work is focused on the mechanism and regulation of this enzyme as it is a key to understanding how plants produce the proper proportion of chlorophyll and heme from a common pathway. We have shown by fractionation of chloroplast extracts and by cloning of the chlD gene that the enzyme in higher plants consists of three subunits (two other genes chlI and H have already been identified). Sequencing of the chlD gene suggests that it encodes a protein of 82.9 kDa (including a putative chloroplast transit peptide). The protein has a nucleotide binding motif and an unusual stretch of prolines followed by a stretch of polyglutamate. It is 58% homologous to the cyanobacterial protein. We have expressed the C-terminal third of the protein in *E. coli*. Although the expressed protein cannot substitute for a fraction containing the authentic pea D subunit in a reconstitution assay, it stimulates (20-25%) activity when added to a soluble extract that contains all three components. Work is continuing on the expression of a full-length D subunit for subsequent analysis of the role of this protein in the reaction.

Plants have a remarkable ability to interact favorably with pollen of the appropriate species, while inhibiting foreign pollen. To clarify the molecular basis for this discrimination, we have identified genes that are critical for pollen communication with female pistil tissues. We have shown that mutations in these genes can affect the abundance of proteins on the surface of pollen and pistil cells. Our work focuses on understanding these surface molecules, and these studies will likely provide valuable tools for manipulating plant resources, both in agriculture and in natural ecosystems.

We are investigating interactions between pollen tubes and ovules by characterizing a mutant with altered pollen tube guidance. This mutation affects the ability of pollen tubes to adhere to ovule cells, and we are exploring the requirements for the adhesive interactions by characterizing the mutant defect and cloning the altered genes. Importantly, the mutant has allowed us to determine that gene expression in both male and female tissues is required for guidance. In parallel, we are exploring the initial interactions between pollen and the receptive stigma cells on the pistil surface by determining the function of a family of related pollen surface proteins. In particular, we are constructing alterations in these proteins to test their roles in binding to stigma cells, in mediating communication with the stigma, or in specifying pollen identity. These studies will expand our knowledge of the unique signaling mechanisms that enable plant cells to communicate, despite their thick extracellular walls.

47. The Iojap Gene in Maize
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Chlorophyll variegation in higher plants is a well known but poorly understood phenomenon. Single recessive nuclear mutations are often responsible for the variegated phenotype although, for a few mutants, defective plastids are transmitted through to the next generation. For more than 50 years the variegated *iojap* mutation in maize has been the model system for such cytoplasmic inheritance. Specifically *iojap*-affected plastids transmitted through the female egg cell remain defective independent of the parental nuclear genotype. The striping pattern of *iojap* plants indicates the Ij protein acts early in leaf development. Mature leaf cells can have all normal chloroplasts, or all mutant ones, or both normal and mutant plastids (heteroplastidic cells). Their arrangement on the

leaf defines the pattern of striping, and may reflect the cytoplasmic inheritance of defective plastids in certain meristematic lineages. Sectors showing nuclear reversion to fully green arise only in heteroplastidic cells and are not observed in white tissue.

We have shown that the protein encoded by Ij is a soluble, chloroplast localized protein that associates with chloroplast ribosomes. Consistent with the proposal that Ij plays an essential role in translation, hypothetical proteins with sequence similarities to Ij have been identified in all sequenced bacterial genomes with the exception of mycoplasmas and archaebacteria. It has long been postulated that chloroplasts are the evolutionary descendants of endosymbiotic bacteria. The combined use of plant and bacterial molecular genetic systems will serve to answer fundamental questions pertaining to *iojap* variegation and cytoplasmic inheritance.

48. Microbial Production of Isoprene
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This research project addresses the microbial production of the volatile hydrocarbon isoprene (2-methyl-1,3-butadiene). Biological systems, especially forest plants, currently emit 100s of millions of tons of isoprene to the atmosphere each year. We argue that capture of this biogenic potential in a regulated system could lead to bioreactors that produce useful amounts of isoprene as a feedstock for the polyisoprene rubber and elastomer industry. While we have made progress in understanding the enzymology of plant isoprene formation, plant systems are less tractable for biochemical and molecular manipulations than a well defined bacterial system. We have discovered that most *Bacillus* species are especially active isoprene producers. We propose that molecular investigations of isoprene synthesis in *Bacillus subtilis* will lead to a basic understanding of how and why bacteria produce this volatile hydrocarbon, and reveal the regulation of its production. We are working to determine by ^{13}C-labeling experiments whether isoprene formation in *Bacillus* occurs by the traditional mevalonate pathway, or by the glyceraldehyde-3-phosphate/pyruvate pathway which gives rise to isoprene in plant chloroplasts. In addition, we are pursuing the idea that isoprene is a signaling molecule produced in exponentially dividing cells, and serving to repress stationary phase genes. The results of these experiments should pave the way for biochemical and molecular investigations of the mechanism and regulation of bacterial isoprene biosynthesis.

49. Sugar Transport and Metabolism in Thermotoga
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We are studying the energetics and regulation of sugar transport and metabolism in the genus *Thermotoga*, hyperthermophilic strict anaerobes that represent the deepest phylogenetic branch of heterotrophic fermentative bacteria known, with the expectation that studies of the comparative physiology and biochemistry of these organisms will yield insights concerning the evolution of these processes. Using non-metabolizable sugar analogs as transport substrates, we have found distinct sodium-dependent active transport systems for D-glucose and beta-D-galactoside in *T. neapolitana* that are energized by ion gradients generated by ATP, derived from substrate-level phosphorylation. While the glucose system is constitutive, both the transport and hydrolysis of beta-galactoside are inducible by galactose and lactose, and subject to a cAMP-independent repression by glucose (catabolite repression). Glucose did not inhibit beta-galactoside uptake by induced cells, however, indicating the absence of inducer exclusion. This absence of cAMP-dependent catabolite repression and inducer exclusion correlate with the apparent absence of the PEP:sugar phosphotransferase system, a multicomponent system with specific proteins that play key roles in catabolite repression and inducer exclusion in certain modern bacteria. The mechanism of the sodium requirement is being investigated to gain insight into how hyperthermophilic organisms generate and maintain ion gradients under conditions of presumed increased membrane permeability at high temperatures. Using ion-specific electrodes, movements of sodium and hydrogen ions are being measured in whole cells during metabolic energy generation and active sugar transport. Also, isolated membrane vesicles obtained by lysozyme-EDTA treatment of cells are being used to study sugar transport energized by artificially imposed ion gradients.

50. Genetic Analysis of Sugar Nucleotide Interconversions in Arabidopsis
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The synthesis of plant cell wall polysaccharides depends on the availability of nucleoside-diphospho sugars representing activated monosaccharides generated via nucleotide sugar interconversions. We have chosen the plant model system *Arabidopsis thaliana* to isolate and characterize genes involved in the *de novo* synthesis of the monosaccharides L-rhamnose, L-fucose and L-arabinose via these biosynthetic pathways. Genes encoding the two enzymatic steps in the synthesis of GDP-L-fucose from GDP-D-mannose have been identified and characterized. The first enzyme in this pathway (GDP-D-mannose-4,6-dehydratase) is encoded by at least two genes (MUR1 and GMD1), while the second enzyme (an epimerase-reductase) appears to be encoded by a single-copy gene. The MUR1 gene is expressed throughout the plant whereas GMD1 gene expression is subject to spatial and temporal regulation within the root. Mutations at the MUR1 locus lead to decreased mechanical strength of elongating inflorescence stems indicating that alterations in precursor availability can change the biophysical properties of the wall. Several genes believed to encode enzymes in the de novo synthesis of L-arabinose and L-rhamnose are currently under investigation to define their role in cell wall synthesis, and to determine their relationship to mutations leading to reduced amounts of these monosaccharides. Our long-term goal is to determine the significance of nucleotide sugar interconversion pathways for the regulation of cell wall synthesis leading to opportunities to alter cell wall compositions by modifying precursor availability.

51. Energy Transduction in Plant Mitochondria
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Cytoplasmic male sterility (CMS), a mitochondrially-encoded disruption in pollen development, is a valuable agronomic trait that facilitates production of hybrid seed. Understanding its mechanism may facilitate the incorporation of this trait into crop species lacking naturally-occurring CMS-encoding genes. Male reproductive development is often disrupted before or during meiosis in cytoplasmic male sterility (CMS) plants. We are using cell biological techniques, including two-photon confocal microscopy and two-photon fluorescence microscopy, to compare cells in reproductive tissues in normal, CMS, and fertile plants carrying the CMS-encoding mitochondrial gene and the fertility-restoring nuclear Rf allele. Membrane-potential sensitive dyes are being used to probe respiratory activity in vivo in CMS and fertile plants. Transgenic plants containing the green fluorescent protein targeted to mitochondria and other organelles are being produced so that subcellular structures can be visualized. Molecular markers closely linked to the fertility restorer locus are being used to identify male sterile and fertile plants with recombination events near the restorer allele.

52. Regulation of Denitrification in Rhodobacter sphaeroides
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Like humans, bacteria respire oxygen to generate energy required for growth. Unlike humans, however, bacteria can respire compounds other than oxygen. Nitrate is one of the many compounds that bacteria can use as an alternative respiratory substrate. Nitrate respiration which leads to the production of nitrogen gas is referred to as denitrification. Our laboratory is interested in identifying and understanding the role of genes whose products are required for denitrification. The bacterium we are using for our studies is a denitrifying variant of *Rhodobacter sphaeroides*. We have focused our effort on understanding the function and regulation of genes whose products are required for nitric oxide metabolism. Nitric oxide is an obligatory intermediate during denitrification and is also a biologically important molecule in higher organisms, including humans. In addition to the genes encoding nitrite reductase and nitric oxide reductase, which respectively produce and reduce nitric oxide, we have identified several other genes whose products are essential for nitric oxide respiration. Several of these encode membrane proteins of unknown function. We have also recently determined that strains of *Rhodobacter sphaeroides* closely related to the strain we have been studying carry the genes for nitric oxide reductase genes as well as for the membrane proteins discussed above but lack nitrite reductase. This

makes them unable to carry out complete denitrification. The reason for this truncated form of denitrification is unclear but such cells may be able to use nitric oxide generated by other bacteria as an alternate respiratory compound when oxygen is limited.

53. Cold Acclimation of Herbaceous Species: Effect of Sugars on Membrane Cryostability
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The ultimate goal of this project is to provide a mechanistic understanding of the cellular and molecular aspects of freezing injury and cold acclimation to provide for rational strategies for the improvement of freezing tolerance of crop species. The current objectives are to determine the mechanism(s) by which cytosolic sugars alter the lyotropic phase behavior of phospholipids and increase the cryostability of cellular membranes. Studies in which the endogenous sugar content of *Arabidopsis thaliana* leaves was artificially manipulated by incubation on sucrose solutions have revealed that low concentrations of sucrose (10-35 mM) are required as a metabolic substrate necessary for low-temperature-induced alterations in the lipid composition of the plasma membrane during the initial stages of cold acclimation and which are necessary to preclude expansion-induced lysis during a freeze/thaw cycle. At higher concentrations, sucrose has a direct cryoprotective effect that results in a decreased incidence of freeze-induced formation of the hexagonal II phase (30-200 mM) and the fracture-jump lesion (100-400 mM). The cryoprotective effect of the sucrose appears to be additive rather than synergistic to the low-temperature-induced alterations in membrane lipid composition and the synthesis of COR polypeptides. Studies of artificial bilayers composed of either DOPE or POPE have revealed that sugars, such as sucrose and fructose, decrease the propensity for the dehydration-induced liquid crystalline-to-hexagonal II ($L_{H_{II}}$) phase transition at moderate levels of dehydration and preclude the liquid crystalline-to-lamellar crystalline (L_{L_c}) at extreme levels of dehydration.

54. Genetic Control of Nitrate Assimilation in Klebsiella oxytoca
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Klebsiella oxytoca (pneumoniae) is an enteric bacterium closely related to *Escherichia coli*. Nitrate and nitrite are important nitrogen sources not only for *K. oxytoca*, but also for many other microorganisms and most plants. In the absence of ammonium, nitrate (or nitrite) induces the synthesis of assimilatory nitrate reductase and assimilatory nitrite reductase, which act in sequence to convert nitrate to ammonium. One aspect of our work has been to identify and characterize the structural genes for nitrate assimilation, which are organized as the nasFEDCBA operon. The nasCA and nasB genes encode assimilatory nitrate and nitrite reductase, respectively. Sequence inspection indicates that the nasFED genes encode the components of a periplasmic binding protein dependent nitrate uptake system. We have constructed and characterized a series of in-frame deletions in the nasFED genes. Growth tests and uptake assays reveal that the mutants are unable to transport nitrate. However, the mutants retain some ability to grow with nitrite, even at elevated pH values where nitrous acid diffusion is negligible. It is possible that the organism expressed a second, nitrite-specific uptake system. Another aspect of our work has been to explore the molecular basis for nitrate and nitrite induction of nasF operon expression. Our previous studies identified the NasR positive regulatory protein, and established the nasF transcribed leader region as a target for NasR-mediated transcription antitermination control. We have purified a maltose binding protein (MBP) fusion form of NasR, and have reconstituted nitrate- and nitrite-responsive transcription antitermination in vitro. The minimal transcription system includes linear DNA template, RNA polymerase, and nucleotide triphosphates. Transcription efficiently terminates at the factor-independent terminator in the nasF leader region. Addition of both MBP-NasR and nitrate (or nitrite) results in efficient transcription readthrough. Other anions, including chlorate and sulfite, are unable to substitute for nitrate (or nitrite). We previously identified leader region deletions that confer uninducible or constitutive phenotypes *in vivo*. Results of *in vitro* transcription with these deletion templates are fully consistent with conclusions drawn from in vivo analysis: uninducible deletions do not support antitermination, and constitutive deletions do not support termination. Current experiments are aimed at defining the regulatory roles played by different segments of the leader region.

55. Studies of the Genetic Regulation of the Thermomonospora fusca Cellulases
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The long term goal of our research is to produce cellulases with higher activity in degrading biomass cellulose to sugar that will allow the economical production of ethanol from biomass. The current goals are: (1) to purify and characterize the activity of a *Thermomonospora fusca* regulatory protein that functions in the induction of six *T. fusca* cellulases and (2) to determine the molecular mechanism of *T. fusca* endoglucanase E2. We have completed a study of the amount of all six *T. fusca* cellulases produced in cells grown on six different carbon sources using quantitative Western blotting. In *T. fusca*, as in *T. reesei*, the two exocellulases made up more than 80% of the cellulase protein and they were coordinately regulated and present in nearly equal amounts. The other cellulases showed similar changes on the carbon sources, but they were not coordinately regulated either with each other or with the exocellulases. Studies of E2 active site mutants have shown that Asp117 clearly functions as the catalytic acid and it has a pK near 10. Asp156 is partially responsible for this high pK as an Asp156 mutant has a much lower pH optimum. Mutation of either Asp79 or Asp265 to Asn reduces the activity of E2, but not to the extent expected if either were the catalytic base. The Asp79/Asp265Asn double mutant retains 0.4% of the wild-type activity on CMC, which is much more than should be present if either or both of these residues functioned as the catalytic base. We have mutated Lys259 to Ala and to His. The Ala mutant has very low activity (0.2%), while the His mutant has higher activity below pH 5 and much lower activity above pH 8. Clearly, residue 259 has to have a positive charge for E2 to be active.

56. Nitrogen Fixation and its Regulation in the Archaeon Methanosarcina barkeri
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Our laboratory's finding that the Archaeon *Methanosarcina barkeri* is capable of nitrogen fixation raises interesting evolutionary questions. Our previous studies have shown that the nitrogenase is a two-component enzyme complex with the greatest phylogenetic similarity to the Mo nitrogenase of the Gram-positive eubacterium *Clostridium pasteurianum*. We have also shown that neither nitrogenase proteins nor mRNA are found in ammonia-grown cells, indicating repression, and that there is a TATA-box element 35 bases upstream of the nif transcription start. Recent results include: 1) The demonstration, using the gel-shift assay, that extracts from N2-grown cells contain proteins which bind to DNA possessing the nif-promoter region. Shifts were not obtained if the promoter region was deleted or if extracts from ammonia-grown cells were used. Ammonia-grown extracts contained a factor which inhibited promoter binding by N2-grown extracts; 2) We have cloned and expressed the TATA-binding protein (TBP) from *M. barkeri*, and demonstrated that antibodies prepared against TBP caused a greater gel shift, indicating involvement of TBP in *nif* promoter binding; 3) We demonstrated that cells accumulate large amounts of alpha-glutamate as an osmolyte when grown in saline medium with either ammonia or N2 as a nitrogen source. Glutamine was only detected in ammonia-grown cells and was transiently present in diazotrophic cells switched off by ammonia and is therefore a good candidate for an N-regulatory signal; 4) We have cloned and expressed ORF105, a nif-associated gene with marked homology to the eubacterial PII protein, which plays a critical role in N-regulation in eubacteria.

57. Plant Growth with Limited Water
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In land plants, growth is more frequently limited by water than by any other environmental resource. The project objective is to identify the underlying molecular mechanisms causing growth limitations by focusing on the process of cell enlargement. Studies so far have shown that, in localized growing regions of germinating soybean seedlings, turgor in most of the cells was completely maintained when water potentials were low enough to inhibit growth. A few h later, the extensibility of the cell walls decreased and an extractable 28kD protein accumulated in the wall fraction. By cloning the cDNA, the protein was found to be an acid phosphatase. There was no change in phosphorylated intermediates likely to be targets for the enzyme. Moreover, the enzyme accumulation and biochemical events leading up to the accumulation were too slow to account for the early growth inhibition.

Therefore, we have begun to explore the earliest events altering the water potential because these correlate with the early growth inhibition. We recently found that local water potential gradients change and cause at least part of the early inhibition. The changes are localized close to the vascular system in small undifferentiated cells that impede the movement of water and deprive the outlying cells of water necessary for enlargement. We have been able to reverse the inhibition by pressurizing the roots thus returning the gradient to a favorable shape next to the xylem. The resumption of growth indicates that water movement resumes. Experiments are underway to investigate whether transport limited growth occurs in fully established plants.

58. Molecular, Genetic and Physiological Analysis of Photoinhibition and Photosynthetic Performance
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Using *C. reinhardtii* as a model, we have examined the relationship between structure/function of the chloroplast encoded D1 protein of Photosystem II in relation to photoinhibitory damage by high light. Mutants with 12 of 20 possible amino acid substitutions at the Ala_{251} residue in the QB/herbicide binding domain of D1 were characterized. Five Ala_{251} substitutions were nonphotosynthetic (*J. Biol. Chem.* 272: 210-217, 1997) whereas seven displayed varied photoautotrophic (*Z. Naturforsch.* 52C, 1997 in press) and photosynthetic (*J. Biol. Chem.*, in preparation) competence. We have isolated 10 nuclear suppressors which reduce the sensitivity of the Leu_{251} mutant to high light and 27 suppressors that enable wildtype to survive under near sunlight intensity (VHL). Nonphotochemical quenching (NPQ), indicative of engagement of photoprotective energy dissipation pathways, was strongly increased in the wildtype- but not the Leu_{251}-suppressors. In both types of suppressors, pigments of the Xanthophyll cycle (Violaxanthin, Antheraxanthin and Zeaxanthin) are upregulated in VHL while Neoxanthin decreases and Lutein strongly increases. The Z+A/Z+A+V ratio increases only in the Leu_{251} suppressors, indicative of photoprotection, and decreases in the wildtype suppressors, suggesting that other photoprotective mechanisms than xanthophyll interconversion provide VHL tolerance. We previously reported a correlation between upregulation of D1 synthesis and transient downregulation in *Rubisco* LSU synthesis when wildtype cells grown at low light are transferred to high light (*Plant Mol. Biol.* 33: 1001-1022, 1997). A search for proteins that bind in a light specific manner to RNP complexes on the leaders of the psbA and rbcL mRNAs to regulate their translation is in progress. Understanding molecular mechanisms involved in photoregulation of the D1 protein in *Chlamydomonas* may facilitate manipulation of land plants to improve efficiency of photosynthesis under light stress.

59. Role of Sucrose in Modulating Stomatal Aperture
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Gas exchange between a leaf and the atmosphere occurs through adjustable stomata, each of which is surrounded by a pair of guard cells. Certain conditions stimulate guard cells to accumulate potassium salts. The special cell-wall architecture forces these cells to bow outward upon the consequent osmotic-water influx; this deformation enlarges the pore. Stomal closure occurs when the guard-cell pair loses solutes. The aperture size is generally a compromise between the opposing priorities of permitting CO_2 uptake and avoiding H_2O-vapor loss. As CO_2 is required for photosynthesis and water is usually the most limiting resource for a terrestrial plant, regulation of stomatal-aperture size is one of the most crucial aspects of a plant's physiology.

The prevailing paradigm is that fluctuations in guard-cell potassium concentrations power stomatal movements. Recently, however, an important osmotic role for sucrose in stomatal regulation has emerged. Under the conditions we have studied, sucrose accumulation in the guard-cell symplast is secondary, and we have focused on the accumulation of sucrose in the guard-cell apoplast, which we study by use of quantitative histochemical methods. Our most recent DOE-supported manuscript on this topic (*Plant Physiol* 114: 109-118) reported that this sucrose originates in the mesophyll. On this basis, we postulated that sucrose accumulation in the guard-cell apoplast, and consequent diminution of stomatal-aperture size, is one means by which plants measure the rate of transpiration. Our current work is directed toward testing this hypothesis.

60. Ethanol-Tolerant Biocatalysts for Fuel Ethanol Production
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Over the past years, our laboratory has genetically engineered biocatalysts for fuel ethanol production from agricultural and woody waste materials by inserting genes (*pdc,adhB*) encoding the ethanol pathway from *Zymomonas mobilis* (naturally producer of ethanol from glucose) into a variety of Enteric and Gram+ bacteria. In these new recombinant organisms, the normal fermentation process for organic acids is completely replaced by the production of ethanol and carbon dioxide. Effective replacement of the native fermentation pathway results from high levels of the recombinant pyruvate decarboxylase and the high affinity (low Km) of this enzyme for pyruvate, a key intermediate in fermentation. Although our engineered strains have a lower ethanol tolerance than *Saccharomyces* yeasts used in commercial starch-based fermentations, our strains have a greatly expanded ability to ferment many different sugars including all which are constituents of biomass (hexoses and pentoses). We are currently attempting to improve the ethanol tolerance of our ethanol-producing recombinant bacteria and to identify genes which are important for this trait. New strains developed during the first year are capable of producing over 60 g ethanol/liter in 72 h, 7.5% ethanol by volume. Approximately 15 different fragments of chromosomal DNA have been isolated which alter ethanol tolerance. Further studies are underway to identify the specific genes which are responsible and to investigate the physiological/biochemical changes which cause an increased tolerance. Additional studies are directed at the genetic engineering of *Zymomonas mobilis*, a naturally tolerant bacterium which produces ethanol very efficiently from glucose. This organism is being improved by the insertion of genes from *Klebsiella oxytoca* to expand the range of sugars fermented to include soluble products from the enzymatic digestion of cellulose (cellobiose and cellotriose).

61. Gene-Enzyme Relationships of Aromatic Amino Acid Biosynthesis in Higher Plants
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The biosynthesis of aromatic amino acids in higher plants is of great significance, not only because of the role of these amino acids in protein synthesis, but because they are precursors of a vast array of compounds of biotechnological and medical interest. Some of the pathway enzymes also offer potential targets for design of new herbicides. Full-length cDNAs encoding five plastid-localized enzymes will be obtained from both *Nicotiana* and *Arabidopsis*. These correspond to four proteins: the bifunctional AroDE (having catalytic domains for dehydroquinase and shikimate dehydrogenase) and the postprephenate enzymes: prephenate aminotransferase, arogenate dehydrogenase, and arogenate dehydrogenase. N-terminal amino acid sequencing will identify the putative transit peptides. The genomic DNAs will be isolated and sequenced. Intron-exon organization will be determined. Molecular studies will include determination of subcellular location using mono-specific antibody and immunogold electron microscopy, analysis of the organ-specific expression pattern of transcript abundance, determination of cell-specific expression in transgenic plants using GUS fusions, effect of antisense RNA and sense RNA manipulations, and demonstration of the import of unprocessed protein precursors into intact chloroplasts. Approaches that might lead to the possible identification of cDNAs specifying cytosolic enzymes of aromatic amino acid biosynthesis are developed.

62. Genetic analysis of abscisic acid biosynthesis
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The hormone abscisic acid (ABA) is synthesized from cleavage of carotenoids and regulates seed maturation and stress responses in plants. The viviparous seed mutants of maize identify genes involved in synthesis and perception of ABA. A new mutant blocked in ABA synthesis, viviparous14 (vp14), has been identified in maize and the gene cloned. Our analysis of the VP14 protein has shown that it is an enzyme responsible for the key regulatory step in ABA biosynthesis in plants. Thus, understanding how this enzyme is regulated in plant tissues is essential to understanding how ABA synthesis is regulated and used as a stress signal in plants. Our ongoing studies indicate that Vp14 belongs to a diverse family of related genes in maize. The Vp14 mRNA is expressed in embryos and roots and is strongly induced in leaves by water stress. A family of Vp14-related genes evidently controls the

committed step of ABA biosynthesis and these genes are likely to play a key role in the developmental and environmental control of ABA synthesis in plants. This project is focused on identifying and determining the function of Vp14-related genes in plants. Key experimental approaches will be to 1) identify mutations in other members of the gene family, 2) characterize the proteins encoded by these genes, and 3) determine how these genes are regulated in normal and stressed plants.

63. The Metabolism of Hydrogen by Hyperthermophilic Microorganisms
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Extreme thermophiles or "hyperthermophiles" are a unique group of microorganisms that grow optimally near 100° C. They have been isolated mainly from marine volcanic environments, including deep sea vents. The majority of them grow by fermenting peptides and carbohydrates and produce hydrogen (H_2) gas. To investigate the nature of the metabolic pathways and the enzymes involved, we are using the archaeon *Pyrococcus furiosus* (Pf, T_{max} 105°C) which is grown in 600 liter culture. From it we have purified an NADP-dependent hydrogenase, redox proteins such as ferredoxin and rubredoxin, a ferredoxin NADP oxidoreductase (FNOR), four different types (POR, KGOR, IOR and POR) of 2-ketoacid ferredoxin oxidoreductases (KAORs), and two ATP-generating enzymes (acyl CoA synthetases I and II), together with three different aldehyde ferredoxin oxidoreductases, all of which contain tungsten, an element rarely used in biological systems. One of the tungstoenzymes (GAPOR) couples glyceraldehyde-3-phosphate oxidation to H_2 production in an unusual glycolytic pathway, while the other two (AOR and FOR) and the KAORs are part of an unusual pathway for peptide catabolism. The reductant that is produced is converted to H_2 via FNOR, NADP and hydrogenase. The genes for ferredoxin, rubredoxin, FOR, AOR, POR and VOR from Pf have been cloned and sequenced and some of them have been expressed in a mesophilic host. In collaborative studies with D. Rees (Caltech), the crystal structures of rubredoxin, AOR and FOR from have been determined. These studies provided the first structure for a hyperthermophilic, a tungsten-containing, or a pterin-containing enzyme. Since H_2 plays a central role in the commercial production of many chemicals, a long term objective of this research is to assess the utility of hyperthermophilic hydrogenases and oxidoreductases in industrial energy conversions.

64. Center for Plant and Microbial Complex Carbohydrates
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The University of Georgia Complex Carbohydrate Research Center (CCRC) has a multidisciplinary faculty and staff who serve as a national resource for basic research in complex carbohydrate science. The CCRC faculty currently has nine tenured or tenure-track members, with two additional members joining the CCRC in 1997-98 (a full professor in nuclear magnetic resonance spectroscopy and an assistant professor in plant biochemistry). A twelfth tenure-track senior faculty member (in the area of synthetic carbohydrate chemistry) is being recruited, and the CCRC expects to grow to a total of 14 to 15 faculty members in the next three to four years. The grant supports research, analytical services, and training in plant and microbial complex carbohydrates. Six of the CCRC's faculty are active participants in the plant and microbial carbohydrate program supported by this grant. Educational activities involve the training of undergraduate and graduate students, postdoctoral research associates, and visiting scientists. Thirty-one undergraduate and 28 graduate students are currently pursuing research projects or graduate degrees (4 M.S. students, 24 Ph.D. students) in the CCRC; 16 undergraduate and 10 graduate students are working in plant or microbial carbohydrate science. Four week-long, hands-on laboratory training courses are held annually for scientists from institutions and industries located throughout the United States; 16 and 15 scientists attended these courses in 1996 and 1997, respectively, some attending more than one course. The plant and microbial carbohydrate program has provided service to 198 individuals by analyzing 974 samples in the time that the service program has been active. These analyses include determination of glycosyl-residue and glycosyl-linkage compositions, and acquisition and interpretation of one-dimensional NMR and mass spectra. The faculty and staff of the CCRC are currently involved in more than 120 internal or external collaborative research projects of which 58 are part of the plant and microbial carbohydrate program. The CCRC has, in one way or another, assisted more than 135 corporations during the same time period.

65. Structures and Functions of Oligosaccharins
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The broad goal of this project is to determine the functions in plants of oligosaccharide signal molecules (oligosaccharins) and to learn in the process more about the interactions of plants and microbes and the regulation of plant growth and development. The importance of oligosaccharins to plants and their pathogens is made evident by the multiple mechanisms plants use to accumulate bioactive levels of these signal molecules and the corresponding mechanisms by which pathogens prevent the signal molecules from accumulating. Many of the biochemical battles that determine whether the plant overcomes or succumbs to the pathogen take place in the cell walls of plants and their microbial pathogens. Several of the subprojects study aspects of host-pathogen interactions, emphasizing the functions of molecules of cell wall origin. These subprojects concern: (I) plant cell wall-localized polygalacturonase-inhibitor proteins and the fungal extracellular enzymes they inhibit; (ii) fungi-secreted *endo*–1,3-glucanase-inhibitor proteins and the enzymes of the cell walls of plants that they inhibit; and (iii) fungi-secreted *endo*–1,4-xylanases and their role in activating defense responses in Gramineae. In addition subproject (iv) studies the bioactivities, in wild-type and mutant plants, of a plant cell wall-derived oligosaccharin that functions in regulating plant growth, and subproject (V) centers on the isolation from plants of endogenous lipo-oligosaccharide growth regulators.

66. CarbBank - A Structural and Bibliographic Database for Complex Carbohydrates
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The computerized Complex Carbohydrate Structure Database (CCSD) and database management system, CarbBank, were created by scientists at the Complex Carbohydrate Research Center (CCRC) in collaboration with scientists in Europe. Building the database and making the software available to researchers worldwide were the initial goals of the project. CarbBank/CCSD is designed to provide a searchable central repository of all carbohydrate structures published in the scientific literature. CCSD records encompass all types of carbohydrate structures that contain tri- and larger saccharide moieties, and include the structure of each molecule along with information on the biological or chemical source, biological activity, antigenicity, and association with particular developmental stages of the organism from which it was obtained or with a disease of the organism. CarbBank is available in a Windows 95/NT program, and CarbBank and the CCSD now reside on the CCRC's Web site, where they can be used directly from all computer platforms by means of a Web browser. The CCRC CarbBank staff is emphasizing software development rather than exerting an all-out effort to keep up with data entry. As new programming tools designed to enhance user interaction with Web sites have become available, they are applied to CarbBank and the CCSD. Plans are proposed to streamline database building by initiating direct author submission of structures as part of the manuscript publication process; to enhance Web browser-compatible systems for author submission and searching the CCSD; to functionally integrate the CCSD with other biosequence and informatic databases including CCRC-Net; to create and coordinate "mirror" server sites in Europe, Japan, and Australia for CarbBank/CCSD so that all users enjoy optimum performance; and to integrate Web-based accounting/user access software. The CCSD now contains ~50,000 records. *Funds provided by the National Library of Medicine.

67. Structural Studies of Complex Carbohydrates of Plant Cell Walls
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Plant primary cell walls control several important properties of plant cells. They control the rate and direction of cell growth that determine ultimately the shapes of cells, tissues, and organs. They form a barrier to pathogens, are the source of oligosaccharins that elicit plant defense responses in host-pathogen interactions, and participate in controlling plant growth and development. Primary wall components are also used in many industrial and medical applications and thus constitute an important natural resource. The goal of this research project is to determine the structures and functions of the non-cellulosic matrix polysaccharides of primary walls that, with cellulose, account for more than 90% of the wall's dry weight. The structural studies emphasize developing analytical methods and using them to elucidate the detailed structures of hemicellulosic (xyloglucan) and pectic

(rhamnogalacturonans I and II) wall matrix polysaccharides. We are also characterizing the interactions between some of the wall matrix polysaccharides by studying the structure of covalent wall cross-links. In addition, we are examining the cell-, tissue-, and species-dependent expression of cell wall epitopes using well-characterized monoclonal antibodies. This analysis may reveal developmentally and spatially regulated variations in the fine structure of the cell wall matrix polysaccharides. Finally, we are studying the structures and epitope localization of matrix polysaccharides in *Arabidopsis* mutants lacking or deficient in specific glycosyl residues. The long-range objective of this research is to better understand the diverse roles of the wall polysaccharides in cell wall function.

68. Fermentation of Cellulose and Hemicelluloses by Clostridia and Anaerobic Fungi
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Biomass consists mostly of cellulose, hemicelluloses, and lignin. Degradation of lignin does not occur by anaerobic microorganisms. It is, however, partly solubilized by the action of specific *p*-coumaroyl and feruloyl esterases produced by anaerobic fungi. On the other hand, cellulose and hemicelluloses are efficiently hydrolyzed by consortia of anaerobic microorganisms to simply components such as sugars, which are fermented to carboxylic acids, alcohols, CO_2, H_2, and methane. Our work deals with the anaerobic bacterium *Clostridium thermocellum* and the anaerobic fungus *Orpinomyces* PC-2, both of which effectively degrade plant tissue. The efficient hydrolysis of the plant tissue (biomass) is attributed to that the two microorganisms produce multiproteiin cellulose/hemicellulases complexes (cellulosomes) containing endo- and exo-cellulases, and hemicellulases (xylanase). In addition, the cellulosome of *Orpinomyces* seems to contain esterases. The catalytic subunits of the cellulosomes are held by scaffolding proteins. This involves interactions between specific domains called dockerins of the catalytic subunits and cohesins of the scaffolding proteins. The dockerins and cohesins of the cellulosomes of the fungus and *C. thermocellum* are functionally similar but structurally distinct. Studies of the cellulosomes will greatly enrich the understanding of the biochemistry of enzymes for the degradation of plant biomass, the most abundant resource on earth, which can be used for the production of biofuels and chemicals. Research is also conducted with *C. thermoaceticum*, which ties into the cellulose degradation by cellulolytic consortia. This bacterium converts the degradation products by the cellulolytic microorganisms to acetate, an important feedstock chemical. Studies with *C. thermoaceticum* involve carbon dioxide fixation by the acetyl-CoA pathway and generation of energy coupled to this pathway.

69. Plant Arginine Decarboxylase, Regulation and Function
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Polyamines are small positively charged compounds, hypothesized to be involved in a wide variety of plant physiological and developmental functions. These include stress response, floral and root development, and senescence. One feature of plant polyamine synthesis is the presence of two alternative pathways to putrescine, from arginine via arginine decarboxylase and from ornithine via ornithine decarboxylase. Ornithine decarboxylase is found in nearly all organisms, however arginine decarboxylase is not found in animals and some other eukaryotes. Arginine decarboxylase is regulated by post- transcriptional and post-translational mechanisms; we wish to determine the mechanisms of this regulation. We are performing molecular and genetic analysis of the polyamine synthesis pathway in the model system *Arabidopsis thaliana*. This builds upon our recent isolation of mutants in the pathway and generation of molecular probes for enzymes in the pathway. There are two structural genes encoding arginine decarboxylase, and we have also identified genes on other chromosomes that regulate this enzyme. A complex pattern of post-transcriptional regulation appears to determine arginine decarboxylase activity. We are continuing our analysis of the regulation and function of these genes using the mutants, clones, and antibodies. We also are isolating several classes of mutants that we do not currently have. The mutants we have isolated allow us to test for the roles of polyamines in processes such as plant stress response and plant senescence.

70. Mechanisms and Determinants of RNA Turnover: The Role of PAB2 in the Post-Transcriptional Apparatus
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In the last decade it has become increasingly clear that post-transcriptional regulation is as important to gene regulation and organismal development as transcription. The operation of post-transcriptional regulation is mediated by the action of a post-transcriptional apparatus (PTA). Poly(A) binding proteins (PABPs) form an integral part of the PTA by binding mRNAs at both the 3' end on the poly (A) tail and to the 5' end on protein factors involved in translational initiation. The presence or absence of PABPs in the PTA effect translational initiation, polysome stability, and mRNA decay. We have shown that Arabidopsis and probably all higher plants have an ancient, diverse, and differentially expressed gene family encoding PABPs. Arabidopsis PAB2 and PAB5 complement cell viability and some molecular functions associated with yeast PABP. PAB2 is strongly expressed in young ovules and the transmittal tissue of flowers, in primordia and young leaves, and in the stele of roots. The five specific aims of this grant are: 1) to localize the tissue and developmental stage-specific expression of PAB2 gene and protein; 2) to elucidate possible molecular mechanisms of PAB2 action in the PTA in yeast; 3) to identify specific transacting factors that bind to PAB2 in the PTA using the dihybrid system and suppressor genetics; 4) to determine if PAB2 binds distinct RNA sequences with specificity; and 5) to identify and characterize PABP mutants in Arabidopsis. Understanding the different biological functions of plant PABPs will help us to dissect important components of plant gene regulation.

71. Identification of Novel Cell Wall Components
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Fungi are superbly adapted to break down organic matter. They are able to scavenge nutrients in environments ranging from leaf litter on the forest floor to soap scum on bathroom tiles. Indeed, one of the defining characteristics of the fungal kingdom is the use of extracellular digestion before absorption of nutrients.

The cell wall is the main interface between the fungus and its environment. Information about nutrient availability, enzymes which break down organic matter, and digested nutrients must all transit the cell wall. In addition to its central position in fungal metabolism, the cell wall is the main determinant of morphogenesis and may contain developmental signals.

The major goals of this proposal are to identify novel cell wall components and clone the genes responsible for their synthesis in the filamentous fungus *Aspergillus nidulans*. Two parallel molecular genetic approaches will be exploited to meet these goals. In the first approach, a group of temperature-sensitive mutants with a swollen cell phenotype (*swo* mutants) will be characterized. The swollen cell phenotype is often associated with cell wall defects. In the second approach, mutants lacking specific cell wall epitopes (*cwe* mutants) will be identified through screening with phage display antibodies. Selected *swo* and *cwe* genes will be cloned by complementation of mutant phenotypes.

72. Nitrogen Control of Chloroplast Differentiation and Metabolism
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Nitrogen deficiency is the most common limitation to plant growth. Our work concerns how nitrogen deficiency affects metabolism, biosynthetic pathways and development of the photosynthetic apparatus. The model system for our work is the green alga, *Chlamydomonas reinhardtii* grown in continuous cultures, in which steady-state levels of nitrogen can be precisely controlled and in which the classical symptoms of deficiency, chlorosis and slow growth rates, are perpetuated. Changes in gene expression and physiological activities in a uniform population of cells are monitored in comparative analyses with nitrogen-sufficient cultures. Primary effects elicited by nitrogen provision, marked by rapid greening and dramatic chloroplast differentiation, are also measured. For example, reversal of chlorosis correlates with selective expression of nuclear genes encoding light-harvesting proteins, which we have shown to directly participate in synthesis of chlorophylls a and b, as well as enzymes involved in early steps in porphyrin

synthesis. At another level, profound changes in respiration and carbon metabolism and the mitochondrial/chloroplast interrelationship accompany long-term adaptation to N-deficiency. Finally, we are employing mutant strains of *Chlamydomonas* for dissection of responses to nutrient limitation; defined disruptions of physiological and biochemical processes impact on the nitrogen-dependent signal transduction and intracellular communication pathways.

73. Structure, Regulation and Evolution of the R transcriptional activators from maize and rice
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The maize *R/B* gene family and their homologs in other plants regulate the anthocyanin biosynthetic pathway by activating the transcription of at least five of the structural genes. Our studies encompass three diverse but related projects. First, a collection of Ds-induced alleles of *R* is being used to identify structural domains required for function *in vivo*. Analyses of two alleles, r-m9 and r-m1, have demonstrated the importance of three nuclear localization sequences (NLS-A,M,C) and the basic helix-loop-helix (bHLH) domain. These results differ dramatically from findings obtained using transient transformation assays. Understanding the mechanism by which an upstream open reading frame (uORF) represses expression of the *R* gene is the second goal. We have succeeded in demonstrating uORF-mediated repression both in a rabbit reticulocyte translation system and in bombarded kernels. Using these systems, we find that repression is not due to the sequence of the 38 codon uORF but, rather, is caused by the intercistronic DNA (ID) which leads to inefficient ribosome reinitiation. Characterization of the *R* gene family of rice and correlation with known genetic loci is the third goal. We have determined that *O. sativa* has at least four *R* genes, at least two of which map to the same two chromosomal regions previously shown to contain most of the genes that determine pigment pattern in rice. Further, the rice gene family has evolved recently and independently of the maize *R/B* family.

74. Biochemistry and Genetics of Autotrophy in Methanococcus
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The methanogenic archaea catalyze the terminal step in the anaerobic oxidation of organic matter to methane, a clean fuel already in wide use. The development of methods for the introduction of genes into methanococci will be essential for the construction of more versatile microbial catalysts and further understanding of the molecular and biochemical basis of methanogenesis. Recent research has developed an efficient transformation system and shuttle vector for moving genes containing novel activities in *Methanococcus maripaludis*. The next step in the development of the shuttle vector is to introduce additional cloning sites next to a strong methanococcal promoter for efficient expression of heterologous genes, research which is in progress. The genetic system is also being utilized for construction of an integration library to mutagenize wild type cells. Screening these mutants has identified several auxotrophs for acetate and other compounds. This method is providing new insights into the pathways of carbon metabolism of these facultative autotrophs. Lastly, isotopic labeling was utilized to establish the pathway of ribose biosynthesis in the methanococci. In contrast to other methanogens, the methanococci utilize the nonoxidative pentose phosphate pathway. Surprisingly, these same experiments suggested that methanococci may utilize a novel pathway of aromatic amino acid biosynthesis. If confirmed, this will be the first report of an alternative to the pathway originating in 3-deoxy-D-arabino-heptulosonate 7- phosphate.

75. Development of Innovative Techniques and Principles That May Be Used as Models to Improve Plant Performance
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The objectives of this project are to: (1) establish the cytoplasmic diversity of germplasm in the weedy subspecies of the primary gene pool and demonstrate its value, (2) identify mechanism(s) for transfer of germplasm from the secondary gene pool to the cultivated species and evaluate the plant breeding potential of this germplasm, and (3) transfer gene(s) controlling apomixis from the tertiary gene pool to cultivated pearl millet for the purpose of producing true-breeding hybrids. Species within the genus Pennisetum

are being used as test organisms. The approach uses plants of wild species with different genetic and cytoplasmic backgrounds, ploidy levels, and chromosome numbers crossed and backcrossed with different genotypes of diploid and tetraploid pearl millet. Research showed that seed loss in apomictic BC6 pearl millet plants was due to chromosome imbalance in the endosperm of the seed and possibly to an inbreeding effect of the backcrossing process. Germplasm from the secondary gene pool (Tift 93 and Tift 8593) used to produce commercial pearl millet forage hybrid, Tifleaf 3, was released and registered with Crop Science. Selection procedures in two exotic gene pools of the primary of the primary gene pool increased genetic rust resistance 20% per cycle while morphological variability was maintained.

76. Mechanisms Regulating psbD Transcription in Higher Plants
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This project investigates the genetic mechanisms controlling the development of the photosystem II reaction center (PSII) in higher plants. Proper PSII development and function are necessary for maintaining photosynthetic efficiency, crop productivity and plant adaptation to high light environments. The *psbD* gene, which is localized on the chloroplast genome of plants and algae, encodes the D2 subunit of PSII. In higher plants, high-fluence blue light, but not red light, differentially activates *psbD* transcription from a blue light-responsive promoter (BLRP). The objective of this work is to understand how blue light regulates *psbD* transcription. To facilitate genetic investigations on the BLRP, we mapped and studied the expression of the BLRP in Arabidopsis chloroplasts. We will determine the nucleotide sequence of the Arabidopsis BLRP and determine which DNA elements are necessary for light-responsive transcription. Electrophoretic mobility shift and DNase I protection assays are being used to define the protein binding sites in each element from Arabidopsis. These binding sites will be used to clone cDNAs for the putative regulatory proteins. The cDNA sequence is expected to assist in identifying the functional domains involved in DNA-binding, protein-protein interactions and transcription. Studying the nuclear-encoded factors and their genes will provide insight into the regulation of *psbD* transcription by blue light, which will give clues into the fundamental mechanisms by which the nucleus controls chloroplast gene expression. Implementation of the Arabidopsis system will enable the detailed genetic dissection of the photosensory pathways regulating *psbD* expression.

77. Violaxanthin De-Epoxidase: Molecular Biology and Physiological Function
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The overall aim of this project is to understand the mechanism and role of violaxanthin de-epoxidase (vde) activity in protecting plants against the potentially damaging effects of high light intensities. Zeaxanthin and antheraxanthin formed by vde enhance the dissipation of excess light energy as heat, thus protecting photosynthetic apparatus from damage. As previously reported, we have cloned vde from lettuce, tobacco and Arabidopsis. The specific aims of the current project are twofold: (1) to determine the physiological significance of vde activity on growth and development by over expression or suppression of vde and (2) to gain insight into the mechanism of vde activity by site-directed mutagenesis studies. Analysis of tobacco transformed earlier with sense and antisense vde constructs were continued. Preliminary results suggest that maximum over expression was around twofold. Constructs of vde using other promoters for higher levels of over expression were initiated. A range of plants with vde activity inhibited up to 95 percent was obtained. Propagation of transformed plants for homozygous selection was initiated. Detailed analysis of wild-type (control) tobacco was completed. The results suggest that vde is developmentally regulated in contrast to xanthophyll-cycle pool size that depends on light environment.

78. Studies on Cytochrome bo3 from E. coli
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The bo_3-type ubiquinol oxidase is the predominant respiratory oxidase present in the bacterial membrane *of Escherichia coli* when the cells are grown with high aeration. This enzyme is a coupling site, which means that the redox reaction catalyzed by the enzyme, i.e., the 4-electron reduction of oxygen to water, is coupled to the generation of a proton and voltage gradient across the cytoplasmic membrane. This enzyme is a very efficient device to convert chemical energy into the form of a transmembrane ion gradient. Our primary interest is to learn how this is done.
Our studies have established this enzyme as a member of the superfamily of heme-copper respiratory oxidases. This superfamily includes the cytochrome c oxidases for which there is now detailed structural information recently available from X-ray diffraction studies. The focus of our work is to combine site-directed mutagenesis with a variety of spectroscopic techniques to identify the functional roles of specific amino acid residues that appear to be involved in the proton pumping mechanism. We have most recently been examining the effects of mutations in residues that appear to be components of two separate channels (D-channel and K-channel) within the enyzme that have been identified by X-ray analysis. We have clearly demonstrated that these residues are critical for oxidase function and that the two channels must play very different roles. In addition, we have demonstrated that different mutations trap the enzyme in different states of oxygenation, providing a useful experimental tool for characterizing these states of the enzyme.

79. Bacterial resistance to silver cations: molecular genetics, physiology and biochemistry
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We are studying the molecular genetics of bacterial resistances to the toxic heavy metals, mercury, cadmium, arsenic and silver. Bacterial cells have highly specific genetic systems for each of these toxic minerals. Each system contains gene(s) for regulation (so that the system is functional only when needed) and gene(s) for the proteins that physiologically and biochemically confer resistance itself. The resistance systems offer promise for "bioremediation" of environmentally polluted settings, while the regulatory genes may be used as components of metal-specific "biosensors" (especially with luciferase gene fusions that respond to "bioavailable" metal ions with increasing light emission). Mercury and methylmercury-detoxifying Bacillus isolates from Minamata Bay, Japan are being analyzed in detailed laboratory studies. In addition, these same strains are being used for studies of the range of arsenic resistance determinants (and their genes) in Bacilli. The CadC transcriptional repressor of the cadmium-resistance cadmium-efflux ATPase has been overproduced and is being studied by protein-DNA interactions in vitro and metal cation-protein binding. The first silver-resistance system was cloned and the 14 kb required for its 7 genes sequenced. Now more in detail on the phenotypes and functions of each gene will continue. Additional environmental and clinical isolates having the silver resistance genes will be characterized by Southern blot analysis, RFLP analysis and PCR of selected genes.

80. Studies on the Microbial Formation of Methane
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The microbial formation of methane is carried out by a unique group of bacteria known as methanogens. These strict anaerobes are widespread in nature, and are found in diverse habitats, wherever active anaerobic degradation of organic matter occurs, such as sediments, the rumen of ruminants, sanitary landfills, and sewage sludge digesters. We are exploring ways of simplifying the culture of methanogens in liquid and solid media, so that these organisms may be more readily employed as research tools by the scientific community. These studies include the sensitivity of methanogens to a variety of factors such as reducing agents, ions, ionic strength, and oxidizing agents. For example, under certain conditions of stress, cells of methanogens may exhibit a bright red fluorescence instead of the typical blue-green fluorescence. We are studying this phenomenon to isolate and characterize the red fluorescent compound, to determine its structure, its role, and its relationship to known coenzymes which may be modified when cells are under stress. Our goal is to define how sensitive, anaerobic methanogenic-cells survive under stress in terms of biochemistry.

81. Function of the Arabidopsis TIR1 gene in auxin response
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Genetic studies in Arabidopsis have led to the identification of a number of genes that are involved in auxin response. The first of these genes to be cloned and characterized was the *AXR1* gene. Mutations in *AXR1* result in a general decrease in auxin response and variety of growth defects consistent with impaired auxin response. The gene encodes a novel protein related to the ubiquitin-activating enzyme and is expressed primarily in dividing cells. Recent results in my group and by others, indicate that the AXR1 class of proteins is found in diverse eukaryotes including representative fungi and animals (including humans). In addition, we now have compelling evidence that AXR1 functions in the ubiquitin pathway. Taken together, these results suggest that ubiquitin-mediated proteolysis has a key role in auxin response. This work addresses the function of a recently identified Arabidopsis gene called *TIR1*. Like *axr1*, the *tir1* mutants are deficient in auxin response and display auxin-related growth defects. Genetic studies reveal that *axr1* and *tir1* interact synergistically, suggesting that the two genes cooperate to mediate auxin response. Molecular cloning showed that the TIR1 protein is related to a human protein called SKP2, as well as a number of other eukaryotic proteins that share a protein domain called the F-box. SKP2 is required for the G1- to S-phase transition in human cells and experiments with several F-box proteins strongly suggest that these proteins function to facilitate ubiquitin conjugation to specific target proteins. Thus the phenotype of the *tir1* mutants and the structure of TIR1 provide additional support for a role for regulated protein degradation in auxin response. To investigate the function of TIR1 and its role in auxin response are the following studies: (1) *TIR1* expression will be analyzed by *in situ* hybridization of RNA probes to tissue sections. (2) The *tir1* mutants have reduced numbers of lateral roots compared to wild type and are impaired in auxin induction of lateral roots. To investigate the basis for this defect we will determine the timing of *TIR1* gene expression during lateral root formation. In addition, we will determine the effects of the *tir1* mutations on the occurrence of early events during formation of the lateral root primordia. (3) To prepare TIR1 specific antibody the TIR1 protein will be expressed in *E. coli* and/or insect cells. ?-TIR1 antibody will be used to determine the tissue distribution of TIR1 and cellular location of the protein. (4) The importance of the F-box to TIR1 function will be analyzed by site-directed mutagenesis. (5) Proteins that interact with TIR1 will be identified using the yeast two-hybrid system. The function of TIR1-interaction proteins will be analyzed using several strategies. (6) To identify new genes that interact with *TIR1* a screen for suppressors of the *tir1-1* mutation will be performed. (7) Three *TIR1*-related genes have been identified in the Arabidopsis genome. We have recovered a T-DNA insert in one of those genes using a PCR-based screen. This mutant (called *flr-2*) will be characterized using established techniques. In addition, we will screen for additional mutants in other members of the *TIR1* gene family. During the last five years, studies in yeast and animal systems have demonstrated that regulated protein degradation plays a key role in diverse aspects of cellular regulation. Our results strongly suggest that this is also the case for auxin response. We expect our experiments to provide exciting new insight into theses fundamental aspects of molecular biology.

82. Analysis of a signal transduction pathway involved in maize epidermis and aleurone differentiation
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The shoot epidermis is a key tissue for plant protection against many biotic and abiotic stresses. The mutant phenotype of the maize *crinkly4* (*cr4*) gene indicates this gene is important for epidermal differentiation. *cr4* encodes a receptor-like kinase suggesting that the CR4 receptor binds an extracellular signal molecular thereby activating a cellular response that leads to proper epidermal differentiation. Little is known of the process of epidermal differentiation, nor of the molecular mechanisms plant cells use to transduce extracellular signals. Analysis of the CR4 signal transduction system will provide valuable insights into both areas. There are three main goals. The first is to identify and analyze genes that are regulated by the CR4 signal transduction pathway. This will be done through an automated robotic differential cDNA library screen done in collaboration with scientists at Pioneer Hi-Bred. Differentially expressed genes will be sequenced to attempt to understand what cellular functions are regulated by CR4. Additionally, OPAQUE2 will be examined as a potential target of the CR4 signal transduction system. The second goal is to identify factors that directly interact with the cytoplasmic domain of the CR4 protein. This will be accomplished using the yeast 2-hybrid system. Interacting factors will then be analyzed genetically using the TUSC reverse genetics procedures available at Pioneer Hi-Bred Intl., Inc. The third objective is to test the hypothesis that the function of CR4 signaling in the epidermis is to specify cell fate. This will be addressed in part through the analyses outlined in the first goal, through the analysis of epidermal specific gene expression in *cr4* mutants, and the ectopic expression of *cr4* in transgenic plants. This research will identify components of the CR4 signal transduction pathway, targets of the pathway and help us understand the developmental significance of this signaling.

83. Characterization of the Membrane-Associated Methane Oxidation System in Methylococcus capsulatus Bath
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In methanotrophs, the oxidation of methane to methanol is catalyzed by the methane monooxygenase (MMO). In some methanotrophs, two different MMOs can be expressed depending on the copper concentration during growth. At low copper to biomass ratios, the enzyme activity is observed in the soluble fraction and referred to as the soluble MMO (sMMO). At higher copper to biomass ratios, methane oxidation activity is observed in the membrane fraction and the enzyme referred to as the membrane-associated or particulate MMO (pMMO). This project focuses on the pMMO. The pMMO consists of two components. Component A composed of three polypeptides with molecular masses of 47,000, 27,000, and 25,000 Da, in a 1:1:1 ratio, and contains 2 - 3 non-heme iron and 2 - 3 copper atoms per 99,000 Da. Component B is a small polypeptide with a molecular mass of 1,218 Da, and contains 1 - 2 copper atoms. As isolated purified enzyme shows an approximate component A to B ratio of 1: 5 with 2 - 3 iron and 14 to 15 copper atoms

Component B also shows a number of siderophore-like properties, and can be isolated from the extracellular fraction in cells expressing the pMMO under copper-limiting conditions.

The overall objective of this project is to characterize the mechanism of methane oxidation in methanotrophs expressing the pMMO. Present work is focused on improvements to the pMMO purification procedure, enzyme characterization, identification of the physiological reductant to the pMMO, and role of component B in methane oxidation.

84. Regulation of Carotenoid Biosynthesis: The Immutans Mutant of Arabidopsis
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The *immutans* mutant of *Arabidopsis thaliana* contains green and white-sectored leaves due to the action of a nuclear recessive gene. The green sectors are homoplastidic for normal chloroplasts, while the white sectors contain plastids with different morphologies (are heteroplastidic). This indicates that the mutation is plastid autonomous and acts independently on the plastids in a cell. The somatic instability in the mutant is modulated by light, temperature and developmental factors. *immutans* is first expressed during seed coat breakage, and plastid phenotypes, once established, cannot be changed. However, defective plastids are not maternally-inherited, indicating that the plastid defect is cured during reproduction. The white sectors of *immutans* accumulate the carotenoid precursor phytoene, but the mutation affects the activity of phytoene desaturase (PDS) only indirectly: *immutans* does not map to the PDS locus, nor does it affect PDS mRNA or protein levels. The fact that PDS expression is normal in the white tissues of *immutans* shows that the expression of this protein is not coupled to pigment content in *A. thaliana*, as it is in other systems. Transgenic plants that express Lhcb promoter/GUS fusions have revealed that *immutans* uncouples Lhcb expression from its normal dependence on chloroplast development, suggesting that *immutans* is an important component in the signal transduction pathways that regulate chloroplast biogenesis. An approximately 30 kb fragment of the *A. thaliana* genome encompassing *immutans* has been subcloned into overlapping cosmid clones that are being used to complement the mutation and define the gene locus.

85. Molecular Biology of Anaerobic Aromatic Biodegradation
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We have been investigating anaerobic benzene ring biodegradation, a process that is critical for the recycling of plant material and the degradation of many environmental pollutants. Benzene rings can be completely degraded in the absence of oxygen only by bacteria. Our emphasis has been on pathways for the degradation of benzoate and 4-hydroxybenzoate, since these aromatic acids are the starting compounds for a central pathway of anaerobic benzene ring reduction and ring fission. We have recently identified and sequenced a cluster of genes from the bacterium *Rhodopseudomonas palustris* that encodes many, and possibly all, of the

enzymes for benzoate and 4-hydroxybenzoate degradation. These genes provide a valuable source of material for purifying and studying several novel enzymes that are known to be involved in ring reduction and cleavage. Assignment of gene products to specific enzymatic steps will also allow us to elucidate the correct sequence of catabolic reactions in the degradation pathways. In addition, the cloned genes will be used to determine how gene expression is regulated in response to aromatic compounds and oxygen. This information will be valuable in designing bacterial systems to mediate biomass conversions or for use in bioremediation.

86. Bacterial Nickel Metabolism for Hydrogenase Synthesis
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The growth of many microorganisms that are beneficial to nutrient cycles in nature requires nickel, as this metal is a component of several microbial enzymes. From studies on bacterial mutants in Ni-containing ureases and hydrogenases, it has become clear that the Ni-requiring organisms contain enzymes to deal with intracellular nickel mobilization and metabolism, and subsequent insertion of the metal cluster into the Ni-enzyme(s). To determine the routes of nickel metabolism and incorporation into a bacterial enzyme (hydrogenase) in the N_2-fixing bacterium *Bradyrhizobium japonicum*, gene-directed mutant strains of the bacterium have been studied, and nickel-binding proteins have been overexpressed and purified. Some mutant strains are specifically deficient within histidine rich areas of the nickel-storage protein we call nickelin, encoded by hypB. Analysis of mutants within the metal-binding domain have shown that the his-rich area, capable of binding 18 nickel atoms per molecule of protein plays a nickel storage role for subsequent hydrogenase expression. Other proteins involved with the nickel-dependent regulation of hydrogenase expression either in sensing nickel or in activating hydrogenase transcription have been identified and specific mutants in each of the genes have been generated. Some of the genes encode domains which act as nickel-binding ligands, and a nickel dependent sensing and regulatory cascade for Ni-enzyme (hydrogenase) expression involving several multi-subunit complexes is proposed. The long-term goal is to understand the number, nature and function of the components involved in bacterial nickel metabolism, especially for hydrogenase expression.

87. Transport of Ions Across the Inner Envelope Membrane of Chloroplasts
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In addition to photosynthesis, chloroplasts carry out a number of essential processes, including nitrite reduction to ammonia and sulfate reduction to the level of sulfide. Nitrite is a source of nitrogen for amino acids, and nucleic acids and sulfate, a source of sulfur for the sulfur-containing amino acids, cysteine and methionine. How nitrite, sulfate and other molecules cross the permeability barrier of the chloroplast, the inner membrane of the chloroplast envelope, has been under investigation in my laboratory for several years. Using a biophysical approach, we have shown that nitrous acid can rapidly cross the inner envelope membrane. The rate of unmediated diffusion of nitrous acid is from five to ten times that of nitrite reduction within chloroplasts. The rate of nitrous acid diffusion across the erythrocyte membrane is, in contrast, slow. This difference may be due to the high cholesterol content of the erythrocyte membrane. Nitrite will, however, enter erythrocytes and we have shown that nitrite transport is mediated by the erythrocyte anion exchange protein.

Carbon dioxide, a substrate of photosynthesis could enter chloroplasts as carbonic acid, bicarbonate or as carbon dioxide. Together with Dr. James V. Moroney, Dr. Shingles developed a sensitive fluorimetric assay for carbonic anhydrase activity and demonstrated that the rate of transport of carbon dioxide across lipid vesicle membranes is accelerated by the presence of carbonic anhydrase on both sides of the membrane. These results are consistent with free diffusion of carbon dioxide across the membrane.
Ca^{2+} influx into inner envelope vesicles was also investigated. In agreement with studies with intact chloroplasts, the inner envelope vesicles exhibit an uptake of Ca^{2+} that is inhibited by the Ca^{2+} channel blocker, ruthenium red. These results suggest that chloroplasts may be involved in Ca^{2+} homeostasis.

88. Macromolecular Scaffolds for Energy Studies
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The Green Fluorescent Protein (GFP) from the jelly fish *Aequorea victoria* is the first known protein that expresses a genetically encoded, autocatalytically formed fluorophore. In the past year, we have discovered that genetically engineered variants of GFP can be attached to micron-sized beads at surface concentrations sufficiently high to enable fluorescence resonance energy transfer (FRET) to occur efficiently. This is surprising because there is an inverse sixth power dependence on FRET efficiency with respect to the distance between donor and acceptor molecules. In such experiments, donor GFP derivatives ("blue", BFP) are typically excited in the violet and fluoresce in the cyan, while acceptor GFP derivatives ("red-shifted excitation", RSGFP) have spectral overlap in the cyan and fluoresce in the green. These fluorescent proteins are typically attached to Nickel ion chelating beads which complex with an engineered N-terminal histidine-tag on these modified GFP derivatives. Since GFP is known to exist as a dimer at high concentrations, there are interesting possibilities for the macromolecular GFP structures on the bead surface. Various models can be envisioned for the FRET-competent macromolecular structure(s): ranging from closely spaced monomers to donor-acceptor heterodimers (BFP/RSGFP). This new bead-based macromolecular scaffold leads to intriguing possibilities for the use of such FRET beads in energy transfer experiments and in the elucidation of the structure of the FRET pair.

89. Acetyl-CoA cleavage and synthesis in methanogens: Mechanistic, enzymological, and metabolic studies
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We are employing biochemical methods to study the acetyl-CoA decarbonylase/ synthase complex found in methanogenic Archaea. The results of our experiments will allow us to elucidate the complicated biochemical mechanism used by the enzyme complex to catalyze the following reaction:

$$CO_2 + 2Fd_{red}(Fe^{2+}) + 2H^+ + CH_3\text{-}H_4SPt + CoA \text{ acetyl-CoA} + H_4SPt + 2Fd_{ox}(Fe^{3+}) + H_2O$$

where H_4SPt and $CH_3\text{-}H_4SPt$ are tetrahydrosarcinapterin and N^5-*methyl*-tetrahydrosarcinapterin, respectively and $Fd_{red}(Fe^{2+})$ and $Fd_{ox}(Fe^{3+})$ are the reduced and oxidized forms of the redox protein ferredoxin. Additionally, we will use the biochemical knowledge gained to better understand how the oxidation-reduction potential of the environment regulates methanogen physiology.

90. Mechanism and Significance of Post-Translational Modifications in the Large Subunit of Ribulose Bisphosphate Carboxylase/Oxygenase
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The methylation of Lys-14 in the large subunit (LS) of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) by Rubisco LS N-methyltransferase (Rubisco LSMT) occurs by an ordered bi-bireaction mechanism. Kinetic and binding analyses of the initial interaction between Rubisco LSMT and des(methyl) forms of Rubisco, revealed a tight (KD 0.1 nM) and specific interaction which was identified as a consequence of a relatively slow kass(7,368 M-1 s-1) and kdiss (8x10-7 s-1). The molecular determinants of this tight and specific interaction were investigated by ELISA analyses of binding between truncated forms of Rubisco LSMT expressed in *E. coli* and immobilized Rubisco. The results demonstrated that among 19 different carboxy- and amino-terminal truncations, representing the removal of 4 to 380 amino acids out of a total of 489, all showed positive signals for binding to Rubisco. While some of the truncated Rubisco LSMT constructs showed noticeably weaker binding to Rubisco than full-length Rubisco LSMT, all maintained a high specificity for Rubisco. Thus, the interaction between Rubisco LSMT and Rubisco appears to be a consequence of multiple binding domains spread over a large portion of the Rubisco LSMT protein. Related studies determined the location of a cross-link between the LS of Rubisco and Rubisco LSMT catalyzed by a homobifunctional sulfhydryl-specific cross-linking reagent (BMH). Cross-linking between Rubisco LSMT and the LS of Rubisco generated a 106 kDa product which was a result of cross-linking between Cys-459 in the LS of Rubisco and Cys-119 or Cys-188 in Rubisco LSMT.

91. Studies of a Novel Pathway for the Biosynthesis of Straight and Branched, Odd and Even Length, Medium-Chain Fatty Acids in Plants
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Fatty acids (FA) of various chain lengths are key components of plant membranes, waxes, reserve oils, and certain compounds volatilized by plants. These natural products are exploited commercially as food components, lubricants, emulsifiers, etc., and are being researched for their use as renewable, biodegradable polymers, biofuels, stimulants for biodegradation of pollutants, etc. We have described a new pathway (termed -keto acid elongation, -KAE) that utilizes enzymes of branched chain amino acid (BCAA) metabolism to synthesize straight-chain, iso- and anteiso-branched, short and medium chain length FA in trichome glands of certain plants. Results of a completed survey indicate that -KAE appears to be restricted to trichome glands of petunia and Nicotiana species. Branched and straight medium chain FA of seeds, acid components of epicuticular wax esters and at least one petal-volatilized, anteiso-branched alcohol are elongated by fatty acid synthase and not -KAE. But, iso and anteiso primers for branched species are provided by BCAA metabolism. A direct assay was established to monitor isopropylmalate synthase (key enzyme of BCAA metabolism) that is 4 times more sensitive than conventional assays. This assay has revealed new properties of this enzyme. Efforts to isolate genes involved in BCAA metabolism and -KAE are in progress.

92. Enzymatic Synthesis and Biomolecular Materials
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The goal of this research is the use of natural biological concepts, processes, structures and molecules as the basis for the synthesis of new materials.
One component focuses on the use of natural, engineered and "created" enzymes to catalyze synthetic reactions. The unique stereochemical control exerted by enzymes and their ability to catalyze reactions at low temperature allows the synthesis of materials with structures and therefore properties that cannot be achieved using conventional synthetic routes. Efforts are focused on the design of reaction conditions, engineering of enzyme structure and activity, and generation of catalytic antibodies for materials synthesis. Other polymers with structures inspired by biological polymers are being synthesized chemically.
Work is also progressing on the synthesis of organic thin films which mimic the biological membrane. Membranes self-assemble, present defined and controllable surfaces, and detect the presence or absence of specific materials. These properties are exploited to alter interfacial and surface properties and to fabricate sensor devices. Thin film sensors have been developed to detect influenza virus, botulism, *E. coli*, cholera toxins, and small molecules, e.g., glucose. Similar films have been used to direct the ordered crystallization of inorganic salts. Research is also focused on the modification of the surface of materials to improve their biocompatibility.
* Funded jointly with the Division of Materials Sciences (DOE).

93. Transferring Photosynthesis Genes to New Cellular Environments
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We seek a genetic and molecular understanding of photosynthetic processes both in bacteria and in plants. In the past our work has centered on the photosynthetic bacterium *Rhodobacter capsulatus*. This bacterium contains a 46 kilobase region in length, which codes for all of the essential genes for photosynthesis, including the genes for the reaction center (RC) proteins, a long wavelength light harvesting antenna (LHI), and the enzymes for the biosynthesis of bacteriochlorophyll (Bchl) and carotenoid (Crt) pigments. Work on the mapping of this gene cluster is complete. The complete nucleotide sequence of this cluster has been determined, denoting a landmark in the molecular biological studies of photosynthesis. We are now directing our efforts toward the genetic transfer of pigment biosynthesis and photosynthesis to organisms which are incapable of performing these functions. The long term goals of our task are i) to gain an understanding of the evolutionary steps which took place in the transfer of photosynthesis to

plants, ii) to gain further understanding of the photosynthetic processes in plants, iii) to use the genetically engineered constructs which are now available to transfer biosynthetic enzyme activity into new cellular environments.

94. Structural Studies of Plant Signal Transduction Proteins
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Ethylene, a gaseous phytohormone, controls a variety of plant developmental processes ranging from seed germination to cell elongation, fruit ripening, and fruit and flower senescence. In addition, environmental stresses such as chilling, wounding, flooding, and pathogen invasion induce ethylene production as part of the defense response of the plant. There are at least two functional ethylene receptors, ETR1 and ERS, in *Arabidopsis thaliana* .

ETR1 forms a disulfide bridged dimer and each monomer consists of at least three distinct domains: an N-terminal ethylene-binding transmembrane domain, a histidine kinase domain, and a receiver domain. ERS is similar but lacks the receiver domain. Usually, the two components of bacterial two-component systems reside on two separate proteins. However, ETR1, like several eukaryotic two-component systems, is a hybrid kinase where both domains are contained in a single polypeptide. We recently subcloned, purified, and crystallized the receiver domain of ETR1 from *Arabidopsis thaliana*. The molecular weight of the purified protein was determined by electrospray mass spectroscopy, and found to correspond to that derived from the DNA sequence of the domain. Preliminary X-ray diffraction experiments show that the flash-frozen crystals diffracted beyond 2.1 resolution. The space group was determined to be P422 with the unit cell parameters of $a=b=48.4$, $c=112.3$. Search for heavy atom derivatives is in progress. Concurrently, a selenomethionine-substituted form has been produced to solve the structure by the MAD phasing technique.
(2) Plant Prenyl Transferase.

The two genes encoding the and -subunits of tomato farnesyltransferase (LeFTase) were cloned from a cDNA library using a PCR approach. The two genes can complement the yeast deleted with FTase- subunit gene*RAM1*. Analysis of the transformed cells revealed that the plant FTase can fully complement the yeast FTase.

A search of the database for protein which terminate with a CaaX-box motif was conducted. Among the proteins which were identified was the Arabidopsis MADS-box transcription factor *Apetalla1* (AP1). In vitro prenylation assays with recombinant AP1 protein showed that this protein can be prenylated, in vitro, by a plant farnesyltransferase with Km value of 3.5 mM.
The gene encoding gerenylgerenyl transferase-I was also cloned by yeast two-hybrid screen using the FTase a subunit as a bait. Expression is carried out in baculovirus infected insect cells. The expressed recombinant proteins are of high quality and are His-tagged at their amino terminus to facilitate purification.

95. Mechanism of Water Splitting and Oxygen Evolution in Photosynthesis
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Photosynthetic oxygen evolution in plants and cyanobacteria is catalyzed by a cluster of four Manganese atoms, the site of water splitting and acts as the locus of charge accumulation driven by the energy of four successive photons through the five S-states. Oxidation of Mn has been established from S_0-S_1 and S_1-S_2 but not from S_2-S_3. Reduction occurs on S_3-S_4-S_0. The structural motif in S_1 and different forms of S_2 is that of a dimer of di-mu-oxo bridged binuclear Mn units. Sr, an active replacement for the required Ca cofactor, is located at 3.5 Å from Mn. Upon advance to S_3, significant structural changes occur with the shorter Mn-Mn distances increasing. Further advance to the most reduced or S_0 state, induces significant but different changes in the complex. Use of highly oriented photosystem II membranes has permitted determination of the orientation of several internuclear vectors relative to the membrane normal. These structural results have been obtained with X-ray absorption spectroscopy which has also been adapted to the study of the Cl cofactor. A new EPR signal has been discovered in the S_0 states and a previously discovered EPR signal in the S_1 state has been confirmed and established to arise from the Mn cluster. A mechanism for the formation of the O-O bond has been postulated on the basis of the structural and oxidation state information.

96. Photosynthetic Pigment Proteins and Photosynthetic Light Reactions
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Light absorption in photosynthetic antenna pigment-proteins is followed by excitation transfer and trapping in reaction centers of photosynthetic membranes. In previous studies we characterized the rapid and extensive excitation transfer in the pigment-proteins C-phycocyanin and the LH2 bacteriochlorophyll (BChl)-protein complex from purple photosynthetic bacteria. In the latter complex, the excitation transfer dynamics is dominated by excitonic interactions among the BChl molecules in LH2. We have explored the relaxation of the excited electronic states of these pigment-proteins using analysis based on an extension of the Kennard-Stepanov relation. These studies show that the low-energy excited states resulting from exciton coupling among BChl molecules in LH2 are energetically stable during the excited-state lifetime (typically several nanoseconds) despite dephasing and apparent delocalization that occurs on the picosecond time scale.

Trans-membrane electric fields generated by salt gradients across thylakoids from higher plants were shown previously in our lab to result in changes in the fluorescence lifetimes and in the kinetics of charge separation in the reaction centers of Photosystem II. The effects were interpreted to result from an increase in the rate of charge separation and a decrease in the rate of charge recombination when the trans-membrane electric field orientation stabilized of the charge-separated state. Chromatophores from the purple photosynthetic bacterium *Rhodobacter sphaeroides* show an opposite behavior with respect to the direction of the applied electric field. Using mutant organisms deficient in either reaction centers or one or more antenna pigment-protein complexes, we find that most of this oppositely directed effect on the fluorescence arises from the antenna complexes. Once the contribution of the reaction center itself is uncovered, it is found to be the same as that seen in PS II. The generality of this effect provides valuable insights into the consequences of the light-stimulated redistribution of electric charge within the reaction centers and their associated membranes.

97. Vanadium Haloperoxidase: Functional organization and regulation of catalysis in Fucus zygote adhesion
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Vanadium halperoxidase (VPx) from marine algae represents a new kind of plant metalooxidase, capable of oxidizing a broad spectrum of organic molecules. We described a role for VPx in substratum adhesion by algal propagules by polyphloroglucinol activation, and VPx was immunolocalized in the extracellular matrix of several algal systems. We recently isolated a full-length 72.5 kDa *Fucus* VPx cDNA clone, which will enable investigation of the functional organization of the multimeric *Fucus* VPx enzyme into catalytic, self-associating and wall-targeting domains. Labeled recombinant VPx fragments will be prepared for domain identification by analysis of the properties and interactions of these expressed VPx peptides. The wall-targeting domain of VPx will then be used to identify the wall receptor. Developmental regulation of extracellular VPx activity will be studied at the egg, preadhesive zygote, initial adhesion, postadhesion zygote, rhizoid germination and 2-cell stages. The temporal and spatial control of VPx mRNA (maternal and/or newly synthesized) and protein will be compared by RT-PCR, activity-stained and Western gels, RT *in situ* PCR, ultrastructural immunolocalization and experimental perturbations. Recombinant VPx activity will make possible studies of the potential of VPx for oxidative crosslinking as well as oxidative degradation applications in biomass energy utilization, such as lignin degradation.

98. Developing Research Capabilities in Energy Biosciences
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Related URL: http://irnpulse.princeton.edu.lsrf/

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99. Identification of Chloride-Binding Domains in Photosystem II
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This project describes research which will elucidate the interaction of chloride with the oxygen-evolving site of Photosystem II (PS II). These interactions will be studied using a combination of physiological, biochemical and molecular tools. First, site-specific protein-labeling reagents will be used to differentially modify various PS II preparations in the presence and absence of chloride. Differentially modified domains will be identified by the use of "Cleveland Mapping", mass spectrometry and N-terminal protein sequencing. Individual modified amino acid residues will be identified using a mass spectrometer equipped with a reflectron allowing the analysis of metastable fragments by the use of post-source decay methods. These experiments will identify regions and individual amino acids on the intrinsic components which interact differentially with the cofactor. Second, a comprehensive analysis of several CP 47 mutants, including R448S and K321G, which exhibit alterations in their chloride requirement for oxygen evolution will be performed. Of particular interest is the examination of the phenotypes exhibited by these mutants during *in vivo* chloride depletion and reconsititution experiments. Oxygen evolution capacity, analysis of fluorescence yield parameters and an analysis of their flash oxygen yield properties are proposed. Third, suppressor analysis will be performed on spontaneous and chemically induced revertants of the CP 47 mutants R448S and K321G. Intergenic suppressors will be identified, mapped and sequenced to determine the identity of proteins interacting with these locations (^{321}K and ^{448}R) which modulate the chloride requirement for oxygen evolution in the CP 47 protein.

100. Investigation into the Metabolic Diversity of Microorganisms as Part of Microbial Diversity
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Related URL: http://www.mbl.edu

For graduate students, postdocs and established investigators who want to become competent in microbiological techniques for working with a broad range of microbes; and, become familiar with modern molecular genetic approaches for recognizing both possible affinities of as yet uncultivated bacteria and for understanding putative phylogenetic relationships.

101. Bacterial lipopolysaccharide structures required for root nodule development
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In most instances of cell-cell interaction in nature, key functions are performed by cell-surface molecules. Development of the nitrogen-fixing symbiosis between leguminous plants and *Rhizobium* bacteria is no exception. One of the bacterial surface molecules essential for infection and nodule development is the O-antigen portion of the lipopolysaccharide (LPS). Recent work in this laboratory has focused on structural alterations in this molecule that are induced by compounds exuded by the host legume. Heretofore, these changes in structure have been analyzed only by changes in antibody binding to the LPS. One of the goals of this project is to determine the actual chemical changes in the LPS. In addition, the LPS structures of three mutant strains will be analyzed. The rationale is that these mutant structures appear to have comparably minor changes with respect to the wild type and yet have profound effects on the symbiosis. Another goal will be to test a hypothesis that Nod factor production or presentation or induction is the basic cause of one of the symbiotic properties of most of the LPS mutant strains. Finally, the genetic regions in

which these mutations have occurred will be sequenced. These studies should provide new insights into the roles of bacterial surface polysaccharides in interactions with plant hosts.

102. A New Pathway for Isopentenyl Pyrophosphate Synthesis in Bacteria and Plants
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A multitude of natural isoprenoid products in plants, more than 22,000 identified so far, including antibiotics, rubber, taxol, carotenoids, sterols, cell growth factors, and cholesterol in animals, have isopentenyl pyrophosphate (IPP) as the building block. The long-term goal of this work is to elucidate the reactions and regulation of the pathways by which this central metabolite is synthesized in plants. Recent evidence suggests that plants have two pathways for IPP synthesis. In the "classical" pathway, also found in animals and fungi, IPP is formed from acetate with mevalonic acid as an intermediate. A second pathway, utilizing pyruvate and glyceraldehyde-3-phosphate as precursors, was recently proposed for IPP synthesis in *E. coli* and plant chloroplasts; however, the sequence of the reactions and the enzymes that catalyze the reactions of this pathway remain to be identified. The major objective of this work is to clone genes for the enzymes involved in the synthesis of IPP in the bacterium *E. coli* and, subsequently, to identify genes for related enzymes in chloroplasts of plants. Mutants of *E. coli* impaired in IPP synthesis will be selected with a colored isoprenoid (?-carotene) reporter assay developed in our laboratory. The mutations will be identified by complementation with a wild-type genomic library of *E. coli* and DNA sequencing. Functional analysis of the gene products will be by sequence comparison with known enzymes, and by analysis of products formed after incubation with labelled precursors. The function of homologous enzymes in cyanobacteria and higher plants will be assessed by complementation of the impaired *E. coli* strains. Results from this approach will contribute to our ability to exploit the immense variety of plant isoprenoids with useful medical and pharmaceutical properties. Further, the basic nature of the findings can contribute to a better understanding of the evolution of higher plants from their prokaryotic ancestors.

103. Molecular and Genetic Analyses of TSO1 in Arabidopsis
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Higher plant cell division exhibits several properties that are distinct from animal cells. However, relatively little is known about the molecular and genetic basis of higher plant cell division control. Our long-term objective is to understand the fundamental mechanisms employed by higher plants to regulate mitosis and cytokinesis. Currently, our research is focused on the genetic and molecular characterization of an Arabidopsis gene TSO1. Single tso1 mutants are abnormal in floral meristem structure, and the mutant cells show an increased DNA content and partially formed cell walls. These defects were only observed in the cells of floral tissues. Thus TSO1 is required for proper mitosis and cytokinesis in the floral tissues. Double mutants will be constructed between tso1 and other Arabidopsis cytokinesis mutations. The TSO1 gene will be isolated using a map-based cloning approach. The mRNA tissue-expression pattern as well as the subcellular localization of TSO1 will be examined using northern analysis, in situ hybridization, immunolocalization, and the Green Fluorescent Protein (GFP) reporter. Through these studies, we hope to define the specific function of TSO1 during cell division cycle, understand the significance and the regulation of the floral-tissue specific effect of tso1, and investigate the relationship between cell division regulation and organ development in higher plants

104. Physiology and Genetics of Catabolic Metabolism in the Aceticlastic Methanogens
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The controlled expression of catabolic gene products is integral to microbial interactions that mediate biomass conversion to methane. The goal of this project is to determine the mechanisms of transcriptional gene regulation in the methanogenic Archaea which are the catalysts of methane production and have a role in global carbon cycling. Although the Archaea have structural gene characteristics that are similar to those of both the Bacteria and the Eucarya, preliminary evidence suggests that they employ

Eucarya-like transcription factors for site-specific transcription initiation. We have shown that transcription of the gene encoding CO dehydrogenase (cdh) from *Methanosarcina thermophila* is highly regulated in response to substrate. A transformation protocol has been developed for *Methanosarcina spp.* and a simple, inexpensive anaerobic plating vessel has been developed that provides a virtually inexhaustible source of growth vessels for colony selection of genetic transformants. A reporter plasmid has been constructed by fusing the cdh promoter with UidA. Regions of DNA promoter sequence and flanking regions are being evaluated for their role in cdh regulation by studying the effects of point mutations and deletions on gene expression using the reporter fusion. Results of this study will determine whether gene regulation in the Archaea functions by mechanisms that are analogous to the other two lineages or by mechanisms that are unique to this phylogenetic line. Regardless of which mechanism(s) is revealed by this investigation, the results will provide further insight into the global molecular strategies of gene regulation.

105. Structure and Regulation of Calcium-Pumping ATPases and Plants
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Calcium is not only an important intracellular signal for many stimuli-induced responses, it is also essential for the proper functioning of organelles and the secretory system. However the transport systems required to regulate Ca concentrations in different cellular compartments are poorly defined at the molecular level. The current goal is to identify high affinity Ca pumps in plants and understand the specific functions of the different Ca-ATPases. Two biochemical types of Ca pumps were detected in plants, though plasma membrane (PM)-type pumps are localized to several different membranes. To identify and characterize each Ca pump, genes encoding Ca-pumps from Arabidopsis are being identified by functional complementation of a yeast mutant (pmr1 pmc1 cnb1). One gene, ECA1, shared high identity with animal SERCA pumps and restored growth of mutants on Ca-depleted medium. ECA1 represents the first plant gene encoding a functional Endoplasmic reticulum Ca-ATPase from plants. The 116 kD protein is localized to the ER and forms a Ca-dependent phosphoprotein that is blocked by cyclopiazonic acid, a SERCA pump inhibitor. Another gene ACA2 complemented yeast mutant phenotype only if the N-terminus was truncated. Ca transport of the full-length ACA2p, but not the truncated ACA2p, was stimulated by calmodulin. Thus ACA2p encodes a novel calmodulin-regulated Ca-ATPase with an N-terminal autoinhibitory domain. The transport properties, regulatory domains and subcellular location of these and other Ca pumps are being investigated in order to understand their cellular functions.

106. Molecular Analysis of the Interactions Between Pollen Tube Components and a Stylar Transmitting Tissue Pollen Tube Growth-Promoting and Attracting Protein
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Pollination, a key process in plant sexual reproduction, relies to a large extent on extracellular matrix interactions between pollen grains and specific tissues and cells within the female reproductive organ, the pistil. Upon landing on the pollen-receptive stigmatic surface of the pistil, pollen tubes emerge and elongate in the extracellular matrix of specialized pistil tissues to deliver sperm cells to the embryo sacs for fertilization. Our research is focused on identifying factors on the pollen tube surface and in the extracellular matrix along the pollen tube growth pathways that contribute to the pollination process. This project aims to isolate and characterize tobacco pollen tube components that interact with a pistil glycoprotein (TTS) which attracts pollen tubes and promotes their growth. TTS protein adheres to the pollen tube surface tips, and are incorporated into the walls of pollen tubes. The sugar moieties on TTS protein are important for its adhesion to the pollen tube surface; its unglycosylated C-terminal domain is adequate for binding to the pollen tube tips. We take advantage of this information and another structural feature (a cluster of histidine residues) on TTS protein to isolate the relevant pollen tube proteins. Pollen tube proteins will be prepared and allowed to interact with various forms of TTS protein *in vitro*. The pollen tube protein-TTS protein complexes will be isolated on an affinity resin that selectively binds histidine residues. The pollen tube proteins thus isolated will be characterized, their genes will be isolated for further analysis and manipulations in order to understand how they interact with TTS protein and to determine their biological roles in pollen tube growth.

107. Cellulose Fermentation by Nitrogen-Fixing Anaerobic Bacteria
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In anaerobic environments rich in decaying plant material, the decomposition of cellulose and other plant polysaccharides is brought about by complex communities of interacting microorganisms. We are simulating processes occurring in natural environments by establishing biologically-defined heterogeneous bacterial communities that we use as *in vitro* systems for quantitative studies of cellulose degradation, especially under conditions of combined nitrogen deprivation. In particular, we will establish cellulose-decomposing Fe(III)-reducing microbial communities, and isolate cellulolytic and Fe(III)-reducing members of these communities that we will use in biologically-defined microbial consortia to study metabolic interactions that may occur among members of these communities. Also, we will examine the effects of humic substances on the degradation of cellulose by anaerobic microbial consortia. A second aspect of our research involves investigations of the multicomplex cellulase/xylanase system utilized by the nitrogen-fixing, ethanol-producing bacterium, *Clostridium papyrosolvens* C7, for the hydrolysis of crystalline cellulose and xylan. This enzyme system consists of at least seven distinct extracellular, high-molecular-weight multiprotein complexes, each with different enzymatic and structural properties. Based on initial studies of this multicomplex system, we hypothesized that a 125,000-molecular weight glycoprotein (S4), found in all complexes and apparently lacking enzyme activity, functions as a scaffolding protein (a "scaffoldin") in the assembly of the multicomplex cellulase-xylanase system. We have cloned a DNA fragment from *C. papyrosolvens* C7 which is homologous to *cipA*, the gene that encodes the scaffoldin of the *Clostridium thermocellum* cellulosome. We will determine whether this cloned fragment encodes a protein that has a structural organization typical of a scaffoldin. Other experiments will be directed toward determining whether the cloned cipA-homologous fragment encodes the S4 protein of the multicomplex system. Also, we will examine the possibility that the multicomplex system is built on more than one scaffoldin. The objective of these studies is to further our understanding of the organization of the many components of the multicomplex cellulase/xylanase system, and generally, to advance understanding of the enzymology of cellulose hydrolysis by anaerobic bacteria. The results of this research will provide fundamental information on the physiology and ecology of cellulose-fermenting, nitrogen-fixing bacteria. This information is intended to fill gaps in our understanding of the complex processes involved in carbon and nitrogen cycling in anaerobic environments. The information obtained will be valuable in the development of practical applications, such as the bioconversion of plant biomass, including agricultural and municipal waste materials, to fuels such as ethanol.

108. Mechanisms for Microbial Reduction of Humics and Structurally Related Compounds
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Microbial reduction of humic substances and other extracellular quinone-containing compounds is a newly discovered form of anaerobic respiration. Studies to date have indicated that this anaerobic metabolism may have an important role in: 1) the natural cycling of organic compounds and metals in a diversity of soils and sediments; 2) plant-microbe interactions; 3) interspecies electron transfer in methanogenic environments; and 4) remediation of organic and metal contaminants. However, there is almost no information on the mechanisms for microbial humics reduction. The purpose of the proposed research is to study electron transport to humics. It is hypothesized, based on preliminary data, that even though there is a strong correlation between the ability of microorganisms to reduce Fe(III) and their ability to reduce humics, that the humics reductase is distinct from the FE(III) reductase. However, it is also hypothesized that the humics and Fe(III) reductase also share some similarities such as localization in the outer membrane and possibly some similar subunits. It is hypothesized that the humics reductase has a broad substrate specificity and is capable of reducing a wide variety of quinone-containing organics, including a variety of humic substances and other aromatics commonly found in soils and sediments. It is suggested that the capacity to reduce humics and other extracellular quinones represents an early form of microbial respiration and that there is a high degree of conservation in one or more of the components of the humics reductase complex, even among phylogenetically distinct humics-reducing microorganisms. These hypotheses will be investigated using biochemical, molecular, and immunological approaches that we have recently developed for purifying and studying the membrane-bound Fe(III) reductase in the dissimilatory Fe(III)- and humics-reducing microorganism *G. sulfurreducens*. The humics reductase will be solubilized from the membrane and purified with chromatographic and/or electrophoretic methods. The physical properties of the reductase will be determined and the localization in the membrane verified with cytoimmunological techniques. Optimized conditions for the humic reductase activity will be established and the range of electron donors and acceptors that the enzyme can utilize will be determined. The genes encoding for the subunits of the hypothesized humics reductase complex will be identified and sequenced. Antibodies and gene probes for the humics reductase in *G. sulfurreducens* will be used to determine if the humics reductase is in fact highly conserved among humics-reducing *Bacteria* and *Archaea*. These studies will provide the first insights into this environmentally significant and novel form of anaerobic respiration. Furthermore, since geochemical and microbiological considerations suggest that the reduction of humics and other extracellular quinones may have

been an early form of respiration that developed as a prelude to the incorporation of intracellular quinones in microbial metabolism, these studies may provide important basic insights into the general mechanisms and evolution of microbial respiration.

109. Regulation of Thylakoid Lipid Head Group Biosynthesis in Arabidopsis thaliana
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Oxygenic photosynthesis of higher plants depends on highly organized pigment protein complexes that are embedded in the polar lipid matrix of thylakoid membranes inside chloroplasts. The lipid composition of thylakoids is highly distinct compared to other cell membranes, but it is also largely conserved between different plants suggesting that the structure of thylakoid lipids is important for photosynthesis. It is the objective of this proposal to investigate the head group biosynthesis and the function of two thylakoid-specific glycolipids, the galactolipid digalactosyl diacylglycerol (DGDG) and the sulfolipid sulfoquinovosyl diacylglycerol (SQDG). The relative amounts of these two lipids increase under phosphate limitation and the elucidation of the underlying regulatory mechanism will provide novel insights into the control of membrane lipid composition. We plan to use a combination of genetical and biochemical approaches based on the DGDG-deficient *dgd1* mutant of *Arabidopsis thaliana* and we will apply our knowledge about *sqd* genes and enzymes essential for SQGD biosynthesis in photosynthetic bacteria. We expect to finish the positional cloning of *DGD1*, possibly encoding the DGDG synthase, and we will attempt to clone a SQDG synthase gene by sequence similarity to a cyanobacterial gene. The genes will allow us to express recombinant proteins in *E. coli*, thus making these available to biochemical analysis. The analysis of DGDG- or SQDG-deficient antisense lines should provide information on the function of these two thylakoid lipids.

110. The Role of the Membrane and Surface Carbohydrates of Rhizobium in Symbiosis
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The primary goal of our research is to determine the role that complex saccharide and membrane lipid chemistry in *Rhizobium* plays in the infection and occupancy of legume plants by these bacteria. Our work so far has turned up a startling degree of overlap between lipid, glycolipid and general carbohydrate biosynthesis in *Rhizobium* and in plants. Hence phosphatidyl choline, diglucosyl diacylglycerol, digalactosyl diacylglycerol, surfoquinovosyl diacyglycerol, phosphatidyl inositol and sulfocholine, all typical lipids in plants and other photosynthetic systems and rare or unheard of in bacteria, have been found in species of *Rhizobium*. This suggests the possibility of some pre-arranged common metabolism between these symbiotic partners. We have also found that the surface chemistry of *Rhizobium* is very variable and very sensitive to environmental factors and can be influenced by host factors and by the environment in the rhizosphere and inside of the plant. A further goal of our work has been to untangle the complex, interdigitized web of carbohydrate metabolism and saccharide biosynthesis in an effort to understand the many pleimorphic effects of mutations that affect biosynthesis and cloud or complicate the picture.

111. Biosynthesis of Triacylglycerol in Developing Oilseeds
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Although plant triacylglycerol (TAG) represents a major form of carbon storage in the biosphere, an understanding of the biosynthetic pathway(s) for triacylglycerol assembly, their subcellular location and regulation is not completely established. Some of the major unanswered questions in oilseed biochemistry are: a) How are unusual fatty acids targeted for storage in TAG and excluded from membrane phospholipids. b) What limits the quantity of oil produced by an oilseed? c) Are there spatially distinct pathways for membrane and TAG biosynthesis? Thus, although the biosynthesis of triacylglycerols in oilseeds has received considerable study, and the basic enzymatic reactions are mostly known, our understanding of how plants control the amount of TAG produced and how fatty acids move from the plastid and are incorporated into TAG is still unclear. The experiments are designed to evaluate the *in vivo* metabolism, regulation, and subcellular organization which lead to TAG assembly in oilseeds. In

particular, we will address the following specific objectives: I) What is the role of phosphatidylcholine in TAG biosynthesis in Brassica? II) Is a deacylation/reacylation cycle required for unusual fatty acid incorporation into oilseed triacylglycerol? III) Are there spatially distinct pools of PC and DAG involved in membrane and TAG biosynthesis? IV) Can TAG production in oilseeds be influenced by fatty acid or nitrogen supply?

112. Minority Summer Research Program in the Plant Sciences
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Because there are so very few minority individuals in plant sciences, minority students tend not to perceive plant sciences as a viable career option. This works to perpetuate their under-representation unless measures are effected to improve their perceptions of this field. To address this problem, we have designed and begun the implementation of a program to acquaint increased numbers of minority students with professional career opportunities in the plant sciences. While the under-representation of minorities in science and mathematics is recognized at virtually all levels of the educational process in our nation, we have chosen to focus our efforts at the college level where we have the most experience and feel that we can be most effective. Our program is comprised of four components: 1. Summer research for minority students in which the students are supported for ten weeks of research with a mentor in plant sciences; 2. Summer research for faculty in which minority faculty are supported for research in the plant sciences community at Michigan State University; 3. Outreach, in which plant science faculty at Michigan State University are supported for the presentation of a one-week short course at a predominantly minority institution; and 4. A graduate level course at Michigan State University on mentoring in a culturally diverse environment. In its four years as a pilot program (1993-96), this program has received considerable recognition and support in the plant science community, at Michigan State University and in the community surrounding Michigan State University

113. Structure-Function Relationships of ADP-Glucose Pyrophosphorylase: Manipulation of the Plant Gene for Increased Production of Starch in Plants
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Structure-function relationships of the ADPglucose pyrophosphorylase from higher plants (potato tuber) and bacteria (*Escherichia coli* and *Anabaena*) will be studied. The *Anabena* structural gene and cDNA clones of the large and small subunits of the potato ADPglucose pyrophosphorylase have been expressed in *E. coli*. The expression of these genes and prior chemical modification studies on the purified spinach leaf ADPglucose pyrophosphorylase enable us to do site-directed mutagenesis at various regions of the plant enzyme to gain more insight on the nature of the catalytic and effector sites and function of the two different subunits in the higher plant ADPglucose pyrophosphorylase. Attempts will also be made to understand why the higher plant enzyme (a heterotetramer, a2b2) requires two different subunits for high activity. Partial proteolysis experiments and molecular modeling programs are being used to predict and determine the secondary structure of the three enzymes. Crystallization and X-ray diffraction studies are major efforts to obtain insight into the three-dimensional structure of the ADP-glucose pyrophosphorylase. "Mutant," active bacterial enzymes that are less sensitive to allosteric inhibition by phosphate have been constructed and it is planned to do the same for the plant enzymes. Also active bacterial or plant enzymes that may not require the allosteric activator for activity; i.e., fully active without activator will be constructed. These could in the future be used to produce transgenic plants having increased amounts of starch.

114. Physiology and Molecular Biology of Lignin-Modifying Enzymes
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Laccases, lignin peroxidases (LIPs) and manganese-dependent peroxidases (MNPs) are the major lignin-modifying enzymes (LMEs) in fungi. While much information is available on lignin degradation by the white-rot fungus, *Phanerochaete chrysosporium*,

relatively little is known on LMEs in other white-rot basidiomycetes. This study focuses on the LMEs in *Ganoderma lucidum*, a widely distributed white-rot fungus in North America, and a soil basidiomycete, AX1. *G. lucidum* produces high amounts of laccase, both in defined media and in wood cultures. However LIP production was not seen in any defined media or wood cultures although LIP gene homology was observed when genomic DNA was probed with LIP gene probes of *P. chrysosporium*. Five major laccase activity bands were seen on SDS-PAGE gel. MNP activity was detected when grown on poplar but not on pine wood or other defined media. We are presently characterizing the laccase and determining its role in degrading synthetic lignin (^{14}C-DHP). Using the Polymerase Chain Reaction technique we have obtained a laccase gene fragment which will be used in isolating the whole laccase gene. AX1 produces high laccase levels, but no LIPs or MNPs. Laccase production varied in different wood cultures suggesting differential laccase gene expression. The laccase enzyme has been partially characterized for substrate specificity, pH optimum, molecular weight and the number of isoforms. Currently, the role of copper in regulating laccase production is also being studied. Furthermore, we plan to clone the laccase gene(s) after purifying and characterizing the laccase enzyme.

115. Molecular Physiology of Succinic Acid-Based Fermentations in Anaerobes: Control of Chemical Yield by CO2 Fixation and Electron Donors
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Fermentation derived succinic acid has many industrial uses as a specialty chemical; and, it is a potential replacement for petro-derived maleic anhydride used for manufacture of engineered plastics and polyesters. This project will develop fundamental and applied understanding on the mechanisms regulating growth and carbon and electron flow in catabolic succinate producing bacteria that utilize CO_2 as an electron acceptor. The physiology, biochemistry and key genes of *Actinobacillus* and *Anaerobiospirillum succiniciproducens* strains will be compared. Both organisms regulate PEP carboxykinase in relation to increasing CO_2 levels required for succinate production. The PEP carboxykinase genes have been cloned and site directed mutants will be utilized to examine the key enzyme structural elements responsible for determination of substrate affinity, and CO_2 fixing activity. Metabolic studies on *Actinobacillus* are aimed at developing a high yield fermentation based on coupling of hydrogen and CO_2 metabolism to "homo" succinate production. Because *Actinobacillus* contains hydrogenase, the influence of electro chemical control of redox potential and electron donor availability on metabolism and growth will be determined. Preliminary studies on the succinate fermentation under conditions of cathodic reduction linked to redox dyes have resulted in major changes in growth and end product levels.

116. Molecular Basis of Symbiotic Plant-Microbe Interactions
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The induction of nitrogen-fixing root and stem nodules on leguminous plants by soil bacteria belonging to the *Rhizobiaceae* is a highly evolved, complex process, requiring a fine-tuned interaction between the bacteria and their host. The persistence and competitive ability of the microbes in the soil and the rhizosphere of plants are important factors in early stages of rhizobial infection. In order to increase our understanding of these early stages, we wish to understand the molecular basis of the microbial response to common environmental stresses and plant factors secreted into the rhizosphere, to develop user friendly methods for the detection and classification of soil microbes and to explore the use of specific nutritional mediators to create "biased rhizopheres". Once the infection process has been initiated, distinct sets of plant genes are induced, which are involved in nodule ontogeny and in symbiotic nitrogen fixation. In order to understand the regulatory circuits responsible for symbiotic control of the expression of these loci, we wish to elucidate the molecular basis of the signal transduction pathways responsible for nodule-(cell-)-specific expression of plant genes encoding symbiosis-specific proteins (nodulins). In addition, we are using the model legume plant *Lotus japonicus* to generate novel symbiotic mutants via chemical mutagenesis and to isolate novel symbiosis-specific genes via differential display of messenger RNA's.

117. Molecular Mechanisms That Regulate the Expression of Genes in Plants
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The steady state level of an mRNA depends both on its rate of synthesis and on its rate of degradation. Rapidly accumulating data indicate that degradation rates of mRNAs in plants and other eukaryotes vary over a wide range and can be differentially regulated. However, relative to transcription, the mechanisms that control mRNA stability are poorly understood. A major goal of our research is to elucidate the mechanisms that target highly unstable mRNAs for degradation in plants because these mechanisms provide plants with a means to make rapid changes in gene expression in response to a variety of stimuli. Our studies have resulted in the identification of specific sequences, (e.g. DST elements or AUUUA repeats), that markedly accelerate the decay of reporter transcripts in stably transformed tobacco cells and decrease mRNA accumulation in transgenic plants. Mutagenesis experiments have begun to provide insight about the regions within the DST element that are required for instability function and similar experiments on other instability sequences are underway. These sequences are also being used as tools in order to develop molecular, genetic, and biochemical approaches aimed at identifying components of the degradation machinery and steps in the corresponding mRNA decay pathways. In addition, we have begun to characterize the RNases of *Arabidopsis* as a first step toward differentiating between the RNases that play a role in mRNA degradation and those with other roles in RNA metabolism.

118. Molecular Biology of Plant-Bacterial Interactions
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This project investigates the molecular basis of interactions between plants and bacteria. Previously, we showed that *Pseudomonas syringae* pathovar tomato strain DC3000 (a pathogenic bacterium of tomato and *Arabidopsis thaliana*) produces Hrp pilus, which is associated with interkingdom transfer of bacterial virulence and avirulence proteins into the plant cell. The Hrp pilus was found to be essential for bacterial pathogenesis and bacterial elicitation of plant disease resistance. We have now raised several antibodies against the HrpA protein or Hrp pili. The HrpA antibody was found to bind to Hrp pili protein or Hrp pili. The HrpA antibody was found to bind to Hrp pili and therefore can be used in future study of temporal and spatial expression of Hrp pili in infected plant tissues. Using Hrp pilus antibody, we have found three additional bacterial proteins associated with the Hrp pilus structure. Experiments are being performed to clone the genes that encode these proteins. The HrpA protein has been expressed in *A. thaliana*. Transgenic plants expressing HrpA were found to allow infection of a P.s. pv. Tomato hrpA mutant strain, which otherwise is incapable of infecting any plants. This result suggests that heterologously expressed HrpA may form a functional pilus. Previously, we showed that a bacterial avirulence protein, AvrB of *P. syringae* pv. *glycinea*, triggers the hypersensitive cell death response when expressed inside the plant cell, but not outside the plant cell or in the bacterial cytoplasm. This result led to the proposal that AvrB must be secreted by bacteria directly into the plant cell. We have now identified several putative *A. thaliana* AvrB-interacting proteins that may play a role in AvrB-mediated pathogenesis or disease resistance response.

119. Regulation of Plant Anti-Herbivore Defense
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The long-term goal of this research program is to understand the molecular signaling pathways that control the expression of plant defense genes. A useful model system for studying these pathways is the systemic induction of plant anti-herbivore defense genes in response to wounds inflicted by phytophagous insects. In tomato plants, the signal transduction pathway that couples wounding to the systemic activation of target genes is regulated by an 18-amino-acid peptide called systemin. Recent results indicate that systemin exerts its effects on gene activity by up-regulating the synthesis of the fatty acid-derived hormone jasmonic acid (JA), a potent regulator of stress-induced genes in virtually all plant species examined. The focus of our current research is to exploit genetic strategies to develop this model and to identify genes involved in systemin-mediated signal transduction.

120. Chloroplast Biogenesis
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We are continuing studies on the biogenesis of plastids. Our current efforts are aimed at understanding the transport of cytoplasmically synthesized precursors into chloroplasts. In particular, we are focusing on identifying and characterizing the components of the transport apparatus responsible for mediating this translocation process. Previous work has identified three proteins in the Toc (Translocon at the outer envelope membranes of chloroplasts) complex, Toc86, Toc75 and Toc34 as well as one protein of the Tic (Translocon at the inner envelope membrane of chloroplasts) complex, Tic110. In addition, we have identified ClpC, a stromal protein in the heat shock 100 family of molecular chaperones, as a component of the transport apparatus. cDNA clones and antibodies for all five proteins are available. Current studies are focused on determining the function of each polypeptide and investigating how these polypeptides interact with each other and with precursor proteins to accomplish protein transport into chloroplasts. We are also employing the antibodies and nucleic acid probes for these putative transport components to investigate their expression in various plant tissues that have different types of plastids. Finally, we have recently identified a cyanobacterial homolog of Toc75 and are currently investigating its role in cyanobacteria. Our goal in these studies is to gain some understanding into the evolutionary origins of the import machinery.

121. Action and Synthesis of Plant Hormones
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The objective of this project is to gain knowledge about the biosynthesis and action of the plant hormone ethylene and about the action of gibberellin (GA) in mediating rapid, submergence-induced elongation of deepwater rice. Our research combines physiological, biochemical and molecular approaches. We are elucidating the regulation of ethylene biosynthesis by determining the expression of genes encoding the two enzymes of this pathway, 1-aminocyclopropane-1-carboxylate (ACC) oxidase and ACC synthase. The evolution of the ethylene biosynthetic pathway is being studied in ferns, which leave evolved the capacity to synthesize ACC but produce ethylene via an ACC-independent route. We are also localizing the expression of ACC oxidase mRNA and transcripts of the ethylene signal transduction pathway with respect to ethylene-mediated asymmetric growth, e.g., formation of the apical hook. Signal transduction of ethylene action is being followed in Arabidopsis, where we have identified a number of ethylene-regulated genes. In deepwater rice, ethylene reduces the level of endogenous abscisic acid (ABA), thereby increasing the sensitivity of the plant to GA. We propose to study the effect of ethylene on ABA metabolism, in particular with respect to the activity of ABA oxidase. Since GA is the immediate growth-stimulating hormone in deepwater rice, we are studying GA-regulated cell wall extension, mainly regarding the role of the wall-loosening proteins, the expansins, in this process, and GA-promoted cell division in the intercalary meristem (IM), using cDNA probes for genes whose products control the progression of meristematic cells through the cell cycle. Using differential display of mRNA, we are also identifying genes in the IM whose expression is regulated by GA the most interesting of which appears to be a novel transcription factor.

122. Photoperiodic Induction and the Floral Stimulus
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A large body of physiological evidence indicates that flower formation is hormonally controlled. In plants exposed to the appropriate photoperiod for flowering, a floral stimulus or florigen is formed in the leaves from where it is translocated in the phloem to the apical meristem. Results of grafting experiments show that the floral stimulus is exchangeable between different response types and is, therefore, very similar, if not identical, in different species. The chemical nature of this floral stimulus is one of the major unsolved problems in plant biology. The research is being conducted with the short-day plant *Pharbitis nil*, strain 'Violet' (Japanese morning glory) which in the cotyledon stage can be induced to flower with a single dark period. mRNA from induced (14 h dark period), or non-induced (8 h dark period, and 14 h period with 5 min red light interruption) cotyledons is used for differential display of mRNA to isolate genes whose expression is changed during induction in comparison with the non-inductive treatments. With 200 primer

combinations, 190 PCR products showed changes in expression during induction, of which about half were upregulated. Changes in gene expression will be confirmed in northern blots to eliminate false positives. Genes that are either up- or downregulated during induction will be sequenced and their possible functions will be determined by comparison with sequences in the data bases.

123. Interaction of Nuclear and Organelle Genomes
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Our studies with plant mitochondria involve how the mitochondria communicate with the nucleus to regulate nuclear-encoded mitochondrial proteins. The alternative oxidase, encoded by the nuclear *Aox1* gene family, is employed to study the nuclear/mitochondrial interaction. Alternative pathway respiration is induced by a number of environmental signals including lowered temperature, salt stress, and wounding. A thread that most of these inducing stresses have in common is oxidative stress generated within mitochondria. We have isolated a large number of cDNA's encoding a variety of genes which respond quickly to mitochondrial oxidative stress. Recently, evidence for the mitochondrial signal for this stress has moved toward reactive oxygen species, specifically hydrogen peroxide. Another aspect of this work has focused on downstream -from respiratory electron transport- regulation of carbon balance through reductive potential generated by the plant TCA (tricarboxylic acid) cycle. Our work indicates a key mitochondrial matrix enzyme, NADP-isocitrate dehydrogenase, may be involved.
Research in photosynthesis is centered on the genetic control of carbon flow through Photosystem I. We have created a number of primary mutants to study electron flow in the "core" of Photosystem I. Recently, we have begun to isolate families of pseudorevertants to these primary mutants. The pseudorevertants appear to "correct" many of the primary mutations. These mutants fall into three classes: Photosystem II mutations, Photosystem I subunit mutations, and mutations in proteins involved in assembly of Photosystem I.

124. Molecular Mechanisms of Trafficking in the Plant Cell
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Maintenance of separate subcellular compartments in eukaryotic cells depends on the correct sorting and targeting of newly synthesized proteins. Thus, mechanisms must exist in the cell to assure that these proteins are targeted to, and subsequently translocated across, the correct intracellular membranes. We are working with proteins destined for different compartments: vacuoles, cell wall and nucleus. We are interested in understanding the molecular determinants of differential protein compartmentalization and identifying the components of the molecular machinery which carry out the sorting process. We have analyzed and characterized sequences responsible for protein sorting to the vacuole and to the nucleus, and have isolated several putative receptors of the endomembrane system and the nuclear import machinery. We are now elucidating the functional complexity of vesicular trafficking and transport to the nucleus.

125. Cell Wall Metabolism
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Our goal in this project is to address two questions of cell-wall metabolism. The first is to understand how xyloglucan, the major hemicellulosic polymer in dicots, is synthesized. The second is to understand the role of expansins, proteins that loosen cell walls, during the growth of rice internodes. The first part of the first objective is to isolate and characterize the fucosyltransferase involved in xyloglucan biosynthesis. Purification of the enzyme has been completed, and protein sequence information should be available soon. The next step will be the isolation of cDNA clones encoding the enzyme and the preparation of antibodies against the protein encoded by the cDNA clone. The second part of the first objective is to investigate the function of a Golgi-localized 40-kD protein that is postulated to have a role in xyloglucan biosynthesis. cDNA clones for this protein have been isolated and antibodies against it prepared. A detailed study of the expression patterns of this gene has been completed. Antisense plants containing suppressed

levels of this protein have been produced and will be used to investigate its role, if any, in xyloglucan biosynthesis. Finally, we are investigating the involvement of expansins in the growth of rice internodes. Expansins are recently discovered cell-wall loosening proteins. We have studied the expression of expansin genes and expansin protein by RNA blot analysis and tissue printing. The cellular localization of expansin gene expression is being studied by *in situ* hybridization. Transformation experiments are being planned to assess the role of expansins in the growth of intact rice plants.

126. Biochemical and Molecular Aspects of Plant Pathogenesis
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The objective of this project is to further our understanding of the biochemical events that are important in the interactions between fungal plant pathogens and their hosts. We are studying examples of pathogen factors that determine basic compatibility (the ability of a fungus to infect any plant) and those that determine specificity (the ability of a fungus to infect a particular plant, i.e, the basis of host-range). As examples of basic compatibility factors, we are studying the extracellular enzymes that degrade the polymers of the plant cell wall. Circumstantial evidence suggests that these enzymes are important for colonization of host tissue and also can act as triggers of plant defense responses. We have purified enzymes, including pectinases, xylanases, glucanases, cellulases, and proteases, from the ascomycetous maize pathogen *Cochliobolus carbonum*, cloned the corresponding genes, and tested their importance in pathogenesis with targeted gene disruption. The host-selective toxin, HC-toxin, is a specificity determinant because it affects only maize of certain genotypes. We established that the maize resistance gene *Hm* encodes a reductase that detoxifies HC-toxin, and have now shown that the site of action of HC-toxin is histone deacetylase, a nuclear enzyme that influences chromatin structure and gene expression by modifying core histones. We are studying why inhibition of this enzyme leads to the establishment of a compatible (susceptible) disease interaction. In the fungus, HC-toxin production is controlled by a single genetic locus, *TOX2*. *TOX2* contains multiple copies of multiple genes necessary for HC-toxin synthesis, including a large cyclic peptide synthetase, a fatty acid synthase, and a toxin export pump. All of these genes are present only in isolates of *C. carbonum* that make HC-toxin and are distributed over 540 kb on the same dispensable chromosome.

127. Developmental Biology of Nitrogen-Fixing Cyanobacteria
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Anabaena and related filamentous cyanobacteria utilize reductant generated by photosynthesis in vegetative cells to fix nitrogen gas within spaced, differentiated cells called heterocysts. We are elucidating the dependency relationships, and the roles, of genes whose collective function allows *Anabaena* to fix nitrogen (an oxygen-sensitive process) in the presence of oxygen. The heterocyst envelope plays a key role by limiting the rate of entry of oxygen into heterocysts. We are studying *hep* genes, required for synthesis or stabilization of the polysaccharide layer of that envelope, and *het* genes, required for morphological differentiation. Overlapping, ca. 100-bp deletions ("windows") were introduced into the region 5' from a *hepA*-reporter fusion, and the constructions transferred to *Anabaena*. Deletions from -534 bp to -446 bp and from -343 bp to -169 bp relative to the *hepA* transcriptional start site greatly reduced expression of the reporter after nitrogen stepdown. Gel-retardation experiments showed the presence of a protein that binds specifically to the latter region, and may control expression of *hepA*. A mutation in a gene that shows great similarity to N-acetylmuramoyl-L-alanine amidases, but not a mutation in the gene 3' from it that shows great similarity to glutamate racemases, blocks formation of the heterocyst envelope polysaccharide. Perhaps export of that polysaccharide requires prior catabolism of peptidoglycan in the differentiating vegetative cell. Gene *hetR* represses expression of *hetC*, which is active early in differentiation after pattern formation, ca. 100-fold. This work will facilitate understanding of cellular differentiation, pattern formation, and biological conversion of solar energy.

128. Frankia Genetics
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Frankia spp., bacteria in the Gram positive actinomycete group, form nitrogen-fixing nodules with a wide variety of non-leguminous woody plants. *Frankia*-nodulated plants have great, world-wide potential as sources of fuel, fiber, shade, and forage, as well as for reforestation. Their potential, and opportunities for understanding the interesting biology of *Frankia*, are strongly curtailed by the lack of methodology for genetic manipulation of these bacteria. Our short-term objective is to develop techniques for stable, reproducible genetic transformation of *Frankia*. Transfer of DNA to *Frankia* requires a suitable replicon, a means of transferring the DNA, appropriate selective markers, avoidance of restriction, and a suitable choice of strain. To tailor a replicon, we sequenced an 8.5-kb plasmid from *Frankia* strain CpI1, and have constructed derivatives of it that bear antibiotic-resistance markers whose codon usage is consistent with that of known *Frankia* genes. Transfer of plasmid DNA to relatively fast-growing strains of *Frankia* has been attempted by electroporation, particle-bombardment, and conjugation. To circumvent possible restriction barriers, we have searched for type-II restriction endonucleases (in collaboration with New England Biolabs, Inc.; none yet found), identified putative clones of DNA methyl transferase genes, and are modifying DNA by a coliphage T4 anti-restriction system (in collaboration with J. Elhai). Longer term objectives include the development of techniques for transposon mutagenesis of, and gene replacement and use of reporter genes in, *Frankia* followed by analysis of the generation of N_2-fixing symbioses of *Frankia* with higher plants.

129. Environmental Control of Plant Development and Its Relation to Plant Hormones
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Plant growth and development are affected by environmental factors such as daylength, temperature, and moisture. Hormones often function as chemical messengers between the perception of an environmental factor and the morphological expression. In long-day rosette plants, stem growth in long days (LD) is caused by an increase in gibberellin (GA) levels, particularly in the shoot tips. The obvious question is: which step(s) in the GA biosynthetic pathway is (are) stimulated by LDs? *GA5* which encodes GA 20-oxidase in *Arabidopsis* was predominantly expressed in the upper parts of stems. With induction of stem growth by LD, *GA5* mRNA gradually increased in the shoot tips. As the plants elongated, *GA5* mRNA in the upper part of the stems increased to a maximum, and then decreased as the growth rate declined. By contrast, expression of *GA4*, encoding 3ß-hydroxylase, showed low expression in stems, and its expression was not correlated with the rate of stem elongation.

Abscisic acid (ABA) is a plant hormone whose synthesis is greatly stimulated when plants experience a water deficit. The objective of our studies is to find out how the biosynthesis of ABA is regulated. In collaboration with D.R. McCarty's laboratory at the University of Florida, Gainesville, the ABA-deficient mutant *vp14* of maize has been characterized. The VP14 GST-fusion protein gene was assayed for cleavage activity, using 9-*cis*-violaxanthin and 9'-*cis*-neoxanthin as substrates. The expected cleavage products, xanthoxin and the C_{25} apo-aldehydes, were identified. All-*trans*-carotenoids were not cleaved. Because the cleavage reaction is thought to be the key regulatory step in ABA biosynthesis, it may be possible to alter ABA levels in transgenic plants that would affect such physiological processes as drought tolerance, cold hardiness, and seed maturation.

130. CLV Signaling in Meristem Development
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The shoot meristem of higher plants is responsible for initiating all above-ground organs. Thus the genes regulating shoot meristem function are required for cell proliferation and differentiation, and for the proper number, spacing and size of primordia such as leaves and flowers. To function as a site of continuous organ formation, the shoot meristem must be able to carry out two fundamental processes. The first function is the maintenance of a population of undifferentiated cells at the center of the meristem. As this population divides, it provides new cells for incorporation into organ primordia. The second process is to direct progeny of the undifferentiated cells towards organ formation and eventual differentiation. This transition from an undifferentiated to differentiated state occurs at the flanks of the shoot meristem, and is regulated in strict balance with the proliferation of the undifferentiated cells.

We have found that the CLV loci (CLV1, CLV2 and CLV3) in Arabidopsis regulate the balance between proliferation and differentiation. clv mutants accumulate over 1000-fold more undifferentiated cells than wild-type plants. CLV1 encodes a receptor-like kinase, and may relay positional information through a signal transduction cascade. Our work focuses on identifying other members of the CLV1 signaling cascade through both genetic and biochemical approaches.

131. Isolation and Characterization of Ammonia Monoxygenase of Nitrosomonas
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Ammonia monoxygenase (AMO) of the autotrophic bacterium Nitrosomonas catalyzes $NH_3 + 2e^- + 2H^+ + O_2 \rightarrow NH_2OH + H_2O$. Electrons pass to AMO from hydroxylamine oxidoreductase (HAO): $NH_2OH + H_2O \rightarrow HNO_2 + 4e^- + 4H^+$. Together with a terminal oxidase, oxidation of NH3 by AMO and HAO provide all the energy for growth in this bacterium. This oxidative step in the N-cycle in nature has a very important role in N-availability to agricultural plants, the production of greenhouse-active and ozone-reactive N-oxide gases and the transformation of ammonia in waste water. AMO oxidizes halogenated aliphatic and aromatic compounds (sometimes producing pure enantiomers) and is thus potentially important to pollutant bioremediation and the production of specialty chemicals.

The prosthetic groups or structure of AMO has not been determined because active enzyme cannot yet be purified. Acetylenic substrates derivatize a protein subunit of AMO allowing isolation and sequence of the genes for three subunits and generating a model containing possible metal binding sites and 12 transmembrane segments, a common property of membrane pumps. This observation and the inhibition of the AMO system in cells by uncouplers and DCCD, suggests that AMO might be driven by an ion gradient, an hypothesis that we are testing. The ultimate goal is to isolate and characterize all protein subunits, the structure of the active site and electron-transfer redox centers by optical, EPR, Mossbauer and other types of spectroscopy and by chemical analysis.

132. Reverse Transposon Tagging of Maize Tubulin Genes
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Microtubules are cytoskeletal elements that are involved in essentially every aspect of plant growth. The ? and ? subunits that comprise microtubules are encoded by large families of expressed genes that give rise to similar but distinct isoforms. Our long-term goal is to determine if there is functional significance to the multiple tubulin genes and isoforms, and if tubulin gene expression can be modified to alter maize growth. The objectives of this project are to determine if disruption of any of the maize ? tubulin genes has an effect on the plant's ability to grow and reproduce normally or on the plant's ability to withstand chilling. We will use molecular genetics to identify plants that have a ? tubulin gene that is disrupted by the present of a *Mutator (Mu)* transposable element, and will analyze offspring of these plants for effects of each mutation. Besides assessing overall growth, we will look for increased or decreased sensitivity to cold, ability to correctly position nuclei in cells that need to become polarized to achieve morphogenesis, ability to undergo asymmetrical divisions necessary for normal morphogenesis, ability to complete meiosis and microsporogenesis, and ability to correctly shape and move sperm cells in pollen tubes. We anticipate that some of our findings could translate into future manipulation of tubulin gene expression to alter growth, development, biomass production, reproduction, or chilling tolerance of an important species.

133. Cellulose, the cell wall and morphogenesis
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This research aims to understand plant morphogenesis. The project studies the relationship between morphogenesis and cellulose microfibrils. Experiments have compared growth of well-watered roots of *Zea mays* to thinner roots grown at low water potential. We measured the spatial distribution of expansion rates, in length and radius, for stele and cortex, and found that expansion in longitudinal and radial directions were regulated independently. To determine whether alignment of microfibrils regulates radial expansion rate, we quantified microfibrillar alignment with electron microscopy of metal replicas of the inner-most wall layer. Microfibrils were transverse to the long axis of the root throughout the growth zone; however, locations with different rates of radial expansion had similar microfibrillar alignment. Quantitative polarized-light microscopy showed that the amount of retardation of the cell walls did not change as a function of position, confirming that different rates of radial expansion occurred without differences in

microfibril organization or abundance. We strengthened the conclusion that rates of radial expansion are independent of the organization or abundance of microfibrils by identifying a mutant in *Arabidopsis thaliana*, rsw7, in which rates of radial expansion were stimulated compared to wildtype but microfibrils were neither depleted nor disorganized. The work points to components other than microfibrils that help to shape plant organs. This research will uncover mechanisms of plant morphogenesis and can thus be expected to enhance applied efforts to optimize plant growth for human benefit.

134. Dosage Analysis of Gene Expression in Maize
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The goal of this project is to understand further the basis of dosage sensitive regulatory effects on gene expression in maize. These effects act directly or inversely on the quantitative level of gene products when the dosage of specific chromosomal segments is varied. It has long been known that addition or subtraction of chromosomal segments relative to the normal diploid has detrimental effects on the vigor of plants. The experiments proposed seek to understand the molecular basis of these aneuploid syndromes as well as to understand the mechanisms of gene expression that are involved. One set of experiments is to test for a correlation between small regions of chromosome arm 10L that produce inverse and direct dosage effects on selected monitored RNAs and that produce aneuploid effects on kernel size. This chromosome arm will be divided into several segments--some with known kernel size effects. These smaller regions will be tested for a correlation with the molecular dosage effects that are found in the larger segment. Also, conditions that make the aneuploid effects more extreme will be tested as to whether the dosage effects are also of greater magnitude. To determine whether the effects can be reduced to the action of single genes, two projects are proposed. First, dosage series including three known regulatory genes (*Vp1*, *C1* and *R1*) effective on the anthocyanin pathway are being examined for effects on the RNAs for the biosynthetic enzymes. One of these, the *C1* locus, is known to give a dosage effect and might well be a single gene example of a direct regulator. Any effects found with the whole arm dosage series will be tested whether they are due to the respective regulatory gene by recombining a loss-of-function allele in each case onto the B-A translocation used to generate the series. If the regulatory gene is responsible for the whole arm effect, then the recombined translocation will no longer be effective. Secondly, modifiers will be induced that increase or decrease the amount of pigment present in kernels that carry a single functional copy of *C2* in the endosperm using the Mutator transposon system. Modulations of this gene encoding chalcone synthase can be recognized phenotypically. Heritable modifiers will be tested for a dosage effect on *C2* RNA. Lastly, run-on transcription assays will be conducted on some of the most extreme inverse and direct effects on *Alcohol dehydrogenase-1* to test whether the rate of transcription is being modulated by these changes in chromosomal dosage. These experiments will provide information on the mechanisms of gene expression involved with these dosage effects and the relationship to the altered vigor of plants that have changes in chromosome number.

135. Epigenetic regulation of phenotypic variegation in maize
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The aim of this project is to understand epigenetic mechanisms of gene regulation. We are studying Pl-Blotched, a gene that controls the synthesis of purple anthocyanin pigments in the maize plant. Plants carrying Pl-Blotched exhibit an unusual pattern of pigmentation characterized by variegation, rather than the usual uniform pattern of pigmentation. Because the nucleotide sequence of Pl-Blotched is nearly identical to the normal Pl-Rhoades sequence but the two alleles are differentially methylated, our hypothesis is that the variegated pattern of Pl-Blotched expression is due to an epigenetic effect. As one way to address this idea, we are looking at the role of development in regulating Pl-Blotched expression. The methylation of Pl-Blotched DNA is progressive through development of the plant from seedling to adult. In seedling leaves, Pl-Blotched DNA is less methylated than Pl-Rhoades DNA whereas in adult leaves, Pl-Blotched DNA is more methylated than Pl-Rhoades DNA. In Pl-Blotched plants carrying the developmental mutation Teopod1, which lengthens the juvenile phase of growth, the change in DNA methylation precedes the change in phase-specific leaf features, such that the latest juvenile leaves display the level of methylation seen in fully adult leaves. If we consider that a change in methylation might occur more rapidly than morphological changes in leaf characteristics, this observation suggests that methylation of Pl-Blotched may be responsive to the same molecular cue that signals phase change. Such developmental influences on gene expression are a hallmark of epigenetic regulation.

136. Genetics and Molecular Biology of Hydrogen Metabolism in Sulfate Reducing Bacteria
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To explore the energy generating processes of the strictly anaerobic bacteria, new genetic tools for the sulfate-reducing bacterium, *Desulfovibrio desulfuricans* G20, have been developed that include a promoter probe vector with *lacZ* as the reporter. Conditions have been established to detect the activity of beta-galactosidase in colonies of the sulfate-reducing bacterium. Procedures for reasonably reliable electroporation of this anaerobe were established. A mutant was constructed in *cycA*, encoding cytochrome *c3*, by plasmid insertion. After confirmation by Southern analysis, preliminary phenotypic characterization of the mutant shows no aberrant features. Although mRNA was dramatically reduced, protein patterns of heme-containing proteins were not significantly different from the wild type. More detailed analysis of the mutation is in progress. Similarly a mutation has been created in a cloned and partially sequenced *hynAB* operon. This operon appears to be poorly expressed, if at all, in G20. The 16S RNA gene sequence has been determined and a phylogenetic comparison made with other such sequences from sulfate-reducing bacteria available in the database.

137. The Respiratory Chain of Alkalophilic Bacteria
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The long term goals of this project are to understand those features of the alkaliphile respiratory chain that are required for this ability of the alkaliphile to grow optimally at pH values that place severe energy demands upon these bacteria, i.e., to elucidate the basis for apparently remarkable energy conservation by alkaliphilic bacteria. The extreme, facultative alkaliphile *Bacillus firmus* OF4 grows on malate at a slightly faster rate and to at least as high a molar growth yield at pH 10.5 as it does at pH 7.5. The robust non-fermentative growth of the alkaliphile at pH 10.5 depends upon its full, pH 10.5-induced complement of a caa_3-type terminal oxidase. Moreover, a second, *bd*-type, terminal oxidase that is up-regulated upon disruption of the caa_3 oxidase-encoding *cta* operon cannot support growth on malate even at pH 7.5. Current studies are focused upon cloning the operon encoding the *bd*-type oxidase so that the effects of its overexpression in the *cta*-disrupted mutant can be tested along-side expression of a cloned *cta* operon. The studies should clarify whether overexpression of the genes encoding the *bd*-type oxidase will render it competent to support non-fermentative growth at pH 7.5, 10.5, both, or neither. If, as hypothesized, the caa_3-type oxidase will turn out to be necessary for oxidative phosphorylation and hence non-fermentative growth at pH 10.5, the properties of this oxidase that are essential for alkaliphily will be probed.

138. The Water Splitting Apparatus of Photosynthesis
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The goal of this research is to understand structural and functional relationships in photosystem II (PSII) with emphasis on the reaction center (RC) and water-oxidization processes that generate photosynthetic reductant. Femtosecond flash-probe studies of the isolated PSII RC using a novel analysis procedure has resolved a long standing controversy regarding the rate of primary charge separation. Biphasic kinetics (8 and 50 ps^{-1}) were detected and represent the effective rate of charge separation within the RC equilibrated core and charge transfer, limited by energy transfer from accessory chlorophyll in the RC, respectively. A new way of investigating the water-oxidation process by limiting the availability of substrate water was also developed. Water content was decreased by adding ethylene glycol to the solvent, and both reversible and irreversible changes in various functional properties of PSII were explained in terms of structural and electronic effects of the increased organic solvent environment. Studies of high affinity Mn ligands (associated with functional Mn) in PSII using the DPC-inhibition assay have identified four ligands (two carboxyl and two histidyl residues). Only one of these ligands, a carboxyl, is specific for Mn, and this Mn is photooxidizable by a single-turnover flash of light. This ligand is probably Asp170 on the D1 RC protein, and its chemical modification uncovers a low affinity, photooxidizable Mn ligand that is neither a carboxyl nor a histidyl residue. The other three ligands (including His337 on D1) bind Mn that is not photooxidizable by a single flash, but they may also bind other metals.

139. Protein and RNA Interactions Involved in the Pathogenesis of Tomato Bushy Stunt Virus
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Our research on Tomato bushy stunt virus (TBSV) has focused on determinants affecting replication, pathogenicity and recombination. TBSV is a small isometric virus that contains a single stranded RNA genome with five major genes. We analyzed the importance of an additional small gene designated pX, at the 3' end of the genome. Bio-assays showed that mutations of the translational initiation codon designed to disrupt translation of the pX encoded protein were unable to accumulate to detectable levels in cucumber protoplasts. Although these results suggested a role for the putative pX protein, introduction of a premature stop-codon to truncate expression of the pX protein were unable to replicate. In contrast, a comparable pX mutation that affected the same nucleotides without changing the predicted amino acid sequence greatly reduced RNA accumulation. Therefore, the pX RNA sequences rather than the predicted pX protein influence genome replication. The requirement for pX also appears to be host dependent because comparisons revealed that subtle pX gene mutations that interfere with accumulation of TBSV RNA in cucumber or *Nicotiana benthamiana*, did not affect replication in *Chenopodium quinoaprotoplasts* or plants. Irrespective of the host, the cis-acting pX gene sequences were not required for replication of defective interfering RNAs that require helper TBSV for replication in trans. These experiments thus suggest that the pX cis-acting element interacts with one or more host components whose composition differ slightly between different plants.

140. Enzymology of Acetoclastic Methanogenesis
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An understanding of the mechanism of methane formation is critical since methane is an important fuel and a significant greenhouse gas whose concentration is rising at a rate of 1% per year. We are studying the mechanism of methanogenesis from acetate, which is the major substrate for methanogens in nature. We are focusing on three important steps in aceticlastic methanogenesis: (i) the initial step of acetyl-CoA disassembly by the molecular aggregate that contains CO dehydrogenase/acetyl-CoA synthase (CODH/ACS) and a corrinoid/iron-sulfur protein (C/Fe-SP); (ii) the methyl-CoM reductase (MCR) catalyzed reaction that generates methane and a heterodisulfide (CoB-S-S-CoM) from methyl-Coenzyme M (methyl-CoM) and Coenzyme B (CoB); and (iii) the heterodisulfide reductase (HDR) reaction that reduces CoB-S-S-CoM to the free thiols, CoB-SH and CoM-SH for the next round of methanogenesis. We are using a combination of kinetics, electrochemistry, and spectroscopy to establish the reaction mechanisms of these enzymes. In the last year, a protocol to generate highly active MCR has been developed and the HDR has been characterized as a heme/iron-sulfur protein. The studies are expected to lead to important insights into how natural gas is formed in nature and into the structure and function of metals in biology.

141. Role of the Rubisco Small Subunit
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Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyzes the rate-limiting step of photosynthetic CO_2 fixation. Because of its low carboxylation efficiency and competitive inhibition by O_2, Rubisco has been viewed as a potential target for engineering enhanced crop-plant productivity. The Rubisco holoenzyme is comprised of two subunits, each present in eight copies. Much is known about the structure-function relationships of the chloroplast-encoded large subunit. It contains the active site. Much less is known about the role of the nuclear-encoded small subunit. There is a family of small-subunit genes in crop plants, making it difficult to substitute engineered small subunits into the holoenzyme. A photosynthesis-deficient mutant of the green alga *Chlamydomonas reinhardtii* has recently been recovered that lacks both members of the small-subunit gene family. Because this mutant can be rescued via transformation with a single small-subunit gene, it is now possible to answer questions about small-subunit function. Alanine-scanning mutagenesis is being used to elucidate the significance of small-subunit structural regions that are unique to the Rubisco of eukaryotes. Most substitutions do not eliminate enzyme function, indicating that these regions are not essential for holoenzyme assembly or catalysis. In contrast, random mutagenesis is being used to define those regions of the small

subunit that are essential. Because Rubisco enzymes from different species display differences in CO_2/O_2 specificity, heterologous small-subunits will also be introduced into *Chlamydomonas* to assess the contribution of small subunits to catalytic efficiency.

142. Asparagine Synthetase Gene Regulation and Plant Nitrogen Metabolism
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We are studying the genes controlling asparagine biosynthesis using a molecular-genetic approach in *Arabidopsis thaliana*. Since asparagine serves to transport and store assimilated nitrogen, these may have significance for improving nitrogen use efficiency. We have uncovered two distinct classes of ASN genes. ASN1, the major expressed gene, is transcriptionally repressed by light and this mirrors the light repression of asparagine accumulation in plants. These results suggest the ASN1 gene controls the synthesis of transported asparagine. The ASN2 gene, cloned by complementation of a yeast asparagine auxotroph, is activated by light and may encode an enzyme with enhanced ammonia-dependent activity possibly involved in ammonia detoxification. The ASN1 and ASN2 genes are also reciprocally controlled by metabolites. ASN2 is induced by light or sucrose and repressed by organic nitrogen. By contrast, ASN1 expression is repressed by light or sucrose and induced by organic nitrogen. We propose that this metabolic regulation of the ASN1 gene serves to channel assimilated nitrogen into asparagine under conditions of carbon limitation (in the dark) or under conditions of organic nitrogen excess. We have begun to study the signal transduction components involved in the light and metabolic regulation of ASN1 expression. We identified cis-elements and nuclear factors involved in the light or sucrose repression of AS1. We are using a transgenic Arabidopsis line (AS1-Hph) in a positive selection to isolate mutants impaired in light or sucrose repression. The phenotypic analysis of such mutants may define how the regulation of asparagine synthesis by light or metabolites affects plant growth and nitrogen use.

143. Functions of the Pseudomonas syringae avrRpm1 Gene During Disease Resistance and as a Virulence Factor in Arabidopsis
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We are interested in determining molecularly how plants sense and respond to pathogens. Recently, we and others have cloned several of the plant genes controlling recognition of pathogens. Products of these "disease resistance" genes, "recognize" the product of a particular pathogen's "avirulence" gene, and this recognition leads to disease resistance. If the plant lacks the correct resistance gene function, or if the pathogen lacks the corresponding avirulence gene, disease results. We use Arabidopsis as a model plant and a bacterial pathogen, *Pseudomonas syringae*, which can cause leaf spot disease on a variety of crop plants. Our DOE funded research concerns specifically how the avirulence gene signal triggers disease resistance. In the last year we have demonstrated that expression of the bacterial pathogen avirulence gene product inside the plant cell can trigger disease resistance when the plant expresses the correct resistance gene. We have also shown that in plants which do not express the resistance gene that this bacterial protein can trigger a delayed, symptom like reaction. We additionally know that some plant genotypes express this reaction and others do not. This result implies that the bacterial protein can act as a plant-genotype dependent toxin, as expected in it has a role in causing disease on susceptible plants. This is consistent with our genetic analysis of this particular bacterial avirulence gene. We also have shown that this protein is localized to the plant cell membrane fraction. We are now using Arabidopsis genetics to identify these novel plant targets.

144. Map-Based Cloning of the ENHANCER GENE SILENCING 1 Locus Which Enhances the Silencing of a Foreign Gene in Arabidopsis
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The ability to introduce foreign genes into plants has changed the way that agricultural technologists can approach crop improvement. However in many cases, genes introduced into plants do not behave as we would expect due to gene silencing. Gene

silencing inhibits expression of some foreign genes and simultaneously inhibits expression of plant genes with strong structural similarity to an introduced gene. It is unpredictable occurring spontaneously in late stages of development or appearing after two genetically manipulated plants are mated. The mechanism that leads to gene silencing is completely unknown. It does not cause permanent mutation of the affected genes since they can become reactivated. Silencing may be caused by a system of gene regulation that scientists could only begin to see working when they started to introduce genes into organisms. We have identified mutations in two Arabidopsis genes that affect the mechanism of gene silencing. These mutations enhance the silencing of a highly expressed foreign gene so that it occurs in embryonic tissues in all plants. The goal of this project is to clone one of these genes called *enhancer of gene silencing1* in order to better understand the mechanism of gene silencing. Taking advantage of the powerful Arabidopsis genome database and the materials collected by the Arabidopsis Stock Center in Ohio, we have mapped the gene to a small interval (4 centimorgans) on the first chromosome. The Stock Center provided us with large Arabidopsis DNA clones that overlap to cover the interval. We are now using sequences from the overlapping clones to reduce the interval in which the gene must be to a region small enough to introduce into plants. The egs1 gene will finally be identified by its ability to restore the expression of the silenced gene when introduced in the mutant.

145. Bioenergetic and Physiological Studies of Hyperthermophilic Archaea
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The objectives of this project are to examine the biochemical characteristics of several hydrolytic enzymes produced by heterotrophic, hyperthermophilic archaea and relate the function of these enzymes to particular physiological and bioenergetic roles. The study focuses on two members of the Thermococcales, *Thermococcus litoralis* (T_{opt} 88°C) and *Pyrococcus furiosus* (T_{opt} 98°C). Both are obligately anaerobic heterotrophs which grow in the presence or absence of reducible sulfur compounds. A novel, intracellular protease (Pfpl) has been isolated from *P. furiosus* which was shown to exist *in vitro* in several functional homomultimeric forms of an 18.8 kDa subunit, including a trimer, a hexamer and a dodecamer, with the largest assembly having the highest specific activity. The gene corresponding to Ppfl is homologous to putative proteins in organisms ranging from *Escherichia coli* to *Homo sapiens*. An affinity-based chromatography method has been developed to facilitate the purification of Pfpl homologs from a variety of cells and organisms. Its function is being examined in relation to archaeal proteosome in *P. furiosus* which was also isolated and characterized biochemically. Bioenergetic and physiological studies focusing on *T. litoralis*, both in pure and co-culture with *Methanococcus jannaschii*, are also underway. *T. litoralis* was shown to produce an extracellular polysaccharide in continuous culture which was composed mainly of mannan and which was involved in biofilm formation. Energetics issues related to biofilm formation, sulfur utilization and co-culture dynamics are under investigation.

146. Transcription Factors in Xylem Development
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Trees form large amounts of wood, or secondary xylem, which is important in both ecological and economic terms. Wood includes a large fraction of the global biomass, and is therefore a key factor in the global carbon cycle as well as the basis of a major industry. Trees are not convenient experimental organisms, and much of the progress made in plant molecular biology using model species has yet to be transferred to woody plants. We are working to unravel the genetic mechanisms that control formation of wood, using as a guide the understanding of gene regulation that has emerged over the past several years from study of model plant and animal species. Proteins called transcription factors are known to control gene expression during both plant and animal development, as well as modulating responses of organisms to environmental stimuli. We are working to characterize transcription factors found in pine xylem, in order to begin defining the regulatory networks that control wood formation. We began working with the Myb class of transcription factors, and identified two different Myb proteins expressed in xylem. Interactions of these proteins with different DNA sequences is being analyzed to test the hypothesis that they control different aspects of wood formation. We are also working with proteins of the Myc family, another major class of transcription factors known to control growth and development in both plants and animals. Members of other families of transcription factors have also been identified, and will be investigated further in the coming year.

147. The Role of Multiple Transcription Factors in Archaeal Gene Expression
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Examination of the archaeal transcription system has indicated that these organisms, while procaryotic in nature, possess an eucaryal transcription apparatus. *In vivo* analysis of the *Haloferax volcanii* transcription system indicated that promoter and termination signals in this organism are similar to those used by the eucaryal RNA polymerase II system, and this organism was found to contain genes encoding eucaryal-like transcription factors, TBP and TFIIB. Unexpectedly, we observed that *H. volcanii* has three TBP and six TFIIB genes, an unprecedented redundancy in these proteins that has not been observed in other Archaea or Eucarya. Gene expression studies also indicated that one of these transcription factors, TFIIB2, is specifically induced during heat shock. This has led to the proposal that this organism uses alternative transcription factor pairing in regulating gene expression. Current studies are focused on determining the role of TFIIB2 in regulating the heat shock response. *In vivo* approaches are being employed to examine the sequences involved in the heat shock regulation of the TFIIB2 gene and to identify regions of this protein that are needed to specifically direct heat shock transcription. *In vitro* approaches will also be used to examine the interaction of this protein with the TBP-DNA complexes and other transcription factors and these studies will aid in the development of an *in vitro* transcription system for this organism. Long range goals include the analysis of the remaining transcription factors and their possible roles in differential gene expression.

148. Biosynthesis of Hydrocarbons and Other Wax Components
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Pea leaf particulate preparation obtained by a sucrose discontinuous gradient centrifugation was solubilized with 0.2% octyl glucoside and subjected to protein fractionation. The purified decarbonylase obtained by this procedure requires phosphatidyl choline for enzymatic activity. SDS-PAGE showed a subunit molecular weight of 67 kDa. Antibodies prepared against this protein showed a single immunologically cross-reacting protein at 67 kDa in solubilized particulate preparation. Anti-Rubisco antibodies did not cross-react with this protein showing that the 67 kDa protein is not derived from cross linking of the large and small subunits of Rubisco. Furthermore, the antibodies prepared against the purified decarbonylase did not cross-react with Rubisco but it inhibited the decarbonylase activity of the purified enzyme. Further characterization of the enzyme and cloning are in progress.

149. Transmethylation Reactions During Methylotropic Methanogenesis in Methanosarcina barkeri
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We have been characterizing enzymes and genes involved in methanogenesis from methylotrophic substrates. These pathways are comprised of highly regulated enzymes and are significant routes of methane formation in natural environments. Methylated amines and thiols are converted to methane with methyl-coenzyme M (CoM) as an intermediate. We have reconstituted CoM methylation from growth substrates like trimethylamine (TMA), monomethylamine (MMA), dimethylsulfide (DMS), and methylmercaptopropionate (MMPA) with highly purified proteins. CoM methylation from methylotrophic substrates involves members of at least two different families of proteins. Each family member display specificity for one or more of the methylotrophic substrates. Generally, each substrate is used by a specific methyltransferase to methylate a substrate specific corrinoid binding protein, which is then demethylated by a substrate specific CoM methylase.

For example, MMA is converted to methyl-CoM via a 29 kDa corrinoid protein (MMCP) which acts only in this pathway. This is one of four known homologous corrinoid proteins involved in the metabolism of methylotrophic substrates which bind their cofactor with the corrinoid binding motif also found in methionine synthase. MMCP is methylated with MMA by a 52 kDa MMA specific methyltransferase (MMAMT). CoM methylation with methyl-MMCP is then catalyzed by a homologue of methylcobamide:CoM methyltransferase, termed MT2-A, which is specific for methylamine utilization. Similarly, TMA:CoM methyl transfer can be achieved with another corrinoid protein (TCP) which appears to be methylated by a TMA specific methyltransferase (TMAMT). Methyl-TCP

can be demethylated by either of two MT2 homologues, MT2-A or MT2-M. Currently, we are isolating the enzymes mediating CoM methylation with dimethylamine (other than MT2-A), isolating the enzymes responsible for activation of MMCP, completing the sequencing of the gene clusters encoding the MMA and TMA methyltransferases, and studying the interaction of MMCP with its two methyltransferases when in the methylated and unmethylated states.

Methanogenesis from DMS or MMPA occurs via a single methylthiol:CoM methyltransferase. *M. barkeri* induces this enzyme during growth on acetate. This 480 kDa protein is composed of one corrinoid binding subunit (homologous to TCP, MMCP, and methionine synthase) tightly bound to larger subunit (homologous to MT2-A and MT2-M methylcobamide:CoM methyltransferases). Unlike the MMA and TMA dependent CoM methylation enzymes, methylthiol:CoM methyltransferase mediates its reaction solely with the corrinoid protein and MT2 homologue, and we are investigating how the corrinoid is methylated by substrate. In addition, we are characterizing the enzyme and gene from other species of methylated thiols utilizing *Methanosarcina* to ascertain if a single two subunit enzyme with similar substrate specificity is employed for growth on both substrates.

150. Structure and Regulation of Methanogen Genes
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Methanobacterium thermoautotrophicum (Mt) generates energy by using hydrogen to reduce carbon dioxide to methane, and assimilates these same gaseous substrates plus nitrogen or ammonium ions and inorganic salts into biomass. The goal of this project is to understand how these catabolic and anabolic pathways are coordinated and regulated. Many steps in methanogenesis are catalyzed by two or more functionally-equivalent enzymes or isoenzymes, and hydrogen availability, the source of energy for Mt, regulates which of these alternative enzymes are synthesized. The molecular mechanisms by which Mt cells sense the concentration of dissolved hydrogen, and communicate this information into gene regulation, are currently under investigation. The rate of hydrogen dissolution in a fermentor sparged with hydrogen plus carbon dioxide depends on the mixing rate. Changing the mixing rate therefore results in methane gene activation and inactivation, and provides a very simple procedure that is being used to manipulate methane gene regulation *in vivo*. As the sequence of the entire Mt genome is now known, hybridization probes are immediately available and are being used to identify and quantitate all methane gene transcripts, with two dimensional gel electrophoresis used to identify and quantitate their encoded polypeptides. In parallel studies, purified Mt RNA polymerase, with recombinant Mt TATA-binding protein and TFIIB are being used to reconstitute and investigate *in vitro* the molecular basis of hydrogen-dependent methane gene transcription.

151. Regulation of Alternative CO2 Fixation Pathways and Control of Redox Status of Photosynthetic Microorganisms
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In addition to serving as a primary source of carbon, CO_2 is used by photosynthetic microorganisms as an important electron sink for the dissipation of excess reducing power. Recent studies in our laboratory have shown that molecular controls for the expression of several processes for redox balancing are tightly coupled in nonsulfur purple bacteria. This control is particularly manifest when the major CO_2 assimilatory route (Calvin-Benson-Bassham or CBB pathway) is abolished after inactivating structural genes that encode essential enzymes of the CBB path. Thus, when grown in the presence of organic carbon, these CBB negative organisms must employ other electron sinks to balance their redox poise. We have found that one way these organisms cope is to derepress nitrogenase and the *nifHDK* genes under these growth conditions. This allows the cells to reduce protons to molecular hydrogen, despite the presence of ammonia in the growth media. Derepression of *nif* in *Rhodobacter sphaeroides* and growth on molecular nitrogen is controlled by a global two-component signal transduction system which also regulates expression of the *cbb* system in this organism. Current studies are directed at elucidating molecular links that exist between the *cbb* and *nif* control circuits in *R. sphaeroides* and related organisms. Our studies also encompass molecular-based studies on an alternative CO_2 fixation route, the reductive tricarboxylic acid (or RTCA) cycle. For these studies, a genetically tractable and fast-growing moderate thermophile, the green sulfur photosynthetic bacterium *Chlorobium tepidum*, has been employed. The RTCA pathway is a route also employed by many extremophiles that use CO_2 as their sole source of carbon. *C. tepidum* thus appears to be an excellent model system for studies of this major CO_2 assimilatory route. All of the key enzymes have now been purified to homogeneity, including ATP-citrate lyase, pyruvate synthase, -ketoglutarate synthase, phosphoenolpyruvate carboxylase, as well as several redox carriers. The two synthase enzymes are iron-sulfur proteins that require a specific reduced ferredoxin isolated from *C. tepidum* and other necessary cofactors for activity. The purified proteins are also being employed to isolate the structural genes so that we may determine how the RTCA pathway is regulated at the molecular level. Isolated structural genes will also be employed to prepare recombinant proteins to better relate their structure to specific catalytic events.

152. Summer Workshop: Molecular Basis, Physiology, and Diversity of Microbial Adaptation
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Graduate programs specializing in microbial biology have declined in recent years, greatly reducing the opportunities for advanced training in this important research area. As one mechanism to rebuild interest in microbial physiology and to train new scientists in the area, we have developed a four week summer course focusing on the theme "Mechanisms of Microbial Adaptation". In 1997 the course contained both lecture and laboratory components targeted to senior level graduate students and industrial scientists who desire an in-depth introduction to research in the area of microbial physiology. Current topics on the mechanisms of microbial adaptation were presented in a 20 lecture seminar series given by the course faculty and 12 distinguished invited guest lecturers. Topics included: adaptive response of microorganisms in fermentations, global response mechanisms, regulation of microbial autotrophism, carbon metabolism in methanogens, and biodegradation and bioconversion by microorganisms. The accompanying laboratory exercises illustrated current practical problems in microbial physiology and introduced the students to modern experimental techniques and conceptual approaches. These experiments provided the students with experience in fermentation technology, characterization and quantitation of proteins and metabolic intermediates, enzyme activity measurements and basic kinetic analyses, genetic approaches for the identification of regulated genes, and the anaerobic techniques needed to purify oxygen-sensitive proteins. A second inherent goal of the course was to establish a significant interactive community of research scientists in the field. Informal presentations by the guest lecturers were used to stimulate discussions between students and faculty about both research problems and career development. A detailed description of the course is available at our web site: http://www.biosci.ohio-state.edu/~microbio/summer/course.html

153. Energetics and kinetics of syntrophic aromatic degradation
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The anaerobic bacterium "*Syntrophus acidotrophicus*" strain SB in coculture with the methanogen, *Methanospirillum hungatei*, degraded benzoate to a minimum threshold concentration at which no further benzoate degradation occurred, even with extended incubation. The addition of sodium acetate but not sodium chloride affected the threshold value; increased acetate concentrations resulted in increased benzoate threshold concentrations. Cocultures that initially contained 2.5 mM benzoate with either 0 or 10 mM acetate degraded benzoate to very low threshold concentrations, less than 500 nM. In contrast, cocultures with 30 or 60 mM acetate added, degraded benzoate to threshold concentrations of 4.4 uM and 1.5 mM, respectively. The final partial pressure of hydrogen in the methanogenic cocultures ranged from 1.5 to 3.0 Pa. At threshold, the Gibb's free energy change under physiological conditions was still favorable, ranging from -14 to -21 kJ per mole. These data show that substrate degradation thresholds do occur under methanogenic conditions and that the threshold values can be substantial under conditions where hydrogen is low if acetate is allowed to accumulate to high concentrations. Similar free energy values obtained under sulfate-reducing and methanogenic conditions when the threshold is reached support the hypothesis that substrate thresholds are thermodynamically controlled and that substrate degradation may be precluded even though the free energy change is still favorable.

154. The Structure of Pectins from Cotton Cell Walls
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In work over the last eleven years on pectins from cotton suspension culture cell walls we have concluded that pectins are based on only two backbone structures. One is a (1-4) linked galacturonan and the other is a repeated disaccharide of galacturonic acid and rhamnose (rhamnogalacturonan).
The homogalacturonan based pectin regions fall into three common types. 1) True homogalacturonans with varying degrees of methyl esterification, 2) Rhamnogalacturonan II, a homogalacturonan section with complex sidechains clustered such that it can be isolated as an ~ 5,000 Dalton fragment after endopolygalacturonase digestion of cell walls, and 3) xylogalacturonan, a homogalacturonan backbone with frequent single xylose substituents linked to the galacturonic acid residues.

The goal of the project is to learn more about the structure, and if possible, function of the various pectic regions and to determine which regions are covalently attached to each other. We also want to determine how they are linked to each other by isolating and characterizing junction zones between them.

To help achieve these goals we are developing highly sensitive methods for carbohydrate analysis and sequencing using capillary electrophoresis, enzyme and chemical digestions, and spectroscopic methods.

In addition, we are comparing the pectins of cotton cotyledons to those of the tissue cultures in case culturing induces major modifications in the structures.

155. Characterization of the Genes Involved in Nitrification
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Nitrification is a bacterial process in which ammonia is oxidized to nitrate. In croplands fertilized with ammonia or urea-based compounds, oxidation of ammonia by these bacteria leads to a loss of available N. Our research is directed at characterizing the genes involved in nitrification with the intention of providing a basis for supporting development of biotechnology aimed at controlling nitrification. The genes in *Nitrosomonas europaea* which code for ammonia monooxygenase (AMO, which catalyzes the oxidation of ammonia to hydroxylamine) and hydroxylamine oxidoreductase (HAO, which catalyzes the oxidation of hydroxylamine to nitrite) were cloned and sequenced. The genes for AMO (amoA and amoB) are each present in two copies. The gene for HAO (hao) is present in three copies. We recently developed a protocol for the transformation of *N. europaea* based on electroporation and homologous recombination of introduced DNA. These observations and developments provide the basis for our proposed experiments. The following specific objectives are proposed: 1) Complete the physical mapping and characterization of the multiple copies of the genes coding for ammonia monooxygenase (amoA and amoB) and hydroxylamine oxidoreductase (hao). 2) Determine which individual copies of amoA, amoB, and hao are dispensable, which are functional and which are expressed. 3) Determine if the individual copies of amo and hao are expressed differentially under various physiological conditions. 4) Identify genes near amo and hao and determine the function of these genes. These studies are expected to provide a better understanding of the genetic basis for ammonia oxidation to nitrite.

156. Genetic Analysis of Chloroplast Translation
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The accumulation of components of the photosynthetic apparatus in the appropriate ratios and within the correct cell types requires the regulated expression of chloroplast genes. This project concerns the control of chloroplast protein synthesis by nuclear-encoded factors. The project is focused on *crp1*, a nuclear gene in maize, mutations in which disrupt the translation of the *petA* mRNA and the metabolism of *petB* and *petD* mRNAs. Thus, the *crp1* gene activates the expression of a small set of chloroplast genes. We recently cloned the *crp1* gene by taking advantage of the Mu transposon tag. The sequence of a full length cDNA clone revealed no significant homologies with any proteins of known function. The cDNA was used to generate recombinant protein and Crp1p antisera. Immunolocalization studies revealed that Crp1p is localized in the chloroplast stroma and is not associated with envelope or thylakoid membranes. The role of *crp1* in activating translation will be investigated by defining the step at which translation is blocked in mutant chloroplasts, by determining whether Crp1p is a component of an RNA binding activity, and by assessing the activities of purified Crp1p in binding RNA, processing RNA, and in activating translation *in vitro*. Towards this last end, we have established methods for the preparation of pea and maize chloroplast extracts that are active in translation. Understanding the mechanism by which *crp1* activates translation will further our understanding of chloroplast translation in general. A related project concerns the role of endonucleolytic processing of polycistronic RNA precursors in chloroplasts. In *crp1* mutants, the absence of monocistronic *petD* mRNA correlates with a loss of *petD* translation, suggesting that *petD* is inefficiently translated in a polycistronic context. Preliminary results of enzymatic RNA structure analyses suggest that the *petD* start codon is sequestered in secondary structures when it is in the polycistronic context but not when it is in the monocistronic context. Experiments in progress will further explore the role of endonucleolytic RNA processing in controlling the translation of chloroplast mRNAs. Finally, a screen is in progress for new *Mu*-induced mutants with defects in the translation of chloroplast mRNAs.

157. Biochemical Genetics of Lignin Degradation by Phanerochaete chrysosporium
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The objective of this research is to further our understanding of the lignin degradative system of the basidiomycete *Phanerochaete chrysosporium*. We are using a variety of approaches to characterize the enzymes and genes involved in this process.
We are studying the structure and mechanism of lignin and Mn peroxidases (LiP and MnP) via spectroscopic, kinetic, and bioorganic methods and in collaboration by crystallography. Using our homologous expression system that produces recombinant MnP in *P. chrysosporium*, we have isolated and characterized by kinetic, spectroscopic, and crystallographic methods several mutants of the Mn binding site of MnP. We also have characterized mutants of Phe 190, an amino acid residue in the heme cavity of MnP, which stabilizes the protein. A similar expression system is being developed for LiP. Finally, we are studying the enzymes and genes involved in lignin degradation by the while rot fungus *Dichomitus squalens*.
We are also characterizing an intracellular quinone reductase which is involved in the further metabolism of monomeric quinones produced as lignin degradation products. We have isolated and sequenced cDNA and genomic clones encoding this enzyme from *P. chrysosporium*, and a variety of studies, including homologous expression, are being carried out to determine the enzyme's mechanism and its role in lignin degradation.

158. Cellobiose dehydrogenase and B-glucosidase from Phanerochaete chrysosporium: Effect on cellulose hydrolysis, cloning, and characterization
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Cellobiose dehydrogenase (CDH) is an extracellular hemoflavoenzyme produced by cellulose-degrading cultures of fungi such as *Phanerochaete chrysosporium*, *Sporotrichum thermophile*, *Trametes versicolor*, and *Coniophora puteana*. In this study, we plan to purify and characterize the thermostable CDH from *Sporotrichum thermophile*. We will also study the interaction of CDH and -glucosidase from *P. chrysosporium* with cellulose using electron microscopy.

159. The Characterization of Psychrophilic Microorganisms and Their Potentially Useful Cold-Active Glycosidases
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Our objective is to characterize psychrophilic microorganisms and to discover, purify, and study novel cold-active glycosidases. Cold-active glycosidases with high activities at low temperatures can be used for low temperature conversions of saccharides in plant biomass, whey, etc. into fuels or fermentation media. Comparisons of cold-active enzymes with their higher temperature counterparts will yield insight into structural features involved in thermal stability. Our goals are to complete the characterization of three -galactosidase isozymes cloned from one Arthrobacter strain, to isolate new psychrophiles producing cold-active glycosidases with even lower temperature optima, to clone and sequence genes for selected enzymes, and to purify cold-active glycosidases for biochemical studies and comparisons. We have completed the analysis of the three Arthrobacter genes. One encodes a lacZ family subunit, except the enzyme has an optimum about 20°C below that of the *Escherichia coli* enzyme. The second gene helped define a new class, which we designated lacG. The third gene encodes a small 52 kDa subunit with homology to the lysosomal acid -galactosidases found in human and mouse. In addition, we have sequenced a new lacZ-like gene from an Antarctic isolate that encodes a -galactosidase with an optimum between 10 and 20°C. The significant similarity of this gene (67%) to the one we examined earlier will permit a careful examination of the amino acid changes that might contribute to the lower temperature optimum of this new enzyme.

160. Energy Transduction in Green Sulfur Bacteria
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The long-term objective of this research program is to develop a detailed understanding of the structure, function and biogenesis of the light-energy transduction apparatus found in green sulfur bacteria. Secondary goals are to analyze their transcriptional machinery, to develop methods for genetic analyses, and to understand the relationship of green sulfur bacteria to other eubacteria. Genes encoding eight (of ten) *Chlorobium tepidum* chlorosome proteins have been cloned, sequenced, and characterized. These eight proteins have been overproduced, purified, and used to raise polyclonal rabbit antisera. CsmA, CsmB, CsmC, CsmD, and CsmE have been shown to be surface-exposed components of the chlorosome envelope. Experiments to demonstrate the location(s) of CsmH, CsmI, and CsmJ are in progress. The latter two proteins are similar in their N-terminal domains, and each protein contains a cysteine sequence motif suggesting each will contain a [2Fe-2S] cluster. EPR measurements on CsmI after *in vitro* reconstitution has confirmed the presence of the anticipated Fe-S cluster. EPR studies of chlorosomes isolated under anaerobic conditions are in progress. Finally, we have recently characterized the genes encoding sigma factors of RNA polymerase in *Chlorobium tepidum* and *Chloroflexus aurantiacus*. Like cyanobacteria, *C. aurantiacus* produces multiple Group 1 and 2 sigma factors. The SigC protein is expressed at similar levels in cells grown photoheterotrophically under anaerobic conditions or heterotrophically under aerobic conditions. Interestingly, the SigA protein is expressed at a significantly higher level in cells grown under aerobic conditions than under anaerobic conditions.

161. Biophysical and Molecular Mechanisms Controlling Plant Cell Growth
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Plant cell enlargement is controlled by wall stress relaxation. This process depends on cell wall structure and the combined activity of wall "loosening" and "rigidifying" proteins. We are using biophysical and molecular approaches to elucidate the nature of wall relaxation and its control of cell enlargement. (A) We have developed a biophysical model of cell wall expansion based on the thermodynamics of hydrogen-bonded polymer networks. Many of the actual properties of expanding walls are described by this model, which predicts that the ratio of cellulose to hemicellulose is a key structural feature that determines wall yield threshold. This prediction will be tested by in-vitro assays of wall extension. (B) The nature of wall rigidification is being examined in several ways. Specific bonds in the wall will be broken or formed by use of chemical and enzymatic agents, and wall extensibility then assayed. The hypothesis that pectin methyl esterase is a significant wall rigidifying enzyme is being tested by a combination of biochemical and molecular approaches. We are also attempting to identify endogenous wall rigidifying activities in protein preparation made from cell walls. (C) The dynamics of cell enlargement is being explored by use of a novel pressure chamber to modulate turgor and record the resulting growth responses. (D) Wall extension properties are being analyzed in brassinolide-deficient mutants (Arabidopsis, pea), which the intention of uncovering the mechanism by which this steroid-like hormone modulates plant form. These studies will deepen our understanding of plant cell wall expansion and its control of plant growth.

162. Elongation Factor 1Alpha and the Plant Cytoskeleton
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The cytoskeleton, and in particular its microtubule (Mt) component, participates in several processes that directly affect growth and development in higher plants. Normal cytoskeletal function requires the precise and orderly arrangement of Mts into several cell cycle and developmentally specific arrays. One of these, the cortical array, is notable for its role in somehow directing the deposition of cellulose, the most prominent polymer in the biosphere. It is therefore important to acquire information regarding the molecules which regulate Mts within the different arrays. Experimental data has been obtained to suggest that plant cells use calcium, in the form of a Ca++/calmodulin complex, to affect the dynamics of Mts within the cortical array. Owing to the importance of Ca++ as a regulatory ion in higher plants we are probing for a putative Ca++/Mt transduction pathway which may serve to integrate Mt activities

within the growing and developing plant cell. We have found that elongation factor 1-a behaves as a Mt associated protein serving to both stabilize, and bundle, microtubules in vitro. Both of these effects are modulated in vitro by Ca++/calmodulin which unbundles and destabilize Mts. Our working hypothesis is that similar events occur in vivo and we are currently undertaking a structure/function approach to identify the regions on this MAP that are involved in these activities.

163. Enzymology of the Pathway for Acetate Conversion to Methane in Methanosarcina thermophila
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Several enzymes identified in the pathway for the fermentation of acetate to methane and carbon dioxide have been purified from *Methanosarcina thermophila* and are under investigation utilizing biochemical, biophysical and molecular genetic approaches. The mechanisms of acetate kinase and phosphotransacetylase are under investigation utilizing site-directed mutagenesis to identify active site residues and mass spectrometry to identify covalent intermediates. Efforts are underway to determine the crystal structures of both enzymes. Crystals of acetate kinase have been obtained in collaboration with Miriam Hasson which diffract to beyond 1.7 angstrom resolution. Procedures are being developed for the overproduction of subunits of the CO dehydrogenase/acetyl-CoA synthase (CODH/ACS) complex in *Escherichia coli and M. thermophila*. Methods are also being developed for overproduction of a protein suspected to be involved in maturation of subunits of the CODH/ACS complex. Electron paramagnetic resonance and Mossbauer spectroscopies, in collaboration with Drs. Steve Ragsdale and Kristene Surerus, have been used to characterize the FMN and iron-sulfur centers of a novel iron-sulfur flavoprotein (Isf) discovered in *M. thermophila*. The results support a role for Isf in donating electrons to cytochrome b in the membrane-bound electron transport chain.

164. Primary Electron Transfer in Green Photosynthetic Bacteria
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Green sulfur bacteria (Chlorobiaceae) represent a group of strictly anaerobic and obligately phototrophic procaryotes which use sulfide as the immediate electron donor in photosynthesis, and have a Type I photosynthetic reaction center which resembles Photosystem I of green plants and cyanobacteria. Much is known about the proteins, pigment organization and energy transfer in light-harvesting system of green sulfur bacteria, but the details of the redox cofactors and the kinetics of the acceptors in the photosynthetic reaction centers are poorly understood. The major gap in our knowledge involves the identity and the kinetic properties of intermediate acceptors participating in electron transfer from the primary pair $P840^{*}$ A_0- to the terminal acceptors F_A/F_B. In this work, we will identify those factors in Chlorobiaceae research which has hindered progress, and we offer experimental strategies to overcome these limitations. Our goal is to measure the optical and EPR spectra of the electron acceptors, and to resolve their kinetics properties. The premise is that the reaction centers of Photosystem I and Chlorobiaceae are similar in the identity of the electron acceptors but they differ considerably in the details of their function. Type I reaction centers are of considerable interest because they generate a long-lived reductant which has the thermodynamic capability to drive the half-cell reaction: $2H^{+} + 2e^{-} \rightarrow H_2$. The green bacterial reaction center combines the most desirable property of Photosystem I, the ability to generate a low potential reductant, with the polypeptide simplicity of a bacterium. Thus, a detailed understanding of the Chlorobium reaction center has the potential to contribute to a molecular device capable of generating molecular H_2, a clean, renewable source of energy.

165. Molecular-Genetic Analysis of Maize Starch Branching Enzyme Isoforms
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Starch is an important energy storage molecule used by all plants. Its synthesis involves the creation of branch points by a class of enzymes known as starch branching enzymes (SBE, EC 2.4.1.18). Biochemical studies have shown that three isoforms of SBE exist in maize endosperm, SBEI, IIa and IIb.

To fully understand the role of the SBE isoforms in starch biosynthesis, a comprehensive molecular-genetic dissection of the SBE gene family is underway. We are in the process of isolating cDNA and genomic DNA clones for all of the maize SBE family members and we will use these sequences to study the expression patterns and regulatory elements of each *sbe* gene. We will also identify maize lines with mutations in each *sbe* gene. To reach these Objectives, we are using a combination of conventional molecular techniques to isolate and study the genes, together with a reverse genetics transposon based approach to identify insertional mutants. Together, these lines of research will advance our knowledge of the importance of the SBEs in starch biosynthesis and in the life cycle of the plant. Knowledge of the regulatory elements which control *sbe* gene expression may be useful in engineering maize endosperm for the production of novel carbohydrate storage products for industrial feedstocks. The knowledge gained and the tangible products of this research (genes, cDNAs and novel germplasm) may also be useful in the production of starches with altered structure for use in industry.

166. Charcterization of Lignin and Mn Peroxidases from Phanerochaete chrysosporium
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The initial steps of lignin biodegradation by white-rot fungi are catalyzed by the lignin and Mn peroxidases. These enzymes were first discovered in the fungus *Phanerochaete chrysosporium* and have now been isolated from a large number of lignin-degrading fungi. Our research has been aimed at elucidating the role of these enzymes in lignin degradation. The enzymology of this process is complicated. The lignin and Mn peroxidases are isozyme families, expressed differentially in response to environmental conditions. These peroxidases are unique in their ability to oxidize substrates which other peroxidases cannot oxidize. The mechanism by which these enzymes act on lignin is not well understood; redox mediators have been proposed to be involved in oxidation of lignin by these enzymes. We have performed site-directed mutagenesis of these enzymes to study the nature of their substrate binding site and the basis of their reactivity. We have also developed a transformation system which is presently being used to generate antisense mutants. These studies are attempting to determine the role of these enzymes in lignin degradation.

167. Light Responses and Photoperiodism in Arabidopsis thaliana
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We have characterized several classes of mutants of *Arabidopsis thaliana* that show alterations in their responses to light. The *pef* mutants (*p*hytochrome/*e*arly *f*lowering) flower early under white light and exhibit elongated hypocotyls when grown under red or far-red light. Because of their selective insensitivity to red/far-red light, these mutants are believed to correspond to mutations in the phytochrome signalling system. A second class of mutants are the *rsp* mutants (*r*educed *s*ensitivity to *p*hotoperiod): these mutants exhibit an alteration in their timing mechanism such that they flower earlier than wild-type plants under certain short-day photoperiods. Furthermore, the *rsp* mutants show an alteration in the periodicity of the free-running rhythm for *CAB* gene expression. This alteration in the timing of two distinct physiological parameters suggest that the *rsp* mutants may correspond to lesions in a central component of the circadian clock. Another class of mutants we have characterized are the *elr* mutants (*e*nhanced *l*ight *r*esponse), which exhibit unusually short hypocotyls when grown under either red or blue light. As the *elr* mutants show no discernible phenotype when grown in the dark, these mutants (like the *pef* mutants) appear to correspond to lesions in a light-signalling pathway.

168. Structural basis of signal and energy transduction in plants
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The aim of this program is to train students in both molecular genetic and structural studies in plant science. Students are trained in graduate programs in Biochemistry, Chemistry, and Biology. These students pursue research in areas ranging from structural studies of plant enzymes involved in terpene biosynthesis to molecular genetic studies of signalling by the plant hormone ethylene.

Each year a retreat is held involving both local researchers and one or more internationally renowned scientists who address the students on molecular genetic or structural studies in plant science. By this mechanism students are being trained with an appreciation of both molecular genetic as well as chemical, biochemical, and biophysical approaches to research in plant science.

169. Membrane-Attached Electron Carriers in Photosynthesis and Respiration
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Our overall aim is the molecular characterization of the structure, function and biogenesis of membrane-associated cytochromes (cyt) that act as electron carriers in photosynthesis (Ps) and respiration (Res). We use the facultative photosynthetic bacteria of *Rhodobacter* species as a model system for these studies. The presence of a dual electron transfer (ET) pathway between the cyt *bc1* complex and the reaction center (RC) in Ps, and the cyt c oxidase in Res, was first revealed by our Ps- and Res-proficient mutants of *R. capsulatus* devoid of the soluble cyt *c2*. We have then demonstrated that the novel membrane-associated cyt *cy* is the molecular basis of this entirely membrane-confined ET pathway that functions both in Ps and Res growth conditions. Using an epitope-tagged and functional derivative of cyt *cy* we purified it for the first time, determined its physicochemical properties and its mode of attachment to the membrane. These studies established that cyt *cy* is composed of an "anchor," a "linker" and a "cyt c" domains, and demonstrated that its "anchor-linker" domains can also attach cyt *c2* to the membrane in a functional form. Our very recent measurements indicate that kinetic characteristics of the cyts *c2* and *cy* are very similar, except that the latter is not diffusion-limited during multiple turnovers. This finding strongly supports the presence of supercomplexes formed of the RC, the *bc1* complex and cyt *cy*. Unexpectedly, an homologue of cyt *cy* was also found in *R. sphaeroides*, its nucleotide sequence was completed, a mutant lacking it was obtained, and its role in electron transport is under study. Current work is now focused on defining the molecular differences between the cyt *cy* molecules of these two closely related *Rhodobacter* species which behave very differently in respect to their ability to use membrane-attached electron carrier cytochromes in photosynthesis and respiration.

170. Molecular and Genetic Analysis of Hormone-Regulated Differential Cell Elongation in Arabidopsis
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The aim of this project is to understand how plant hormones act coordinately to regulate the elongation of cells. Ethylene and auxin are known to modulate cell elongation in the hypocotyl and apical hook in etiolated seedlings. The hook is formed by asymmetric elongation of cells on opposite sides of the hypocotyl. For genetic studies, the apical hook provides an excellent model system to identify genes required for hormone-regulated growth since this structure is dispensable for plant viability. Mutants in Arabidopsis that exhibit altered patterns of differential growth in the hypocotyl have now been identified and include those lacking an apical hook or showing constitutive apical hook curvature. One of these genes, HOOKLESS1 (HLS1) a putative N-acetyltransferase, is required for apical hook formation and for normal expression of auxin response genes in the hypocotyl and apical hook. Interestingly, HLS1 transcription is regulated by ethylene, thus providing a link between the ethylene and auxin response pathways. This novel protein may regulate cellular responses to auxin by one of several mechanisms, such as by alteration of gene transcription, or a change in hormone sensitivity or transport. In order to further explore this connection, we are now identifying transcription factors that interact with the promoter of HLS1. Through the identification of additional mutants defective in differential growth and by characterization of their corresponding gene products, we hope to gain new insight into this little understood process. It is expected that the general mechanisms found to be important for controlling cell growth in this model system will also be utilized in other plants.

171. Biochemical Basis of YCF1-Dependent Vacuolar Glutathione-S-Conjugate Transport
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The *Saccharomyces cerevisiae YCF1* (yeast cadmium factor) gene was originally isolated according to its ability to confer cadmium resistance. *YCF1* encodes a 1,515 amino acid, cystic fibrosis transport regulator- (CFTR-) like ATP binding cassette (ABC)

transporter which is closely related to the human multidrug resistance-associated protein (MRP1 or GS-*X* pump) implicated in the resistance of a number of cancer cell lines to chemotherapeutic drugs. Recent experiments by our group demonstrate that YCF1 is both a functional and structural homolog of the mammalian GS-*X* pump. YCF1 participates in the MgATP-energized vacuolar uptake of *bis*(glutathionato)cadmium ($Cd.GS_2$) and a wide range of organic toxins after their *S*-conjugation with glutathione (GSH).

This program of research is concerned with several fundamental aspects of the mechanism of YCF1: (i) Its mode of interaction with GS-conjugates. We have shown that YCF1 is necessary for MgATP-energized vacuolar GS-conjugate transport but we have not yet determined if it directly interacts with these compounds. (ii) Its sufficiency for transport. Although the inferred structure of YCF1 is consistent with its direct participation in MgATP-energized GS-conjugate transport, it is not known if YCF1, alone, is sufficient. (iii) Its facility for transporting metal ions other than Cd^{2+} after their complexation with GSH. While the wild type *YCF1* gene is required for Cd^{2+} resistance, nothing is known of its involvement in the vacuolar sequestration of other GSH-complexable metal ions. (iv) Its regulation by phosphorylation. YCF1 is unusual in possessing a CFTR-like regulatory domain containing dibasic PKA phosphorylation motifs whose modification may modulate activity. (v) The necessity of the N-terminal extension unique to it and other members of this subclass of ABC transporters for substrate recognition and/or transport.

In view of the involvement of YCF1 in both heavy metal and organic toxin transport and its equivalence to MRP1 and the vacuolar GS-*X* pumps of plants, these studies are likely to provide fresh insights into the membrane transport phenomena associated with mammalian tumor biology and plant herbicide resistance, in particular, and heavy metal tolerance and bioremediation, in general.

172. Molecular Studies of B-D-Glucan Synthesis and Turnover in Cereals

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We continue our search in rice for genes that encode the Golgi-localized synthases of the (1AE3),(1AE4)–D-glucan (-D-glucan), a cell-wall polysaccharide unique to the cereal grasses. -D-Glucan is synthesized transiently during the expansion phase of cell growth and is hydrolyzed during cell differentiation once cells have ceased growing. The glucan is synthesized again in the endosperm walls of the grain. The -D-glucan constitutes a majority of the cell wall mass of the endosperm, whereas cellulose content is very low. Based on several enzymic properties which are shared by the synthases of -D-glucan *in vitro* in isolated Golgi apparatus and callose at the plasma membrane (Gibeaut, D.M., and N.C. Carpita, 1993, Proc.Natl. Acad. Sci USA 90, 3850-3854), we proposed that the genes that encode the cereal -D-glucan synthases are derived from ancestral cellulose synthase genes. We have sequenced five rice cDNAs with high homology to cotton and Arabidopsis CelA genes, each of which likely encode the cellulose synthase catalytic subunit. These rice cDNAs fall into three groups, and we are using a PCR-based approach to identify members of these three and other CelA-like genes from a genomic library of rice. Each of the cDNAs contains a hypervariable region between regions of high conservation that are predicted to encode highly conserved UDP-Glc binding domains of the deduced polypeptides. These regions are suitable for construction of gene specific probes, and experiments are designed to differentiate between cellulose synthase and -D-glucan synthase by differential expression in seedling and endosperm. Further proof will come from the immunolocalization of unique peptides of the -D-glucan and cellulose synthases at the Golgi or plasma membrane, respectively.

173. Ferulate-5-hydroxylase: requirements for expression and activity

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Lignin is a phenolic polymer that imparts strength and decay resistance to the plant secondary cell wall. In flowering plants, lignin is composed mainly of two aromatic monomers which differ in their methoxyl substitution pattern. These monomers are derived from ferulic acid and sinapic acid and give rise to guaiacyl- and syringyl-substituted lignin monomers, respectively. The balance between guaiacyl and syringyl units in lignin varies among plant species, within a given plant, and even within the wall of a single plant cell. Two cytochrome P450-dependent monooxygenases are required for the synthesis of lignin monomers, cinnamate-4-hydroxylase (C4H) and ferulate-5-hydroxylase (F5H). Ectopic overexpression of F5H in Arabidopsis under the control of the C4H promoter abolishes tissue-specific lignin monomer deposition and generates a novel lignin that is almost entirely comprised of syringyl units. These data demonstrate that the composition of the lignin polymer is dictated by the temporal and tissue-specific expression pattern of F5H. We have further shown that the CaMV 35S promoter fails to promote F5H gene expression in cells undergoing or providing precursors for lignification. The promoter of the C4H gene used in this study is far more efficient in this regard. Finally, we have shown that it is possible to genetically engineer plants to accumulate lignin that is highly enriched in syringyl residues. Thus, it seems possible to increase the syringyl content of crop species and trees, thereby generating lignins that are easier to digest or extract without detrimental consequences on agricultural performance.

174. Structure and Function of the Lumenal Proteins of the Photosystem II O2-evolving Complex in Cyanobacteria
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The primary objective of this project will be to determine the proteins in the cyanobacterium *Synechocystis* sp. PCC6803 that interact with the Mn-stabilizing protein (MSP) and help form the lumenal component of the PSII O_2-evolving complex. We have cloned the *psbU* gene (encoding the 12 kDa protein) and the *psbV* (encoding cyt C550) and have produced insertional mutations. We have also produced the double mutants *psbO psbU*, *psbO psbV* and we will analyze the characteristics of all of these mutations. We will be particularly interested in O_2-flash yields and fluorescence properties, as well as the growth characteristics of these mutants. We have also utilized a digital imagining spectrometer (DIS) to isolate a series of randomly-induced mutants of *psbO*. The DIS permits many colonies to be analyzed for fluorescence and we have utilized the high sensitivity of this instrument to obtain some interesting mutations. We will continue this analysis throughout the course of the year to determine which residues provide particularly interesting lesions in MSP. One goal is to determine the domains within MSP that give rise to specific categories of mutations.

We are also analyzing the characteristics of PSII in the unicellular, diazotrophic cyanobacterium *Cyanothece* sp. ATCC 51142. This strain demonstrates great heterogeneity in PSII organization throughout the N_2-fixing cycle, which we interpret as a means of downregulation of PSII. We will concentrate on factors which affect the oxidizing side of the photosystem, including the status of the lumenal proteins and factors which lead to the degradation of PSII.

175. A Molecular-Genetic Approach to Studying Source-Sink Interactions in Arabidopsis thaliana
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Sources (such as leaves) and sinks (such as roots and seeds) of carbohydrate interact in complex ways that play an important role in plant development and physiology as well as in determining crop yields. The mechanisms by which sources and sinks of carbohydrate interact are poorly understood, but are likely to involve the regulation of key genes by soluble sugar levels. Our research is focused on determining the role of sugar-regulated gene expression in plant development and on elucidating the molecular mechanisms by which this type of gene regulation occurs in plants. Towards this end, mutants that are defective in sugar-regulated gene expression have been identified in the model plant *Arabidopsis thaliana* and are currently being characterized. This work should lead to a better understanding of source-sink interactions, which is a pre-requisite to developing more rational approaches to improving crop yields.
The final aspect of our work is focused on characterizing fumaric acid accumulation in a wide variety of plant species. Our interest in this area stems from our discovery that fumaric acid accumulates to high levels in a number of plant species, including model plants like *Arabidopsis thaliana* and economically important plants like soybean. Fumaric acid is of interest because it can be used by plants to synthesize sugar and starch. Therefore, understanding fumaric acid accumulation could be critical to understanding sugar and starch metabolism in plants, a topic of great economic importance to agriculture.

176. Reconstitution of the Clostridium thermocellum Cellulosome
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Funding: $80,000 12 months

The *Clostridium thermocellum* cellulosome is a multicomponent cellulase complex. Its largest subunit, CipA (formerly called CelL), contains nine repeated domains (R's), each functioning as a binding receptor to various cellulosomal catalytic subunits, and a cellulose binding domain (CBD) in between the second and the third repeats. We have previously reported that CelS, an exoglucanase and the most abundant catalytic subunit of the cellulosome, forms a stable complex with CipA. Furthermore, binding of CelS to crystalline cellulose, as its activity toward the same substrate, is enhanced by CipA. Thus the activity of a cellulosomal exoglucanase subunit can be enhanced by the anchorage function of CipA.

In this work, we examined the effect of anchorage on the activity of CelD, an endoglucanase subunit of the cellulosome, using recombinant CelD (rCelD) and the CipA functional domains, R3 (the repeat next to CBD) and CBD/R3, expressed in Escherichia coli. rCelD formed a stable complex with CBD/R3 as analyzed by a gel-shift assay on a nondenaturing polyacrylamide gradient gel. Binding of rCelD to crystalline cellulose, as its activity toward both phosphoric acid-swollen and crystalline cellulose, was dependent on CBD/R3. These results indicate that the activity of an endoglucanase subunit of the cellulosome, as that of the exoglucanase subunit, is enhanced by the anchorage function of CipA. Such anchorage function may thus augment the potential endo-exo synergism in the cellulosome. The results shed more light into the structure of the cellulosome and are important for engineering the cellulase complex for biomass energy conversion.

177. Regulation and Function of Salicylic Acid Immediate-Early Induced Genes in Plant Disease Resistance
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Plant disease resistance is a phenomenon which, in part, requires the biosynthesis and perception of salicylic acid (SA) for the induction of defense gene expression. We have used differential display analysis of tobacco cell cultures to identify SA early response genes. We cloned fragments of 15 genes with this behavior and estimated the total number of SA early response genes at 30-60. The kinetics of induction and response to cycloheximide (CHX) treatment allowed classification of genes into four groups. Classes I-III are characterized by immediate-early responses, showing increased accumulation of mRNA within 30 minutes of SA treatment. Moreover, CHX did not block induction of these genes, indicating that latent cellular factors mediate the SA response. Class IV genes were induced more slowly, but still within 2-3 hr of SA treatment, and required protein synthesis for expression. Although identified in this study as SA-responsive genes, several could also be induced by other compounds. Two genes were characterized in more detail, including isolation of cDNA sequences and additional analysis of gene expression. Sequence analysis revealed that one is the previously identified Ethylene Response Element Binding Protein 1 (EREBP1), an ethylene induced transcription factor for basic Pathogenesis-Related (PR) genes, whereas the other, G8-1, is a novel sequence. *EREBP1* was found to be activated not only by SA, but also by auxin and methyl jasmonate. In contrast, *G8-1* was found to be strongly induced only by SA and its active analogs and was exquisitely sensitive to low SA concentrations. In addition, these and other genes were found to be activated at early times following TMV infection of resistant tobacco genotypes.

178. Corn Storage Protein - A Molecular Genetic Model
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Corn is largely used to produce animal protein. Therefore, livestock receives its protein from corn meal. The major proteins in corn meal are a family of proteins, called zeins, whose main function is to store amino acids in the seed. These proteins are encoded by a multigene family. The organization of this gene family in the genome and its regulation by various trans- and cis-acting mechanisms provide not only an opportunity to reach a deeper understanding of the regulation of gene expression, but also new tools and products for plant genetic engineering. Multigene families are also an example of genetic redundancy that has been far more difficult to tackle from a genetic and a molecular point of view. But recent advances in the manipulation of large DNA fragments has made it possible to make long-range physical maps. This has allowed us to map a tandem array of 22-kDa zein genes within 250 kilobases of genomic DNA. This array is divided into subclusters separated by about 60 kilobases containing mainly highly repetitive retroelements, the latter preventing us from forming a contiguous set of genomic clones. This was solved with DNA probes from a closely related cereal, Sorghum, whose repetitive DNA does not cross-hybridize to the corn genome, while its gene sequences do. Furthermore, the smaller genome size of Sorghum seems to correlate with shorter distances of gene sequences. Therefore, the synteny between a smaller and a larger cereal genome will facilitate new approaches to genome analysis.

179. Signal Transduction Pathways that Regulate CAB Gene Expression
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The process of greening, or chloroplast differentiation, involves the coordinate regulation of many nuclear- and chloroplast-encoded genes. The cues for the initiation of this developmental program are both extrinsic (e.g., light) and intrinsic (cell-type and plastid signals), but very little is known of the signaling pathways that regulate nuclear photosynthetic gene expression. The research program focuses on the genetic, biochemical, and molecular characterization of new *Arabidopsis thaliana* mutants in which a photoregulated promoter (CAB) is expressed aberrantly with respect to light, intrinsic developmental signals, and signals from the chloroplast. These genetic screens have allowed us to identify 18 new genes that play a role in the signal transduction pathways controlling photoregulated gene expression and chloroplast development in Arabidopsis. Specifically, we have obtained mutants in which CAB is expressed either: (1) in the light at abnormally low levels, defining positive regulators (9 genes); (2) in the dark at abnormally high levels, defining negative regulators (3 genes); or (3) in the presence of inhibitors that eliminate chloroplast function, defining an intracellular signaling pathway from chloroplasts to the nucleus (6 genes). During the past year, we have cloned three genes, mutations which result in lower CAB mRNA levels in response to light. One of the mutations lies in phytochrome B and two of the genes encode proteins that are localized to the chloroplast envelope. These genes thus represent a step toward elucidating how information is transmitted from photoreceptors and organelles in the cytoplasm to regulatory factors in the nucleus.

180. Regulation of the floral homeotic gene AGAMOUS
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The importance of flowers for human sustenance cannot be overstated. Not only are they organs of plant reproduction, but their immediate products are fruits and seeds, including cereal grains, which provide the majority of calories consumed by humans. Thus, understanding how plants regulate the formation of flowers is likely to have long-ranging practical applications. The long-term objective of this project is to understand the mechanisms underlying the patterned differentiation of cells within the flower. The experimental system is the model plant *Arabidopsis*, which is particularly suited for genetic and molecular studies because of its short generation time, small genome, and facile DNA-mediated transformation. The specific problem to be investigated is how a global regulator, *LEAFY*, which encodes a master switch for the initiation of individual flowers, controls the region-specific expression of a homeotic gene, *AGAMOUS*, whose expression and activity are limited to the central region of the developing flower. A special gain-of-function allele of *LEAFY* has been created, whose phenotype suggests not only that the activity of LEAFY protein is regulated during normal flower development, but also that the interaction between *LEAFY* and *AGAMOUS* is direct. To study this interaction in more detail, an interrelated set of experiments is proposed. First, *AGAMOUS* cis-regulatory sequences mediating the genetic interaction with *LEAFY*, which encodes a new type of nuclear protein, will be identified. In parallel, it will be determined whether LEAFY protein can bind in vitro to *AGAMOUS* sequences. *LEAFY*-response elements will then be used in genetic screens in yeast for other factors involved in *AGAMOUS* regulation. The proposed experiments will advance our understanding of how widely expressed, early acting genes participate in the regulation of later-acting genes that are expressed only in restricted regions of an emerging organ primordium. Apart from contributing to the general knowledge about pattern formation, the detailed analysis of *LEAFY* might reveal new general insights into transcriptional regulation during development, since this gene encodes anew type of transcriptional regulator.

181. Regulating expression of cell and tissue-specific genes by modifying transcription
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Rice tungro bacilliform virus (RTBV) is one of two viruses which together are responsible for causing rice tungro disease. In previous studies we characterized the viral genome, including the major promoter, and several proteins encoded by the virus, including the reverse transcriptase (RT) and viral protease that is responsible for processing the viral polyprotein. The major promoter regulates

accumulation of the genome length transcript: the transcript serves as template for production of viral DNA (by the reverse transcriptase) and as messenger RNA for synthesis of viral structural and non-structural proteins.
In transgenic rice plants the RTBV promoter is expressed in elements of the vascular system, including primary xylem and primary phloem; this is in agreement with the observation that RTBV infection is limited to vascular tissues in plants. After identifying the DNA sequences (*cis* elements) to which rice nuclear proteins bind, we determined the importance of each *cis* element for expression of the promoter in transgenic plants. One of the *cis* elements, referred to as Box II, was used in a yeast one plasmid system to isolate the protein that binds the element. The protein, referred to as RF2a, was cloned and expressed in *E. coli*, and following purification and renaturation was shown to bind Box II as homodimer; we also determined that a heterodimer was likely responsible for regulation *in vivo*. RF2a is active in an *in vitro* transcription assay system derived from rice cells, and in such assays promotes expression from the RTBV promoter but not selected other promoters. Transgenic plants which contain reduced levels of RF2a due to transgenes that produce (-)sense RNA derived from the RF2a gene exhibit abnormal vascular development in seedlings, but not in adult plants. Research in progress is directed to characterizing sub-domains of RF2a that regulate expression of the promoter, and to develop mutants of RF2a that restrict infection by RTBV.

182. Membrane Targeting of P-type ATPases in Plant Cells
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Our long term goal is to understand how integral membrane proteins are targeted to different subcellular locations. This knowledge is needed to engineer plants with new solute transport systems. Such systems include pumps for heavy metal uptake and salt efflux, which may be useful for bioremediation and salt tolerance, respectively. The general focus of our research is on a family of ion pumps called P-type ATPases. We are carefully examining the subcellular localization of two proton pumps (AHA2 and AHA10), two calcium pumps (ACA2 and ACA3) and a putative copper pump (AXA2) in a model plant system, Arabidopsis. Our approach is to assess localization using immunocytology, cell fractionation, and imaging of pumps fused to a green fluorescent protein. Our primary focus is on an unusual pump, ACA2, which is most similar to a plasma membrane-type calcium pump in animals, but is distinct since it is localized to an internal membrane. We are testing three hypotheses using mutant pumps expressed in transgenic plants. 1) Does the N-terminal domain contain targeting information? 2) Does the tonoplast become a "default" destination when normal targeting information is deleted? 3) Can ACA2 be retargeted to the plasma membrane by making chimeras with different regions of a plasma membrane proton pump?

183. Nuclear Genes Regulating Translation of Organelles mRNAs
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We have identified a set of proteins that bind with high specificity and affinity to the 5' untranslated region (UTR) of the chloroplast psbA mRNA. Binding of these proteins to the 5' UTR is required for ribosome association of the mRNA and hence for translation of the downstream coding region. We have also identified the RNA elements contained within the 5' UTR of the psbA mRNA that are recognized by this protein complex and that are required for high levels of translation. Cloning and characterizing of the RNA binding proteins has revealed that one of these proteins is a member of the poly(A) binding protein family. Binding activity of this protein is regulated in response to light, resulting in light regulated translation within the chloroplast. Regulated binding is achieved by altering the redox status of the RNA binding protein by an associated protein disulfide isomerase. Protein disulfide isomerase are involved in the formation and reduction of disulfide bonds normally associated with protein folding. We have constructed chimeric genes containing the psbA 5' RNA elements and the coding region of either a bacterial luciferase gene or a single chain antibody gene. Both of these heterologous marker genes are translated at high levels within the chloroplast. These marker genes should allow us to precisely define the factors that are involved in translational regulation of psbA mRNA, and provide important insight into the molecular mechanism by which protein/RNA interactions trigger translational activation of specific chloroplast mRNAs.

184. Regulatory role of ANT in organ initiation and growth
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Genetic and molecular experiments in *Arabidopsis thaliana* have contributed much to our understanding of the specification of organ identity in flowers. However, many other processes in the development of a flower, including the determination of organ number and position, the development of particular cell-types, and the acquisition of the final appearance (shape and size) of floral organs, remain poorly understood. One gene that affects both the number and the appearance of floral organs is *ANT*. *ant* mutants possess a reduced number of sepals, petals, and stamens, narrow floral organs; and ovules without integuments. Thus, ANT is required for proper organ growth and in some cases, organ initiation. The common bond between these different processes is cellular proliferation, and we propose that ANT is a critical factor controlling the number of cell divisions in floral organ primordia. In addition, ANT may also influence the plane of cell division. This is suggested by the effects of *ant* mutations on the shape of floral organs. ANT is thought to function as a transcription factor due to the presence of two AP2-domains, which have been shown to bind DNA in related proteins. In addition, ANT contains several putative activation domains and a potential nuclear localization signal. We will examine the proposed ability of ANT to regulate cell division by characterizing transgenic plants containing high levels of *ANT* expression. These transgenic plants produce larger flowers than wild-type plants, providing strong evidence that ANT is a key regulator of cell division in flowers. In order to understand how ANT acts in this process, we will identify and characterize functional domains of the protein. This will include investigating the function of the putative DNA-binding and transcriptional activation domains. Knowledge of the DNA binding site and activation properties of ANT will be important in future studies to identify target genes. The proposed experiments will be extremely useful in understanding the regulatory mechanisms used by ANT in the control of plant growth and organ initiation.

185. Regulation of Alcohol Fermentation by Escherichia coli
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We are studying the regulation of alcohol and lactate fermentation in the facultative anaerobe *Escherichia coli*. The protein encoded by the adhE gene expresses both alcohol and acetaldehyde dehydrogenase activities and is responsible for ethanol synthesis. Its N-terminal half is homologous to other aldehyde dehydrogenases and its C-terminus to Fe-activated alcohol dehydrogenases. The mechanism of anaerobic induction of adhE has been characterized by means of gene fusions. The buildup of reduced NADH during anaerobic conditions plays the major role in regulating the adhE gene. The regulatory gene, adhR, has been cloned and preliminary work indicates that the AdhR protein binds to DNA in the adhE upstream region. We are presently isolating and characterizing regulatory protein mutants which result in high expression of adhE even when the level of NADH is low. Other mutations resulting in constitutive expression of the adhE gene, even in air, are known. The upstream regions of several of these have now been sequenced, confirming that they are in the adhE promoter region. The ldhA gene, encoding the fermentative lactate dehydrogenase has also been cloned and sequenced. Although it is induced by low pH, the ldhA gene does not respond to the same regulators as other known acid-inducible genes. The mechanism of ldhA induction in response to acidic conditions and growth phase is being investigated using ldhA-lacZ gene fusions. The ldhA upstream region is presently being dissected by PCR to locate the promoter and regulatory sites.

186. Nodulation Genes and Factors in the Rhizobium-Legume Symbiosis
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We study the bacterium *Rhizobium meliloti* and its legume host, alfalfa, which interact to form symbiotic root nodules. Within nodules, *Rhizobium* carries out nitrogen fixation; the plant thus can grow and produce protein without requiring nitrogen fertilizer. Because fertilizer manufacture requires intensive fossil fuel use, improvement of biological nitrogen fixation is an important strategy for maintaining productive agriculture as fossil fuels become scarce. We have defined and cloned the nodulation (*nod*) genes in *Rhizobium* that cause specific host plants to develop root nodules, providing a site for bacterial colonization and nitrogen fixation.

The *nod* genes direct the synthesis of a chemical signal emitted by the bacteria that influences plant development, and we have recently defined early stages in the plant response to the bacterial signal. These early responses include calcium oscillation, a mechanism for signal transduction known in animal cell biology. What other bacterial and plant genes are involved in the symbiosis? We have begun several new projects to define these. We have purified the DNA for the symbiosis plasmid of *R. meliloti* for the purpose of creating two libraries of random DNA fragments, to be used to determine its sequence. We have also used a set of four independent strategies to discover the genes involved in invasion of the plant by the bacteria. In future work, we hope to define the plant genes involved in symbiosis, through generation of a library of expressed plant genes, and through classical and molecular genetics of the model legume *Medicago truncatula*.

187. Plant Recognition of Bradyrhizobium japonicum Nod Signals
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We are studying the agronomically important, nitrogen-fixing symbiosis between *Bradyrhizobium japonicum* and soybean. *B. japonicum* infects soybean roots and induces the formation of a nodule, a new organ, in which the bacteria reside. Organogenesis of the nodule is induced by substituted lipo-chitin molecules synthesized by the products of the bacterial nodulation genes. The potency of these molecules, as well as their high specificity, suggests the presence of plant receptors. However, past work has shown that nod signal perception is complex requiring at least two, structurally distinct lipo-chitin signals. These data argue for the presence of two plant receptors with differing structural specificity. In collaboration with other research groups, we have isolated cDNA clones of a nod signal binding protein from soybean. The encoded protein shows significant similarity to a family of lectin-like proteins that possess NTP phosphatase activity. A major objective of our work is to characterize this soybean lectin and ascertain its role in nodulation. In addition, we are pursuing a biochemical approach to identify nod signal binding activity in soybean tissue. The purpose of this work is the biochemical isolation of a nod signal receptor. Our eventual goal is to elucidate the complete signal pathway involved in the soybean nodulation response. Detailed knowledge of legume symbioses is important for the possible extension of biological nitrogen fixation for energy conservation.

188. Molecular Mechanism of Light Regulated Protein Transport Across the Nuclear Envelope
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The compartmentalization into nuclear and non-nuclear compartments affords a straightforward means by which eukaryotic cells limit the access of transcriptional regulators to their nuclear target genes. However, little is known about the cellular mechanisms that regulate nuclear uptake and depletion of proteins. The Arabidopsis COP1 protein, a light inactivated repressor of transcription essential for plant development, can confer a light regulated nuclear localization pattern on the fused reporter protein beta-glucuronidase (GUS). We have determined by genetic complementation that COP1, when fused to GUS or green fluorescent protein (GFP), can function normally in Arabidopsis. We are now using protein tagging with the reporter proteins GFP and GUS to delineate domains in the COP1 protein that are essential and sufficient for light regulated nuclear accumulation. In addition, we are determining why and how COP1 localizes to specific subnuclear domains (speckles), reminiscent of diverse animal proteins involved in chromatin-mediated processes. First, specific mutations are introduced into the COP1 protein. The mutant proteins are then examined for their ability to undergo light regulated nuclear localization. The localization data are correlated with data on the proteins' ability to function properly during plant development. We have identified a constitutive nuclear localization signal in COP1 and are now defining a domain required for cytoplasmic retention of the protein under light conditions as well as a domain that targets COP1 to subnuclear speckles.

189. Novel Biomaterials: Genetically Engineered Pores
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An extensive collection of nanometer-scale pores is being constructed by remodeling -hemolysin (HL), a protein secreted by the bacterium *Staphylococcus aureus*. The single polypeptide chain of 293 amino acids self assembles to form heptameric pores in membranes ~14 Å in internal diameter. By examining the properties of mutant and chemically modified HLs, and the three-dimensional structure of the pore a working model for assembly has been devised. Monomeric HL first binds to lipid bilayers and then aggregates to form a heptameric prepore complex. Finally, the open pore is formed when subunits in the complex undergo a cooperative conformational change, involving both the central glycine-rich loop and the N-terminus of the polypeptide. The central loop lines a section of the transmembrane channel in the fully assembled pore. Continuing studies of assembly and function are allowing point mutagenesis, combinatorial mutagenesis and targeted chemical modification to be used to create pores with new properties. For example, triggers and switches have been built into HL to gain control over the opening and closing of the pores. Inputs that actuate the reengineered molecules can be biochemical in nature (activation by specific proteases), chemical (modulation of activity by covalent and non-covalent interactions with small molecules) and physical (activation by light). We have also begun to build pores based on the HL barrel, by *de novo* design. The new pores will be used to confer novel permeability properties upon materials such as thin films, which might then be used as components of energy conversion and storage devices, selective electrodes, electronic devices, and ultrafilters.
*Funded collaboratively with Division of Materials Sciences (DOE).

190. Regulation of Development and Nitrogen Fixation in Anabaena
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The regulation of development and pattern formation is a fundamental biological problem. During development cells must collect and integrate external and internal information with a regulatory network that controls gene expression. This regulation is important because the decision to commit some cells to a terminal developmental pathway affects the overall fitness of a multicellular organism. The nitrogen-fixing filamentous cyanobacterium *Anabaena* sp strain PCC 7120 was chosen as a simple model of development and pattern formation. *Anabaena* PCC 7120 reduces atmospheric nitrogen to ammonia in highly specialized terminally differentiated cells called heterocysts. When grown on dinitrogen, a one-dimensional developmental pattern of single heterocysts separated by approximately ten vegetative cells is established to form a multicellular organism composed of two interdependent cell types. This is an important model system because of the multicellular growth pattern, the distinct phylogeny of cyanobacteria, the suspected antiquity of heterocyst development, and the contribution of fixed nitrogen to the environment. Several genes involved in heterocyst development have been identified but no integrated regulatory network has yet emerged. The goal of this project is to understand the signaling and regulatory pathways that commit a vegetative cell to terminally differentiate into a nitrogen-fixing heterocyst. Several genes identified by us and by others were chosen as entry points into the regulatory network. These genes will be studied and, as part of our long term goals, used to identify additional genes in the pathway. The specific objectives are: 1) The *hetS* gene, a 54-bp ORF that suppressed heterocyst differentiation, will be studied. A nearby gene, *hetY*, encodes a small nucleotide-binding protein and it will be tested for involvement in heterocyst development. 2) An *ntcA* knockout mutant has the unexpected phenotype of being Het- and may be required for *hetR* expression. *ntcA*'s position in the regulatory pathway will be determined. 3) *hetC* encodes an ABC transporter and a knockout mutant is Het-. The hypotheses that *hetC* exports a differentiation inhibitor or imports a putative cell density pheromone will be examined. 4) A new *Anabaena* sigma factor gene was recently cloned with PCR in our lab. Reverse genetics will be used to test this gene for a role in development and efforts to identify new sigma factor genes will be continued. 5) Finally, new genes in the developmental pathway that are epistatic to the genes described above will be identified. Extragenic bypass suppressors of the *hetS, hetN, ntcA, and hetC* mutations can be selected for by growth on nitrogen-free media.

191. Regulation of Chloroplast Division in Higher Plants
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The long term goal of this research is to understand how chloroplast division is regulated in higher plants. This is important because the number and size of chloroplasts influences many functions of plants including photosynthesis. In addition, activation of chloroplast division is an early event in the differentiation of mesophpyll cells. Therefore, isolation of genes regulating chloroplast division may provide insight into the early steps in chloroplast and mesophyll cell differentiation. The proposed research will focus on a chloroplast division mutant that exhibits a dramatic decrease in chloroplast number and an increase in organelle size specifically in mesophyll cells. The gene identified by this mutation, *CDM1*, has been isolated by map-based cloning. Chloroplasts in mutant mesophpyll cells are either very large or relatively small. This type of division phenotype is also observed in some bacterial cell division mutants that are impaired in the ability to select the cell midpoint for cell division. This possible parallel between chloroplast and bacterial cell division will be examined in this study. Additional insight into the function of CDM1 will be obtained by

characterizing CDM1 localization in cells and by searching for proteins that interact with CDM1, screening for second site suppressor mutants of *cdm1*, and determining the developmental and cell type specific expression of *CDM1*.

192. Mutants of the Legume Medicago truncatula Defective in Root Hair Development and Infection by Rhizobium
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A major focus of research in our laboratories is to isolate and understand the function of plant genes that control early interactions between *Rhizobium meliloti* and *Medicago truncatula*. One aspect of this research involves defining cellular, physiological and genetic determinants of rhizobial infection and nodule initiation. The experiments described in this project involve detailed phenotypic and molecular-genetic characterization of two classes of nodulation mutants that are defective in rhizobial infection and/or induction of nodule primordia. In addition to their nodulation phenotypes, both classes of mutants are pleiotropic for aspects of root and/or root hair development. Class I mutants are exemplified by the mutant *dmi* (doesn't make infections), which does not become infected by the *Rhizobium* symbiont or by mycorrhizal fungi. In addition to the absence of infections, the *dmi* mutant does not develop root hair curling in response to *Rhizobium*. Instead, upon inoculation with *Rhizobium*, *dmi* root hairs loose polar growth and become bulbous. We hypothesize that *dmi* can perceive one or more rhizobial signals, but may be defective in later parts of the signal transduction pathway, preventing cellular events or gene induction required for infection and root hair curling. Our working hypothesis is that the isotropic growth phenotype of *dmi* is associated with the loss of polar organization of actin microfilaments. Class II mutants are exemplified by the mutant *pdl*, where the number of infections is similar to wild type, but all infections are arrested in the root epidermis. The *pdl* mutant is pleiotropic for excessive root hair proliferation, the absence of organized nodule primordia, and short, unevenly thickened roots. *pdl* appears to be defective in control of cell shape and enlargement, perhaps due to defects in microtubule orientation and/or altered perception or production of an ethylene signal. Genetic analyses indicate that *dmi* and *pdl* represent recessive mutations at different loci. Through extensive screening of multiple seed bulks, we have identified eighteen additional mutants with *dmi*-like (class I) or *pdl*-like (class II) phenotypes. The focus of this proposed research is the detailed phenotypic and genetic characterization of these mutants, including the initiation of map-based cloning of the responsible genes.

193. Molecular, Genetic, and Biochemical Analysis of Cellulose Biosynthesis in Arabidopsis thaliana
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We have been continuing the use of the multidisciplinary approach in the area of structure, biochemistry, genetics, and molecular analysis for our studies of cellulose biosynthesis in *Arabidopsis thaliana*. *Vigna radiata* has been used as a pilot research organism for development of suitable biochemical protocols. For the first time, a complete separation of *in vitro* (1,3)– and (1,4)–glucan synthase activities has been achieved in mung bean using non-denaturing electrophoretic conditions. Several polypeptides are common to cellulose and callose activities, and some polypeptides very specific for each separated fraction. Particles with the "rosette" organization are exclusively associated with cellulose assembly, while in callose synthesis, smaller particles never aggregated into the "rosette" structures. Similar solubilization conditions and native gel electrophoresis were tested with *A. thaliana* membrane fractions. The enzymes and product bands were successfully separated. Identification of proteins involved in cellulose synthesis is in progress. Recent application of isoelectric focusing in combination with native gel electrophoresis allowed for the first time exclusive synthesis of cellulose *in vitro* from mung bean enzyme fractions. We plan to apply the same conditions to *A. thaliana* to produce fractions active in cellulose assembly for kinetic studies, antibody production, sequence analysis and TEM observations. Using a sequence motif for processive -glycosyl transferases we have identified an EST from *A. thaliana* which could be a cellulose synthase. We anticipate a combined methodology will enable purification of the proteins and eventual isolation of the genes involved in cellulose biosynthesis and its regulation.

194. Phosphorylation of Plant Protein Synthesis Initiation Factors
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Our goal is to identify plant protein synthesis factors that may be phosphorylated and determine their potential as sites of regulation for plant protein synthesis. The phosphorylation of eIF-2a in mammals and yeast and the phosphorylation of cap-binding protein in mammals both play a critical role in the control of protein synthesis, gene expression and signal transduction in mammalian cells. The complexity of the regulation translation by phosphorylation of mammalian cap-binding protein and eIF-2a, underscores the need for greater understanding of translational control mechanisms in plants. The unique presence of two forms of the cap-binding protein in plants (p26 of eIF-4F and p28 of eIF-(iso)4F) offers an excellent system to study the role of phosphorylation of cap-binding proteins in translational control of plants.

We propose to determine the phosphorylation state of initiation factors from wheat by *in vivo* labeling wheat sprouts with [32P]orthophosphate and establish which initiation factors, particularly eIF-2a, eIF-4A, eIF-4B and the subunits of eIF-4F and eIF-(iso)4F, are (or are not) phosphorylated. The effects of growth and heatshock on the phosphorylation state of the initiation factors will also be determined.

The completion of the goals will open an area to be explored by both *in vitro* and *in vivo* methods. Of particular interest will be what kinases/phosphatases will be found to be involved in regulation and their roles in gene expression.

195. Ferredoxin-Linked Chloroplast Enzymes
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The ferredoxin-binding site of spinach nitrite reductase has been partially mapped using differential chemical modification of the enzyme and of the enzyme/ferredoxin complex with arginine- and lysine-modifying reagents. Two arginine residues, Arg 375 and Arg 556, and one lysine residue, Lys 436, are protected by ferredoxin against labeling by phenylglyoxal and pyridoxal-5'-phosphate, respectively. Positive charges are present at these three positions in all ferredoxin-dependent nitrite reductases for which sequences are available, suggesting that these amino acids are directly involved in electrostatic binding of ferredoxin to the enzyme. Characterization of the ferredoxin-dependent spinach glutamate synthase after treatment of the enzyme with the tryptophan-modifying reagent N-bromosuccinimide, suggests that at least one (and perhaps two) tryptophans are directly involved in electron transfer from reduced ferredoxin to glutamate synthase. Experiments with site-specific ferredoxin mutants, using a cyanobacterial ferredoxin as a homolog for spinach ferredoxin, have established the importance of a negative charge at the position equivalent to Glutamate 92 in spinach ferredoxin for efficient electron transfer from ferredoxin to spinach glutamate synthase. The midpoint potential of the regulatory disulfide/dithiol couple of spinach chloroplast phosphoribulokinase (PRK), an important regulatory enzyme of the carbon fixation pathway, was shown to be -295 mV at pH 7.0. Oxidation-reduction titrations of PRK mutants confirmed that Cysteine 16 and Cysteine 55 form the regulatory disulfide.

196. High-Throughput Technologies for Functional Analysis of Archaeal Genomes
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This work focuses on the development of high-density DNA microarrays for two methanogenic archaea, *Methanococcus jannaschii* and. *Methanobacterium thermoautotrophicum*. In this one year program we will lay the foundation for high-throughput array construction that will be essential for subsequent functional genomic studies. The specific aims of this project are as follows: 1. To design primers to each predicted open reading frame (ORF) in *M. jannaschii* and *M. thermoautotrophicum* that will allow the amplification of a unique target sequence. This target will represent the corresponding coding region on a complete genomic chip. 2. To amplify each target sequence from *M. jannaschii* and *M. thermoautotrophicum* and verify that these PCR products are the

expected DNA fragment. 3. To establish a relational database that will track the production of target DNAs and the nucleotide sequence used to represent each ORF.

197. Tandem-pore outward-rectifying K+ channels: Molecular partners of the proton ATPase in membrane potential regulation
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Ion and nutrient transport across the plasmalemma of living cells is governed by membrane potential. The goal of this project is to provide a comprehensive understanding of the bioenergetic principles that influence membrane potential in plants and fungi. Several lines of evidence indicate that two distinct transport proteins, the outward-rectifying K^+ channel and the H^+-pumping ATPase, set the limits of membrane voltage and maintain negative cell polarity in these organisms. Our recent cloning of the yeast outward-rectifying K^+ channel, TOK1, lets us test this hypothesis directly by investigating the molecular, biophysical, and biochemical properties of the channel protein and by characterizing its interaction with the yeast H^+-ATPase. To determine the factors that regulate TOK1 activity, we are conducting two-electrode voltage-clamp recording and patch-clamp recording of the channel expressed in *Xenopus laevis* oocytes. We are investigating the voltage-dependence of channel activation and deactivation along with the regulatory effect of extracellular monovalent ions, cellular pH, and cytosolic second messengers. To evaluate TOK1 function *in vivo*, we are disrupting the coding sequence of the TOK1 gene in wild type yeast and in the pma1-105 hygromycin B-resistant mutant yeast strain that has altered membrane potential and K^+ channel activity. By these means, the role of this channel in cell physiology can be analyzed and its interaction with other yeast transport proteins may be determined. Finally, to assess the broader merits of these experiments in plant and fungal physiology the electrophysiological and pharmacological properties of the outward-rectifying K^+ channel(s) from a closely-related fungus, *Neurospora crassa*, and the flowering plant *Arabidopsis thaliana* are being compared with those of TOK1. This research will elucidate the role of K^+ channel currents in maintaining electrical homeostasis and pave the way for a better understanding of membrane transport phenomena.

198. Arabidopsis Genome Sequencing Using Random Shotgun Sequencing of BAC Clones
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The objective of this program is to produce high quality, annotated genomic sequence within the framework of the international *Arabidopsis* Genome Initiative (AGI). We have focused our initial efforts on BAC clones from chromosome II, submitting fully annotated sequence to GenBank when each clone is complete (over 1.3 million base pairs to date), as well as releasing preliminary 1sequence for those BAC clones in progress (over 1 million base pairs to date). In addition, we have developed a high throughput protocol for generating BAC end sequences which provide a powerful resource for both mapping and selection of minimally overlapping BAC clones. Initially we focused on chromosome II-associated clones, but recently switched to a genome wide strategy in collaboration with the French Genome Center. We have produced over 6,000 end sequences from two different BAC libraries, and it is expected that the sequence for each end of 22,000 BAC clones will be completed in the coming year. The combination of the BAC end sequence database and the production of high quality finished sequence will facilitate the completion of the genome sequence and will make a critical contribution to the worldwide plant biology community.

199. Enzymology of Acetone-Butanol-Isopropanol Formation
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Several species of *Clostridium* produce acetone, butanol and isopropanol (solvents), which are important industrial chemicals and fuel additives. Commercial production of acetone and butanol by fermentation began with the starch-based Weizmann process using *C. acetobutylicum*. After the 1930s, *C. acetobutylicum* was replaced by newly isolated clostridia that produced high levels of solvents from sugars. Using DNA reassociation and other tests, *C. beijerinckii* and two other *Clostridium* species were identified as

the organisms used in industrial solvent production from sugars. *C. beijerinckii* does not require an acidic pH for solvent production, and some strains produce isopropanol in addition to acetone. We have been using *C. beijerinckii* in our study with an aim of establishing mechanisms and strategies for regulating the expression of solvent-production genes and the flow of metabolites. The long-term goals are to prevent the degeneration of strains, to regulate the product ratio, and to prolong the solvent-producing phase of growth. Our present study has an emphasis on characterizing the aldehyde and alcohol dehydrogenases that convert butyryl-CoA to butanol under different conditions, on determining the structure and organization of solvent-production genes in *C. beijerinckii*, and on studying the control of expression of the solvent-production genes. The organization of genes (*ctfA*, *ctfB* and *adc*) for acetone formation and the promoter regions differ between *C. beijerinckii* and *C. acetobutylicum*, suggesting mechanistic differences in the regulation of expression of solvent-production genes in the two species.

200. Structural Domains in NADPH: Protochlorophyllide Oxidoreductases Involved in Catalysis and Substrate Binding
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Chlorophylls are major components of the energy absorption and transduction apparatus in all photosynthetic organisms. In vascular plants, chlorophyll biosynthesis is regulated in part at the level of protochlorophyllide (Pchlide) to chlorophyllide (Chlide) catalyzed by NADPH: protochlorophyllide oxidoreductase (POR). POR is completely dependent upon light for its activity. In dark-grown seedlings, POR accumulates along with its substrates, Pchlide and NADPH, in stable ternary complexes in the prolamellar bodies of the etioplasts. Illumination of POR ternary complexes with stoichiometric amounts of light quanta result in the trans-reduction of the C17-C18 double bond in the D ring of Pchlide and the formation of the Chlide product. The action spectra for the enzyme closely matches the absorption spectra for Pchlide indicating the Pchlide is the primary light-absorbing species involved in the reaction. We are interested in understanding the mechanism of light-dependent Pchlide reduction and the structural determinants in POR proteins required for substrate (NADPH and Pchlide) binding, catalytic activity, and proper intraorganellar assembly within the plastid. We have generated and characterized a series of site-directed and clustered charged-to-alanine scanning mutants of the POR from PEA and have identified potential residues and domains required in the enzyme for Pchlide photoreduction. In addition we have located some of the structural factors required for proper association with the plastid membrane. Work is currently underway to define in considerably more detail the effects of various mutations on the kinetic properties (i.e., Km for substrate or cofactor, turnover rate, etc.) of the enzyme and to refine our analysis of the structural factors required for enzyme function. We are particularly interested in clarifying the role of the various conserved cysteine residues as well as the role of the C-terminal region of the POR protein in forming the active site pocket or in binding/stabilizing the substrate. We are also working towards solving the three-dimensional structure of the protein by using X-ray crystallography. These studies should provide significant information on one of the most crucial biosynthetic steps in the development of photosynthetic organisms.

201. Membrane Function in Lipid Mutants of Arabidopsis
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Our investigations of the biochemistry of wild-type and mutant plants have provided us with new information about the enzymology and regulation of lipid metabolism in higher plants. More importantly, our isolation and characterization of *Arabidopsis* mutants laid the groundwork for important discoveries about the role of membrane lipids in the cell biology and physiology of plants as well as the means to clone genes that encode fatty acid desaturase enzymes. Recently, the scope of our work has been expanded by the isolation and cloning of desaturase genes from the model nematode *Caenhorabditis elegans*. The many genetic tools available in *C. elegans* means that it may be possible to develop this worm as an additional model for studying the relationship between lipid composition and membrane function.

202. Regulation of Terpene Metabolism
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Terpenoid oils, resins and waxes from plants are important renewable resources with a range of pharmaceutical, agricultural and industrial uses. The objective of this project is to control terpenoid production by targeting regulatory steps for genetic engineering to increase the yields and expand the types of terpenoid natural products that can be made available for commercial exploitation. Model systems used are (+)-camphor metabolism in sage and (-)-menthone metabolism in mint. Developmental studies indicate that the yield of these terpenoids is controlled by the balance between biosynthetic and catabolic capacity. Experiments utilizing antibodies directed against, and cDNAs coding for, key metabolic enzymes indicate that regulation of terpene biosynthesis resides primarily at the level of gene expression, that several compartments within the secretory gland cells (plastids, endoplasmic reticulum and cytosol) participate in terpene biosynthesis, and that the bulk of terpene production occurs at the early stages of gland development. Two genes have been selected for overexpression in transgenic plants to increase terpene yield, and the experiments to test this possibility are in progress. Experiments are also underway to examine flux control within, and communication between, the cytosolic mevalonate (acetyl CoA-dependent) pathway and the plastidial non-mevalonate (pyruvate/glyceraldehyde-3-phosphate-dependent) pathway to the central precursor isopentenyl diphosphate. The cDNA encoding the first dedicated enzyme of the non-mevalonate pathway (catalyzed by 1-deoxy-*D*-xylulose-5-phosphate synthase) has recently been isolated from mint.

203. Carbon Metabolism in Symbiotic Nitrogen Fixation

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Nitrogen fertilizer is an important input for increasing crop yield because nitrogen deficits often limit plant growth. However, producing nitrogen fertilizer is expensive and energy intensive and excess fertilizer can increase nitrate in groundwater and soil acidity. Some plants are able to obtain nitrogen through symbiotic associations with nitrogen-fixing bacteria in which the plant exchanges carbon compounds for ammonia produced by the bacteria. Enzymes of the plant and bacterial tricarboxylic acid (TCA) cycles are at the center of this exchange, generating energy, reductant and biosynthetic intermediates from the catabolism of photosynthate. We are investigating the genetics and biochemistry of symbiotic carbon metabolism in both soybean and alfalfa. Our immediate goal is to define the role of the decarboxylating leg of the TCA cycle, a series of reactions needed to synthesize amino acid precursors that may also be required to generate energy and reductant for nitrogen fixation. We are investigating TCA cycle mutants, including those with defects in citrate synthase, isocitrate dehydrogenase and oxoglutarate dehydrogenase, and are developing conditional TCA cycle mutants to probe the relationship between the TCA cycle and nodule development and metabolism. Recent studies suggest that rhizobia use the decarboxylating leg of the cycle much more in the symbiosis with alfalfa than they do when associated with soybean. The difference may be related to the form of nitrogen exported from the nodules or with the way in which energy is generated by the bacteria.

204. The Energy Budget for Steady-State Photosynthesis

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The photosynthetic apparatus must balance its output of reducing power (NADPH) and phosphorylation potential (ATP) to match the biochemical demands of the plant. In addition, the input of excitation energy into the reaction centers must be regulated to prevent photodamage, particularly of photosystem II (PS II). The way in which control of energy balance is linked to regulation of photosynthesis in living plants is not completely understood, though several possible mechanisms have been proposed. These proposals include changes in the H^+/e^- ratios for photosynthetic electron transfer, changes in the partitioning of electron flow through linear and cyclic pathways, and a variable bypass of the coupling between H^+ efflux and ATP synthesis (proton slip). It is generally agreed that the primary mechanisms for down-regulating photosynthesis involve decreasing the efficiency of exciton transfer to PS II via modification of its associated antenna system. However, it is not clear how this is coupled to the control of chloroplast energy balance, particularly under conditions where the availability of $NADP^+$ limit linear electron transfer. Confounding these issues are uncertainties in the steady-state stoichiometries of H^+/e^- for linear and cyclic electron transfer pathways and of H^+/ATP for the chloroplast ATP synthase. Resolution of these issues is essential for understanding the overall energy budget of photosynthesis, its regulation and response to environmental changes, and is therefore important for efforts to increase crop plant ranges. Past attempt to address these questions have been limited by the lack of non-invasive probes for specific photosynthetic partial reactions in the steady-state. To address this, we have developed instrumentation and techniques that allow us to non-invasively measure, in intact plants and isolated chloroplasts, the steady-state fluxes of electrons through PS I, PS II and the cytochrome b_6f complex as well as coupled fluxes of protons into the lumen. We propose to use these techniques along with gas exchange and biochemical assays to compare light-driven fluxes of electrons and protons with substrate uptake and product formation under steady-state conditions designed to alter the relative biochemical demands for ATP and NADPH. These comparisons will allow us to assess the extents of linear and cyclic electron transfer as well as proton slip and fluxes of electrons to alternate sinks. They will also yield estimates of

relative H^+/e^- ratios and H^+/ATP ratios for steady-state photosynthesis. We will then extend these studies to intact plants under conditions where the biochemical demands for ATP and NADPH are expected to vary, e.g. using leaves of varying developmental stages with different levels of nitrate reduction and by varying light intensities and the concentrations of CO_2 and O_2.

205. A New Perspective on Phenoxy Radical Coupling Reactions In vivo and Phenylpropanoid Pathway Regulation
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Lignification, suberization and lignin-forming systems in plants are exquisitely orchestrated yet poorly understood biological processes involving phenolic coupling, and account for up to 40% of all organic carbon in living systems. The first example of control of phenolic coupling was described this year, in our discovery of dirigent proteins. The dirigent protein is thought to function by trapping and orienting free-radical intermediates in such a way as to engender both regio- and stereoselective coupling. In this first case of a presumed new class of proteins, only (+)-pinoresinol is obtained from E-coniferyl alcohol, rather than diffusion-controlled non-specific coupling. The dirigent protein has no homology with any other protein of known function, nor does it possess oxidative capacity to generate the free-radical species from coniferyl alcohol. Free-radical generation involves one-electron oxidizing enzymes, such as laccase. The gene encoding the dirigent protein has been cloned, and the recombinant functional protein obtained using a Spodoptera/baculovirus system.

Studies are now directed towards establishing the existence of other members of this class of protein, with particular attention being placed upon lignification. Emphasis is being placed upon determining how preferential formation of the beta-O-aryl linkage within native lignin polymers occurs, and how this is controlled at the protein level.

Related studies have established that during active phenylpropanoid metabolism in lignifying plants, suberizing tissues and fungi, the ammonium ion released during phenylpropanoid metabolism is reassimilated via GS/GOGAT to regenerate glutamate, which is used as amino donor for phenylalanine regeneration and hence completion of the nitrogen cycle.

208. An Advanced Course in Plant Biochemistry
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Like much of biological research, plant biology has flourished and undergone a rapid increase in scientific discovery, which has contributed to the many applications that have become part of the radical advances occurring in the field of biotechnology. The enormous success of molecular genetic techniques have had a profound effect on plant biology and attracted many established as well as younger investigators into research on plants. While many general aspects of metabolism and biochemistry are common to all biological organisms, most universities teach biochemistry from a medical point of view. As a result, there is a lack of knowledge in the training of most biologists about the unique biochemical aspects of plant systems. Few universities are large enough to support the number of faculty needed to keep at the forefront of plant biochemistry advances. By periodically offering a special summer course in plant biochemistry that includes lectures and demonstrations by top experts in the field of plant biology, an important and unique service will be provided to scientists in plant biology.

207. Washington State University Interdisciplinary Plant Biochemistry Research and Training Center
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The Plant Biochemistry Research and Training Center at Washington State University has this year extendud the range of programs from 13 to 17 participating faculty. In its third year, research training opportunities have been provided for 7 undergraduate students for both academic year and summer student research appointments. Additionally, a total of 15 graduate students (Ph.D. and M.S.) and 3 post-doctorals are currently enrolled in the Center activities and training. The multi-and interdisciplinary programs have been

extended to include: the Plant Biochemistry Seminar Series; the biennial Plant Biochemistry Summer Course (held this year); and the annual review of the Center by eminent plant biochemists. The areas of strategic importance emphasized in the Training Program include: regulation of biochemical pathways; plant cell wall formation; and signal transduction mechanisms. Each trainee overlaps research activities between at least two laboratories in order to gain the necessary interdisciplinary insight and experience.

208. Enhancement of Photoassimilate Utilization by Manipulation of ADPGlucose Pyrophosphorylase
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The goal of this project is to increase the conversion of photoassimilate into starch via manipulation of ADP-glucose pyrophosphorylase (AGP), an enzyme which controls one of the key steps in starch biosynthesis. Using a powerful mutagenesis-glgC complementation system we have generated and identified several up-regulatory enzyme mutants that display increase sensitivity to activation by 3-phosphoglyceric acid (3-PGA) while simultaneously having increased resistance to Pi inhibition. For example, one mutant, UpReg-1, requires only 2 uM of 3-PGA to achieve 50% of maximum enzyme activity. This level of 3-PGA is 50-fold less than that required by the wildtype enzyme. Moreover, when measured in the presence of 250 uM 3-PGA, UpReg-1 requires more than 4700 uM of Pi for 50% inhibition, or 67-fold greater amounts of Pi than that required by the wildtype enzyme. The study of these AGP mutants will not only increase our knowledge on the structure-function relationships of this enzyme and the role of this enzyme in carbon partitioning in source and sink tissues but will lead to novel strategies of increasing starch production and, in turn, increasing overall productivity for many crop plants.

209. Targeting and Processing of the Thiol Protease Aleurain
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We study a plant vacuolar thiol protease, aleurain, that is structurally and functionally equivalent to a mammalian lysosomal protease, cathepsin H. These unique enzymes are poor general endoproteases but have active aminopeptidase activity; they undergo unusual processing such that a short minichain is cut from the enzyme prosequence and disulfide-linked to an extra cysteine in the mature large chain. Aleurain is expressed in most cells in barley but its functional importance to the plant is unknown. We have shown that aleurain is a marker for an acidified, lytic vacuolar compartment distinct from the protein storage vacuole compartment in plant cells that contain storage proteins. As the vacuolar compartment increases in size, the two separate types of vacuoles appear to merge; this may be a mechanism by which storage proteins are exposed to proteases to initiate their degradation. An understanding of mechanisms regulating targeting of aleurain and of storage proteins to their separate vacuoles, and mechanisms by which the two compartments may merge, may provide new approaches towards more effective mobilization of protein reserves. An understanding of mechanisms that control the activation of protease proenzymes may enhance our ability to protect or mobilize those reserves at specific times during plant development. We have identified and cloned a potential receptor protein that binds proaleurain and directs it into the clathrin-coated vesicle pathway to the acidic, lytic vacuole. Future work will expand an understanding of that pathway by identifying and characterizing other soluble vacuolar proteins that are bound by the same receptor. Cytoplasmic proteins that interact with the cytoplasmic tail of the receptor, and thereby participate in the process by which the receptor with its ligand is segregated into vesicles for transport to the vacuole, will be identified and characterized. Another marker for the specific acidic, lytic vacuolar compartment will be obtained by purifying the protease that specifically "clips" proaleurain upon its entry into that compartment. The functional importance of aleurain will be investigated by constructing mutant *Arabidopsis* plants that do not express the *Arabidopsis* equivalent of that enzyme.

210. Genetics in Methylotrophic Bacteria
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The purpose of this project is to study the transcriptional regulation of methanol oxidation (Mox) functions in the facultative methylotrophic bacterium, *Methylobacterium extorquens* AM1. Mox functions include a 14-gene operon (mxaFJGIRSACKLDHEB) containing genes required for production, assembly, and regulation of methanol dehydrogenase, a gene immediately upstream of the mxa cluster, but divergently transcribed (mxaW), a 4-gene cluster (pqqABC/DE) and a 2-gene cluster (pqqFG) required for production of the methanol dehydrogenase prosthetic group, pyrroloquinoline quinone (PQQ), a two-gene cluster (mxdRS) of unknown function, and two additional sets of Mox regulatory genes, mxbDM and mxcQE. Sequence analysis of these regulatory genes suggests that they comprise two sets of sensor protein kinase/response regulator systems. In addition, mxaB appears to encode a third response regulator, but the sensor protein kinase that couples with mxaB is unknown. Methanol-inducible promoters have been isolated for mxaF, mxaW, pqqA, and mxbD, and reporter gene fusion experiments have demonstrated that mxcQE are required for expression of mxbDM, and mxbDM are required for expression of the mxaF, mxaW and pqqA promoters, but not for the mxbD promoter. mxaB is required for expression of only the mxaF and pqqA promoters. Experiments are underway to purify each of the putative response regulators and map their binding to the four methanol-inducible promoters.

211. Biochemical Characterization of the Ethylene Receptor ETR1 in Arabidopsis thaliana
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Ethylene regulates a number of developmental processes in plants including fruit ripening and abscission. The goal of our project is to elucidate the sensory system that utilizes ethylene as a signal. The ETR1 gene codes for an ethylene receptor in Arabidopsis. We are seeking to understand how this receptor senses and transduces the ethylene signal. Using recombinant receptor protein expressed in yeast, we have shown that the receptor binds ethylene with high affinity. A recombinant protein in which the first 128 amino acids of ETR1 are fused to a bacterial glutathione-S-transferase sequence binds ethylene, indicating that the N-terminal domain of the receptor is necessary and sufficient for ethylene binding. Binding activity of the recombinant protein is increased 20-fold when copper ion is supplied in the buffer, supporting the long-standing hypothesis that ethylene interacts with a transition metal at the receptor binding site. We have shown by affinity purification that the copper ion co-purifies with the recombinant protein using wild type ETR1 binding site, but not in the etr1-1 mutant protein that does not bind ethylene. We are developing a molecular model of the ethylene binding domain which accounts for the coordination of a copper ion at the active site. We are also developing procedures to purify the full length ETR1 protein to determine whether ethylene binding regulates activity of the C-terminal kinase domain of the protein.

212. Molecular Genetics of Ligninase Expression
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In addition to playing a key role in the carbon cycle, lignin-degrading fungi have demonstrated potential in a number of emerging technologies. These include biomechanical pulping, bleaching and otherwise improving chemical and mechanical pulps, converting lignin to useful chemicals, effluent treatments, and remediation of contaminated soils. The mechanism(s) involved in these processes are poorly understood, and this represents a barrier to further development.

The long term goals of this research seek to elucidate the basic genetics and physiology involved in the degradation of lignin and related aromatic compounds. Fungal genes expressed in wood and in organopollutant-contaminated soil are being identified using specialized cloning techniques. Focus has been on regulated genes which encode extracellular enzymes of *Phanerochaete chrysosporium* and *Ceriporiopsis subvermispora*. The structure, genomic organization and regulation of these genes are under investigation. Key genes are expressed in heterologous systems and recombinant proteins prepared for basic biochemical investigations.
This research furthers our understanding of mechanism(s) involved in the degradation of lignin and related aromatic compounds. These studies also provide insight into lower eukaryote genome organization with particular emphasis on complex gene families and chromosome dynamics. Development of commercial processes will be greatly facilitated by the identification of key genes and the production of recombinant enzymes.

213. Microbial Formaldehyde Oxidation
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Funding: $92,000 12 months

Our research dissects microbial pathways for formaldehyde oxidation and sensing. This work capitalizes on our discovery of a glutathione-dependent formaldehyde dehydrogenase (AdhI) in the facultative phototrophic bacterium *Rhodobacter sphaeroides*. In photosynthetic cells, AdhI is required to use methylated compounds like methanol as a sole carbon source. Under respiratory conditions, AdhI removes the formaldehyde produced when methanol is co-metabolized. Our experiments also take advantage of mutations that alter *adhI* expression. Under respiratory conditions, the sensor/histidine kinase-response regulator pair (GfdRS) represses *adhI* transcription. A second class of *trans*-acting mutations appear to alter function of an activator of *adhI* transcription (SpdA).

One set of experiments is asking how cells generate energy and one-carbon skeletons from formaldehyde oxidation. Specifically, we are characterizing mutants that are defective in either respiratory methanol co-metabolism or photosynthetic utilization of methanol as a sole carbon source. By determining where individual lesions block formaldehyde metabolism, we will trace the flow of carbon and identify enzymes that remove this compound under respiratory conditions or assimilate it in photosynthetic cells. Simultaneously, we will determine how cells sense formaldehyde and control *adhI* transcription. Specifically, we are testing the prediction that GfdS is a sensor/histidine kinase, asking if formaldehyde controls phosphorylation of this presumed sensor, monitoring GfdR phosphorylation by GfdS, and testing if GfdR phosphorylation increases its binding to a presumed *adhI* operator. To test if SpdA is an activator of *adhI* expression, we are defining presumed gain-of-function mutations that increase transcription of this gene.

214. Identification of the Primary Mechanism for Fungal Lignin Degradation
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Microbial ligninolysis is an essential link in the terrestrial carbon cycle that is thought to be carried out almost entirely by wood- and litter-decomposing basidiomycetous fungi. These organisms produce a variety of extracellular oxidative agents, including lignin peroxidases (LiPs) that have the unusual ability to cleave the recalcitrant nonphenolic structures that predominate in lignin. However, many ligninolytic fungi appear to lack LiP activity, and the mechanisms by which lignin is initially degraded in wood remain unclear. Work on this project has shown that one LiP-negative fungus, *Ceriporiopsis subvermispora*, is able to cleave the nonphenolic arylglycerol-2-aryl ether lignin structure that predominates in lignin. The fungus was given new polyethylene glycol-linked ^{13}C-labeled arylglycerol-2-aryl ether lignin model compounds, and the high molecular weight products that resulted from extracellular fungal attack were analyzed by ^{13}C nuclear magnetic resonance spectrometry. The data showed that the lignin structure underwent two major degradative reactions: cleavage between C1 and C2 of the propyl side chain and cleavage of the 2-aryl ether linkage. These results suggest that *C. subvermispora* produces a ligninolytic agent other than LiP and that this agent oxidizes lignin structures either by ionizing them to cation radicals or via oxyradical addition to an aromatic ring. Further investigation has shown that these reactions are strongly dependent on the presence of Mn: when *C. subvermispora* cultures were grown in the presence of low (0.5 uM) Mn and a polymeric lignin model compound, they exhibited almost no degradative activity towards the compound, but the addition of 150 uM Mn to these cultures elicited rapid degradation within less than 24 h. Since past work on this project has demonstrated that the Mn-dependent peroxidases of *C. subvermispora* are able to initiate lipid peroxidation reactions that cleave nonphenolic lignin *in vitro*, it may be that these Mn-requiring enzymes are responsible for the ligninolytic activity found *in vivo*. Current work is aimed at determining which Mn-dependent enzymes are involved in this process, and whether they are induced at the level of gene transcription.

215. Epigenetic Silencing of the Maize r Gene
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The maize *r* gene product activates expression of structural genes in the 3-hydroxy anthocyanin pathway. Different *r* genes (individual alleles) confer pigmentation on different plant parts. In some strains multiple *r* genes are clustered on chromosome 10 forming complexes. In certain heterozygotes the level of *r* action is heritably silenced, i.e., paramutated. We want to know how the potential of one *r* complex to silence genes in the homologous chromosome is organized and how the change is brought about. Is silencing facilitated when the inciting and responding genes are in the same rather than in homologous chromosomes? When two responding genes and one inciting gene are present in trisomic plants, are the responding genes affected coordinately or independently? When during plant development does silencing occur? We also want to know what other loci are necessary for *r* silencing to occur. Our general strategy is to dissect silencing genetically, then characterize selected aspects molecularly.

216. The Biochemistry, Bioenergetics, and Physiology of the CO-Dependent Growth of Rhodospirillum rubrum
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Rhodospirillum rubrum is a purple, non-sulfur photosynthetic bacterium that is capable of growth with carbon monoxide as the carbon and energy source. CO-dependent growth requires Carbon Monoxide Dehydrogenase (CODH), a NiFeS-containing enzyme that oxidizes CO to CO_2, and a CO-induced, CO-insensitive hydrogenase. The coo regulon includes the cooFSCTJ operon and the cooMKLXUH operon, both under the control of CooA, which serves as the transcriptional regulator for the system. Alterations of specific amino acid residues by site-directed mutagenesis of the cooS gene have yielded important information on the residues suspected of serving as ligands to the NiFeS prosthetic group (C cluster) and the FeS group (B cluster) involved in the redox reactions of the enzyme. Continued characterization of mutant forms of the enzyme will be a goal of the coming grant period. The cooCTJ gene products have been implicated in processing of Ni for the C cluster, and the CooJ protein has been purified and characterized. Its role in binding and processing Ni for CODH will continue to be investigated. The CooC protein has been overexpressed in *R. rubrum* and antibodies to the protein have been generated. The protein will be purified and characterized in the coming grant period. Anti CooC antibodies will be employed in the study of its role in Ni processing. Crystals of CODH have been obtained and the structural analysis of CODH continues.

217. Feedback Regulation of Photosynthetic Processes
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Funding: $97,854 12 months

In this project the interaction between photosynthesis and sucrose synthesis are studied using naturally occurring mutant plants and transgenic plants. Using nuclear magnetic resonance we have shown that during darkness carbon is exported from the chloroplast primarily as hexose phosphate. The hexose transporter must be highly regulated; it must be off during the day, forcing carbon through cytosolic FBPase, and active at night. Characterization of the hexose transporter has been started. The export of hexose phosphate at night bypasses the cytosolic FBPase allowing FBPase-lacking plants to survive. The effects of increasing the capacity for sucrose synthesis on yield are studied using plants expressing sucrose phosphate synthase (SPS) on either a rubisco small subunit promoter or the cauliflower mosaic virus 35S promoter. The SSU promoter increases SPS in the leaf 6 fold and in the fruit 2 fold. The 35S promoter increases SPS in the leaf 2 fold and in the roots 2 to 10 fold. We are manipulating the SPS level using classical breeding techniques to test whether there is an optimum SPS level for yield. To test whether SPS in roots affects yield, young plants were grafted so that either the root or the shoot only was transgenic. Most, but not all, of the effect of transformation results from the extra gene in shoot material. One tomato line routinely exhibits substantially increased fruit yield, as do potatoes with excess SPS. This work will contribute to our understanding of how photosynthesis works and to increased plant yield through biotechnology.

218. Molecular Mechanism of Energy Transduction By Plant Membrane Proteins
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Proton pumps are membrane-bound proteins that act as biological energy transducers. The plasma membrane of plants and fungi contains an ATP-coupled proton pump (H^+-ATPase) that converts chemical into electrical energy, and provides the driving force to catalyze the uptake and accumulation of solutes. The plant/fungal plasma membrane H^+-ATPase contains a single polypeptide of Mr=100,000. Its simple polypeptide structure makes this enzyme an attractive candidate for studying the molecular basis of energy transduction. A major aim of this project is to identify aspects of the enzymes' primary structure that are essential for converting chemical into electrical energy. DNA cloning and sequencing techniques are being used to obtain the complete amino acid sequence for twelve H^+-ATPase structural genes present in *Arabidopsis thaliana*, a model higher plant with a small genome and a rapid generation time. Mutant *A. thaliana* plants in which each of these ATPase genes is disrupted are being isolated. The phenotypes of these plants will help to determine the biological role of each pump isoform *in situ*. Expression of the genes is being studied using Northern blots and 'epitope-tagging' to produce isoform-specific polypeptide probes. Overall, these studies are essential for testing hypotheses concerning the biological role of ion pumps and the molecular mechanism of protein-mediated energy transduction in plants.

219. Analysis of Structural Domains Required for Phytochrome Function by In Vitro and In Vivo Mutagenesis
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Phytochrome is a red/far-red photoreversible photoreceptor that has a central role in light-regulated plant development. To determine how phytochrome functions at the molecular level, we have exploited a biological assay for active chromoproteins that involves the expression of chimeric phytochrome genes in transgenic tobacco. Such ectopic overexpression induces a striking "light exaggerated" phenotype that can be used as an *in vivo* assay of receptor function. The goal of the project is to combine this transgenic system with *in vitro* mutagenesis to identify phytochrome domains important to synthesis, dimerization, chromophore attachment, Pr/Pfr phototransformation, Pfr-enhanced degradation, and biological activity. Preliminary mapping has discovered two distinct domains near the N-terminus of phytochrome A, one required for the structural integrity and biological activity of the chromoprotein, and a second, serine-rich domain that modulates phytochrome activity. Modification of this second domain creates a hyperactive photoreceptor that is 10-50 more sensitive to light. Biochemical and genetic approaches are now in progress to determine the mechanism(s) that underlie this hyperactivity since it may reflect a novel post-translational step that attenuates phytochrome A function. The first 600 amino acids also was found to contain element(s) responsible for the rapid degradation of phytochrome A as Pfr. Appending these domains to the C-terminal region of the more stable phytochrome B confers instability to this chimeric protein as Pfr. Additional sets of phytochrome A/B chimeras are under construction to localize this degradation signal. Completion of this work will represent an important step in understanding the function of this essential photoreceptor.

220. Molecular Characterization of Bacterial Respiration on Minerals
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Aerobic respiration on reduced iron is a principal metabolic activity exhibited by certain chemolithotrophic bacteria that inhabit ore-bearing geological formations exposed to the atmosphere. Each phylogenetically distinct group of iron-oxidizing bacteria expresses one or more unique acid-stable, redox-active biomolecules in conspicuous quantities during aerobic respiration on iron. Structural and functional studies continue on two such novel biomolecules that have been purified to electrophoretic homogeneity, rusticyanin from *Thiobacillus ferrooxidans* and cytochrome$_{579}$ from *Leptospirillum ferrooxidans*. The aim of these studies is to determine the role of each protein in the iron respiratory chain of its respective organism. Other redox-active components present in cell-free extracts of iron-oxidizing organisms continue to be sought, isolated, and investigated with regard to their roles in the same respiratory chains. Another aim is to investigate the mechanisms, consequences, and principal features of bacterial adhesion to insoluble minerals. The specific, selective adhesion of *T. ferrooxidans* to pyrite was recently shown to be mediated by aporusticyanin located on the outer surface of the bacterial cell. Efforts are in progress to identify, isolate, and characterize other mineral-specific receptors expressed in different genera of the chemolithotrophic bacteria that respire on insoluble minerals. It is anticipated that this project will provide useful information toward manipulating *T. ferrooxidans* and related organisms for commercial use.

221. Special Regulation of C4 Genes in C3, C4 and C3/C4 Intermediate Flaveria Species
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The dicot genus Flaveria includes species utilizing C3, C4, and C3/C4 intermediate schemes of carbon fixation. In the C4 scheme, leaf bundle sheath (BS) and mesophyll (M) cells cooperate to make carbon fixation more efficient. CO_2 is assimilated in M cells, then reduced in BS cells, via a C4 pathway that relies on expression of the genes for pathway enzymes in M- or BS-specific patterns. In less efficient C3 species, M cells independently fix CO_2, and the BS is not generally photosynthetic. C3/C4 intermediate species exhibit anatomical and biochemical characteristics between the C4 and C3 extremes. In both C3 and C3/C4 species, C4 pathway genes are present, but used in different spatial patterns. The variety of Flaveria species provides an opportunity to compare the structure and regulation of C4 pathway genes in closely related C3 and C3/C4 species, and to determine the feasibility of increasing the efficiency of the non-C4 species through reregulation of their C4 pathway genes. We have isolated and characterized genes encoding malic enzyme (ME), ribulose bisphosphate carboxylase (rbcS), and malate dehydrogenase (MDH) from C3 and C4 species of Flaveria. We are characterizing the DNA elements associated with the different activity patterns of these genes in Flaveria species, by means of both transient and stable introduction of reporter constructs into C3, C4 and C3/C4 species. The individual C4 pathway genes appear to be spatially regulated via distinct rather than common DNA elements.

222. Calcium Mapping and Signalling in Yeast
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The long-range object of these experiments is to develop a comprehensive and quantitative picture of calcium transport and signalling in a model cell, *Saccharomyces cerevisiae*, whose ion-physiology closely resembles that of higher plant cells. Recent public release of the complete genome sequence for the organism has yielded ~45 genes which may be directly or indirectly involved with calcium metabolism. This number is small on the scale of most eukaryotic genomes, and invites both systematic investigation and surgical engineering. The basis of quantitative functional models must necessarily be quantitative measurements, of intracellular calcium localization and of both intra- and extra-cellular Ca^{++} fluxes.
To facilitate such measurements, we are constructing giant yeast cells in which light-microscopic localization should be simpler than in haploid yeast, and on which electrophysiological measurements should also be greatly simplified. In addition to cytoengineering, patch-clamp measurements are being carried out on a tonoplast calcium channel (YVC1; *P.N.A.S.* 87:7824, 1990), which is not yet identified with a structural gene. A systematic search for this coding sequence is being conducted by motif-scanning, test deletion, and patch-clamping of isolated yeast vacuoles. Two other potential approaches are the identification of high-affinity inhibitors, to be used as protein-purification tags; and drop-out searches of 2-D protein gels following deletion of genes for YVC1-associated proteins. A systematic patch-clamp search is also being conducted for the electrophysiological counterpart to a major plasma-membrane calcium channel, YGR217w.
An important spinoff of the plasma-membrane Ca^{++}-channel search is proving to be the detailed electrophysiological description of other small ion currents in the yeast plasma membrane, including a conspicuous low-affinity inward K^+ current.

223. Essential Cysteine Metabolism in Archaea
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This project will investigate the unique enzymes in archaea which are responsible for the biosynthesis and recruitment to translation of cysteine. Cysteine is one of the twenty canonical amino acids and is required for translation in all known organisms. Despite this ubiquitous requirement for cysteine, no open reading frames encoding cysteinyl-tRNA synthetase and enzymes of cysteine biosynthesis have yet been identified by any of the archaeal genome sequencing efforts. Since these enzymes are essential for cysteinyl-$tRNA^{Cys}$ formation and free cysteine biosynthesis respectively, no adequate explanation yet exists as to how cysteine codons eventually give rise to cysteine residues in archaeal proteins. This suggests that either these enzymes are sufficiently divergent in archaea to prevent their detection by conventional sequence analysis methods or that they are absent. The aim of this

project is to determine the mechanisms of, and possible relationship between, archaeal cysteinyl-tRNA and free cysteine biosynthesis. We have previously shown that tRNA-dependent amino acid transformations are able to functionally replace key enzymes in both translation (aminoacyl-tRNA synthetases) and intermediate metabolism (aminolevulinate synthase). This gives rise to the possibility that archaeal cysteinyl-tRNA synthetase and the enzymes of cysteine biosynthesis are replaced by a novel enzymatic activity capable of converting Ser-tRNA^{Cys} to Cys-tRNA^{Cys}. A biochemical precedent for such an activity is the selenocysteine synthase which catalyzes the tRNA-dependent transformation of serine into the cysteine analogue selenocysteine. We propose to test for biochemical and genetic evidence of both (1) the enzymes of cysteine biosynthesis which have been described in bacteria and eukarya and (2) a direct route for Cys-tRNA^{Cys} formation or an indirect, tRNA-dependent, pathway which may also provide the means of cysteine biosynthesis in archaea. The experimental system of choice will be the archaeon *Methanobacterium thermoautotrophicum* as it is readily amenable to conventional biochemical and genetic analyses and its genomic sequence has recently been completed. Purification, cloning and overexpression of the enzymes necessary for essential cysteine metabolism from this organism will allow us to address the enigmatic evolutionary origin of cysteine biosynthesis in archaea.